ANNUAL MEETING
SFRR-E 2021
Belgrade, Serbia, 15-18 June

Redox Biology in the 21st Century: A New Scientific Discipline

MEETING ABSTRACTS
PRESENTED VIRTUALLY

ORGANIZED BY
Society for Free Radical Research Europe (SFRR-E)

SPONSORED BY

Republic of Serbia
Ministry of Education, Science and Technological Development
Dear Colleagues,

“Those who were fortunate to wake up this morning in Belgrade may believe that they have accomplished enough in their lives. To insist on more than this would be merely immodest” (Serbian poet and writer Dusko Radovic).

With these words, we would like to warmly welcome you to the virtual SFRR-E 2021 annual meeting “Redox Biology in the 21st Century: A New Scientific Discipline” from June 15-18, 2021, presented from Belgrade, Serbia.

Belgrade (Serbian: Beograd, meaning “white city”) is the capital of Serbia and one of the oldest cities in Europe. It lies at the confluence of the Sava and Danube rivers, the position that defined Belgrade as the Door to Europe, the meeting point between East and West, North and South. In its 7000-year-old history, our city was demolished more than forty times, each time reborn and resurrected, like the Phoenix. Today, Belgrade unites diversity, creating a unique spirit of time. In the words of another Serbian writer, Momo Kapor: “Belgrade is not even in Belgrade, because Belgrade, in fact, is not a city; it is a metaphor, a way of life, a perspective on things”.

There are a number of reasons one can say that the 21st century has given birth to a new scientific discipline – Redox Biology. And Redox Biology is also, like any other aspect of science and life, a perspective on things, with the cooperation of opposites in its basis. With a goal. Harmonized in health, out-of-balance in illness. Studying Redox biology: oxidants, antioxidants, redox active molecules and redox regulation is a multilayered endeavor to comprehend the complexity and uniqueness of this regulation. Understanding this complexity will allow for a greater understanding of biology and, life.

This Conference is an attempt to get to know more deeply the core of Redox Biology, the core of life.

With these warm thoughts, we are waiting to virtually meet you in June 2021.

On behalf of the Organizing Committee,

Bato Korac

LOCAL ORGANIZER
Serbian Society for Mitochondrial and Free Radical Physiology
Bato Korac, Aleksandra Jankovic, Andjelika Kalezic
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Keynote Lecture – 1

My encounters with free radical biology: a summing up

Salvador Moncada

University of Manchester, Manchester, UK

Four areas of research have dominated my interest over the years, each of them arising from a finding in our laboratory. Those are, the discovery of the mechanism of action of aspirin-like drugs; the discovery of thromboxane synthase and prostacyclin; the identification of endothelium derived relaxing factor (EDRF) as nitric oxide, the mechanism of its biosynthesis and the study of its biological functions and; the role of mitochondria as signalling organelles in physiology and pathophysiology. In each of these projects we have come across interfaces with free radical biology, some of which we have pursued, some have been pursued by others and some lay until today dormant in the literature. I will during my presentation endeavour to describe some of this work and the areas where I think further research will clarify some significant points.

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Keynote Lecture – 2

An ageing free radical: still moving forward

Barry Halliwell

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In a recent talk “Reflections of an Ageing Free Radical” at the SFFRI online symposium on 18 March 2021, I looked back at my interest in and contributions to the field of antioxidants / free radicals / reactive oxygen species. Indeed, my interest in them began during my D.Phil. in Botany at Oxford in 1971-73, during which I identified considerable production of H$_2$O$_2$ by plant organelles, and its involvement in leaf metabolism. H$_2$O$_2$ is often argued to be a cytotoxic molecule, but actually it is widespread in the environment, including in human urine, water and some of the beverages we drink. Indeed, reactive oxygen (ROS) and related species (RNS, RCS, RSS etc.) play key roles in Biology: they helped drive human evolution and they still shape human development from fertilization onwards. These concepts will be explained, and the major challenges in the field going forward will be presented. H$_2$O$_2$ is an important signaling molecule in many biological processes in vivo, especially in signaling. It can become problematic when it encounters “catalytic” transition metal ions, whereupon much more reactive species such as hydroxyl radical (·OH) are formed. Such metal ions play key roles in neurodegenerative diseases, atherosclerosis and many other conditions. Indeed, an essential antioxidant defence is to sequester them in non-redox-active forms. Yet many antioxidants have failed in clinical studies. Reasons for this will be discussed and novel approaches presented, including discussions of iron chelation and the potential role of ergothioneine, as I transition from “Reflections” to “Still Moving Forward”.

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Basic science award lecture

Arterial redox signaling controlling blood pressure during inflammation

Roland Stocker

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A key determinant of blood pressure is the tone of resistance arteries, which itself is determined by relaxant and constrictor stimuli. Under physiological conditions, arteries constrict readily to endocrine stimuli just as they relax in response to endothelium-derived mediators. Relaxation of resistance arteries is mediated in part by H$_2$O$_2$-induced oxidative activation of protein kinase $\alpha$ (PKG1$\alpha$). During inflammation, however, indoleamine 2,3-dioxygenase 1 (Ido1) becomes expressed in the endothelium of resistance arteries. This enzyme metabolizes the essential amino acid L-tryptophan (L-Trp), and its activity is commonly linked to the innate and adaptive immune systems. In addition, increased endothelial expression of Ido1 is associated with hypotension in experimental models of systemic inflammation as well as in patients suffering from severe sepsis. Using a combination of in vitro and in vivo techniques including mass spectrometry, NMR, photoluminescence, immunoblotting, myography and micromanometry, we observed that Ido1 uses H$_2$O$_2$ to generate singlet molecular oxygen ($^1$O$_2$) that stereo-selectively oxidizes L-Trp to a tricyclic hydroperoxide (cis-WOOH). cis-WOOH then induces arterial relaxation and hypotension in mice via oxidation of cysteine residue 42 of PKG1$\alpha$ in vascular smooth muscle cells. Investigating the presence of the major hydroperoxide-metabolizing enzymes in mouse resistance arteries, in combination with biochemical and kinetic studies, indicate significant differences between H$_2$O$_2$ and cis-WOOH. Specifically, cis-WOOH redox signals in part as a result of stereospecific ‘escape’ from reductive activation. Our observations suggest stereoselectivity as a novel principle underlying in vivo redox signaling that together with conversion of H$_2$O$_2$ into an organic hydroperoxide may help explain the previously reported kinetic conundrum for H$_2$O$_2$ as a redox signaling molecule in vivo.

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Redox-metabolic synergy – a backbone interface for adipocentric approach to metabolic diseases

Aleksandra Jankovic

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Understanding mechanisms that enable/restrict the metabolic plasticity of adipose tissue (AT) is crucial for reducing the global burden of obesity and metabolic diseases. We consistently showed that metabolic reprogramming of adipose tissue is inevitably tied to redox reprogramming in numerous physiological (cold re-acclimation, hibernation) and pathophysiological states (obesity, metabolic syndrome, diabetes, cancer). Cumulative evidence suggests that AT’s redox-metabolic regulatory feedback loop is under pressure in overnutrition states. Namely, an increase in glucose and fatty acid flux via adipocytes shifts the balance of redox systems (NAD(P)/NAD(P)H, thiol - S-/SH and glutathione - GSSG/GSH, antioxidant/prooxidant enzymes), thus increasing the levels of reactive oxygen and nitrogen species (RO(N)S). When produced in a controlled manner, RO(N)S regulate the activities of numerous proteins increasing the lipid buffering capacity. Persistent nutritional overload, however, and consequent oxidative pressure may at any moment exceed the antioxidant capacity of the adipocyte leading to irreversible damage of proteins and other biomolecules, impairing metabolic and endocrine function of AT. Understanding how the “redox milieu” limits AT capacity for lipid storage and oxidation is vital for designing treatment strategies in obesity. Our results show that investigating specific panels of redox biomarkers can clearly “mark” AT depots with disrupted lipid buffering capacity, thus identifying the risk of metabolic syndrome in obesity. Besides, our research aims to target the AT contribution to metabolic diseases such as diabetes, atherosclerosis, and cancer by shifting the AT redox homeostasis. We showed that setting the “catabolic redox milieu” in AT by targeting nitric oxide (NO) and glutathione (GSH) signaling can “ignite” AT by inducing a brown AT-like thermogenic phenotype. Such redox-based approaches (NO supplementation, GSH depletion) show great therapeutic potential as an alternative to natural stimuli such as cold exposure or adrenergic stimulation. In summary, obesity and related metabolic diseases are redox diseases of AT that could be targeted by advancing selective AT-specific redox-based approaches.

Investigations partially Funded by Science Fund of the Republic of Serbia, Program for Excellent Projects of Young Researchers, grant # 6066747.

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Leopold Flohé redox pioneer young investigator award lecture

Redox regulation at the heart of RNA Polymerase III gene transcription machinery

Alessandro Vannini

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RNA Polymerase (Pol) III is responsible for the transcription of short essential RNAs, such as the entire pool of tRNAs, the 5S rRNA and the U6 snRNA. Uniquely in higher vertebrates, the gene of the selenocysteine (SeCys) tRNA is under the control of a unique promoter, whose architecture is distinct from the bulk of Pol III genes named type III promoters. At type III promoters, TFIIIB bridges RNA Pol III to an extragenic TATA box and is composed of Bdp1, TBP and Brf2. Brf2 shares structural and functional features with TFIIB, the canonical Pol II factor but we discovered that Brf2 uniquely contains a redox sensitive structural element. Under oxidative stress conditions, assembly of functional TFIIIB complexes is impaired, resulting in lower Pol III transcriptional output, with a concomitant decrease of SeCys tRNAs levels, culminating in lower expression of the selenoproteome. Thus, in normal cells Brf2 acts as a cellular blockade that in case of prolonged or acute oxidative stress induces apoptosis. In contrast, amplification and overexpression of Brf2, as observed in breast and lung cancers, allow cells to tolerate higher levels of ROS before triggering apoptosis. In summary, we uncover a redox-sensitive module at the heart of the RNA Pol III transcription machinery that impacts basic cellular functions in health and disease.

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Clinical science award lecture

OxInflammation in Rett Syndrome

Giuseppe Valacchi

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Rett Syndrome (RTT) is a progressive neurodevelopmental disorder resulting from mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2). With an estimated incidence of one case per 10,000 live births, RTT is the second most commonly recognized genetic cause of mental retardation in females. Although brain is the main organ affected by MECP2 mutation, RTT manifest a complex multisystem nature with a wide range of clinical manifestations. Among a different variety of metabolic alterations, an oxinflammatory condition characterized by the positive feedback loop between unbalanced redox homeostasis and atypical immune function appears to be a critical player in the onset and progression of RTT pathophysiology. Oxinflammatory biomarkers show a strong correlation with RTT clinical stage and severity in brain and periphery of both animal models and RTT patients. Investigations on the underlying molecular mechanisms reveal an impaired enzymatic defensive activity coupled with an uncontrolled activation of NADPH oxidase in RTT patients. Moreover, alterations in mitochondrial bioenergetics, dynamics and quality control pathways by mitophagy link the redox imbalance to immune dysfunction, by promoting the accumulation of damaged mitochondria able to induce an aberrant NLRP3 inflammasome signalling. In addition, increased levels of lipid oxidation end-products such as 4-HNE and HODEs, oxidized arachidonic and linoleic acid metabolites respectively, can affect RTT immune responses as indicated by the proinflammatoty plasma milieu together with morphological abnormalities of immune cells. Altogether, our data provide new insights in elucidating the altered biological processes associated with the oxinflammation phenomenon in RTT and, furthermore, can help to identify new potential therapeutic targets for a disorder that, at today, is still orphan of therapies.

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Annual award lecture

Epigenetics in clinical practice. Examples in oxidative stress-related diseases

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As it is known epigenetics is defined as the changes in gene expression without modification of the DNA sequence that can be heritable. The pillars of epigenetic regulation are DNA methylation, histone post-translational modifications, histone variants (histone isoforms) and RNA regulation (mainly miRNAs and long non coding RNAs). Epigenetic regulation of gene expression is one of the most powerful homeostatic mechanisms in human physiology that has allowed human beings to survive and adapt for thousands of years to the environmental changes. This robust mechanism of adaptation takes place both in human physiology or disease. Any cellular imbalance that modulates homeostasis has the potential to trigger molecular changes that result either in physiological adaptation to a new situation or pathological conditions. These effects are partly due to alterations in the functionality of epigenetic regulators, which cause long-term and often heritable changes in cell lineages. Oxidative stress has proved to act as a modulator of epigenetic agents and is capable of inducing physiopathological and even deleterious effects. In my dissertation I will present some examples of mRNAs, DNA methylation and histone post-translational modifications that contribute to modification of gene expression in different diseases that are oxidative stress-related. Examples will range from age-related neurodegenerative pathologies to cancer, and include respiratory syndromes, infertility, and systemic inflammatory conditions like sepsis. We will also give some information on the potential use of epigenetic factors as disease biomarkers and also as therapeutical tools. The era of theragnostics is approaching.

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Early bird educational lecture – 1

Genetic variants as modulators of human (patho) physiology

Fabio Virgili

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If we take two randomly selected individuals, we find that more than 99% of their DNA has an identical sequence. The remaining fraction contains stable variations of sequence, the most common (but not the unique one) type of which is constituted by single-nucleotide polymorphism (SNP). These variations are associated with the evident morphological diversity in the population, individuality, and to the susceptibility to diseases. In fact, over the last 20 years, genome-wide association studies (GWAS) have identified more than 10,000 genetic variants significantly associated with various “complex traits” including important pathologies such as CVD, cancer and other degenerative diseases. The presence of multiple variants associated with a diseased trait determines the “risk” of the occurrence of a specific pathology, emerging in particular in the presence of the exposure to also specific environmental/lifestyle characteristics. Even though several variants have been identified within genes involved in redox status, quite surprisingly, studies addressing their effect on oxidative stress and pathologies associated with redox imbalance are very scarce. This lecture will be dedicated to the description of human genetic variability and to gene variants potentially affecting redox status.

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Biomarkers of redox biology in human studies

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Oxidative stress is considered to be an important symptom of various diseases and a driving factor of the aging process. Interestingly, no unique definition exists of oxidative stress and how it contributes to the clinical worsening. Furthermore, no common strategy exists about its measurement. However, a vast number of methods have been developed and used to measure oxidative stress. Many methods of measuring oxidative stress have proven unreliable and no single method exists enabling objective determination and characterization of oxidative stress in human studies or clinical settings. Strategies to measure oxidative stress within the settings of human studies are limited. Major problems are the sample availability, sample stability and cost-, time- and man-power intensive methods. It seems today that sample preparation and stabilization as well as standard preparation are key steps in the accurate quantification of oxidation-related products and examination of physiological/pathological processes. Nevertheless, countless human intervention and observational studies were carried out in both, healthy subjects and patients, and describe oxidative stress measured by various biomarkers, often using also nonspecific methods. However, the literature is very heterogeneous. It is often difficult to draw general conclusions on the significance of the used oxidative stress biomarkers. In order to overcome these current problems, several markers of oxidative stress biomarkers of oxidative stress should be used, addressing different aspects of oxidative stress and the conclusion from the measured biomarkers should be carefully drawn, since oxidative stress might not be a single symptom, but rather reflect various physiological or pathophysiological conditions.

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Multiparametric imaging approaches to dissect the role of ROS and RNS signaling pathways using chemogenetic tools and genetically encoded biosensors

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Nitric oxide (NO) and hydrogen peroxide (H₂O₂) are versatile signaling molecules that belong to the most studied reactive oxygen species (ROS) and reactive nitrogen species (RNS) in biology. The short half-life and high reactivity of NO and H₂O₂ undermine its accurate detection in living cells representing a significant analytical challenge. We exploit powerful biosensors for multispectral measurements of NO and other key signaling molecules in living cells with high spatial and temporal resolution using fluorescence microscopy. In addition, we employ a yeast-derived D-amino acid oxidase (DAAO) as a chemogenetic tool to generate on-demand intracellular H₂O₂ production in distinct cellular locales to dissect the relationship of intracellular H₂O₂ and NO in cultured endothelial cells. Our high-content and multiparametric imaging approaches allow the manipulation of intracellular signaling pathways on subcellular levels and provide critical insights into the molecular mechanisms whereby RNS signaling pathways are regulated by chemogenetically induced ROS.

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Regulation of redox signaling mediated by H$_2$O$_2$ transporters

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The main sources of H$_2$O$_2$ production are physically separated from its targets in and between cells. This condition sustains the formation of gradients that permit the transmission of redox signals by this second messenger. Passive diffusion was long assumed to allow H$_2$O$_2$ transport across lipid bilayers. However, in the last decade it has become clear that H$_2$O$_2$ is routed by a subgroup of aquaporin (AQP) channels, (peroxiporins) whose permeability can be modulated to adapt fluxes and hence signal strength and duration. Thus, the plasma membrane peroxiporin AQP8 is regulated by redox-mediated mechanisms impacting a conserved cysteine, and its open or closed state fuels or inhibits H$_2$O$_2$ downstream pathways, respectively. More recently, we found that AQP11 acts as a resident peroxiporin in the membrane of the endoplasmic reticulum (ER), maintaining organelle redox-tasis. Altogether, our results suggest that different peroxiporins are used to activate different targets depending on their subcellular distribution, interactors and permeability. Therefore, peroxiporins provide to redox signaling an extra layer of spatio-temporal specificity, assuring timed release of redox signal transducers in topologically restrained intra- or inter-cellular locations.

Supported by AIRC, Spanish Ministry of Science and Innovation-UC3M and Marie Skłodowska-Curie program-Polish Ministry of Science and Higher Education.

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Symposium 1-3

Imaging and functional monitoring of redox signaling and metabolism in cardiovascular tissues

Massimo M. Santoro

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Veneto Institute of Molecular Medicine, Padua, Italy

Detecting and measuring the dynamic redox events occurring in vivo is a prerequisite for better understanding the impact of metabolism and redox signaling in physiological and pathological conditions. This aspect is particularly relevant in the cardiovascular tissues wherein alterations in the redox balance are associated with stroke, aging and pharmacological intervention. A still ambiguous aspect in redox biology is represented by how redox changes are occurring in subcellular organelles, mitochondria and nuclei as the most important ones. Here we discuss the use of the zebrafish model together with its easy genetic and optical availability to study redox biology of hearts and blood vessels in living animals. We provide evidence for the usefulness of this model for pharmacological compounds screening by addressing the blockade of glycolysis, pentose phosphate pathway, glutaminolysis, fatty acid oxidation and glutathione synthesis for altering subcellular redox state in vivo. These transgenic zebrafish lines may represent tools useful to characterize the impact of redox changes in living tissues and might offer new exciting opportunities for studying metabolic driven antioxidant response in biomedical research.

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The dynamic redox language of the cell – peroxiredoxin mediated signaling

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The 2019 Nobel Prize in Medicine demonstrated that it is vital to understand how cells adapt to and sense oxygen availability. Failure to do so is linked to nearly every disease and delineating the redox signaling involved is key to developing appropriate therapies. If redox signaling would be compared to a racetrack where racecars partake in a relay race to the “finish line” (i.e. the resultant cellular outcome in response to the signal), peroxiredoxins (Prdxs) would be the racecars at the start receiving the “go” signal that sets their wheels in motion, that then speed to pass the baton to the second racer. This second racer is a binding partner for Prdx, and the signal is peroxides. However, the cellular landscape has kinetic barriers and compartments that could alter the shape of the racetracks. There are also different types of Prdxs, several possible binding partners for each one, and different peroxide signals. Thus, complex coordination is required for accurate navigation and successful completion of these signaling races to get the peroxide signal to the finish line.

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Symposium 2-2

Protein persulfidation: the oldest solution for oxidative stress

Milos R Filipovic
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Life emerged in hydrogen sulfide (H2S) environment and flourished for millions of years before switching to oxygen, but signaling by H2S became recognized as an important new way to control diverse cellular functions only two decades ago. One of the main mechanisms through which this gasotransmitter conveys its message is protein persulfidation, i.e. transformation of protein thiols (PSH) into persulfides (PSSH). Being "one sulfur away" from a thiol, persulfides are not easy to monitor. However, recent developments of new chemical tools for persulfide labeling permitted deeper understanding of the mechanisms of their formation and their role in the cell signaling. Here, we explore the possibility that protein persulfidation represents the oldest and evolutionarily conserved modification of cysteine residues used by the cells to protect proteins from hyperoxidation and loss of function caused by oxidative stress. Using different models (bacteria, yeast, worms, mice, rats and human cells) we demonstrate the inverse correlation between protein persulfidation and oxidative stress-induced cell death and aging.

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Understanding the role of coenzyme A and protein CoAlation in the function of peroxiredoxins and redox regulation

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Coenzyme A (CoA) is an essential cofactor present in all living cells. The discovery of CoA by F. Lipmann in the middle of the last century uncovered its critical role in cellular metabolism and warranted a Nobel Prize. Metabolically active CoA derivatives such as acetyl-CoA are at the center of cellular metabolic and signaling pathways, mediating the citric acid cycle, synthesis of amino acids and fatty acids, the degradation of fatty acids, protein and histone acetylation, gene regulation, neurotransmitter synthesis, and post-translational modifications. Dysregulation of CoA biosynthesis and homeostasis is associated with neurodegeneration, cancer and metabolic disorders. CoA has been recently found to function as an important antioxidant in cellular response to oxidative and metabolic stress, mediated by protein CoAlation (disulfide bond formation between CoA and protein cysteine residues). Protein CoAlation alters the molecular mass, charge, and activity of modified proteins, and protects them from irreversible sulfhydryl overoxidation. To date, more than 2000 CoAlated proteins have been identified in mammalian cells and bacteria exposed to oxidative or metabolic stress. Amongst them are three members of the peroxiredoxins family of antioxidant proteins: peroxiredoxin 3, 5 and 6. Peroxiredoxins are important peroxidases, which reduce a range of peroxide substrates and participate in redox signaling, cell proliferation, differentiation, and hormone signaling. Peroxiredoxins are involved in the pathogenesis of cancer, neurodegenerative, and inflammatory diseases through the regulation of cell growth, metabolism and immunity. There are six family members of mammalian peroxiredoxins which are widely expressed in cells and tissues, but differ by mechanism of enzymatic activation, subcellular localization, and substrate specificity. Using a combination of biochemical, structural, mass spectrometry, and cellular studies, we have exploited the physiological implications of CoAlation on the function of peroxiredoxins 5 and 6.

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Symposium 3-1

Gasotransmitters and thiol redox signaling: a focus on regulated cell death

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The gasotransmitters nitric oxide (NO) and hydrogen sulfide (H₂S) are versatile signaling molecules that are involved in a large number of physiological processes including cell survival and death. While it is appreciated that NO and H₂S can act in an autocrine or paracrine fashion to influence cell survival/death processes in diverse cell types, the underlying molecular mechanisms remain incompletely understood. S-nitrosylation, the formation of S-nitrosothiol (RSNO) by covalent addition to cysteine residues of a NO moiety regulates the function of a broad spectrum of proteins. Similarly, H₂S-dependent S-sulfhydration, the formation of persulfide (RSSH), is emerging as a common mechanism of protein regulation. Endogenous thiol antioxidants such as the thioredoxin system, reverse S-nitrosylation/sulfhydration. Growing evidence indicates that S-nitrosylation and S-sulfhydration are involved in the regulation of multiple cell death modalities, including apoptosis, necroptosis and pyroptosis. In addition, recent research suggests that S-nitrosylation/sulfhydration and thiol antioxidant systems affect the crosstalk between apoptotic and necrotic forms of regulated cell death. Hence, the picture that emerges is that NO and H₂S, largely acting through thiol based mechanisms, influence when and how cells die. The effects of NO and H₂S on cell death decisions may play an integral role in both normal physiology and disease processes.

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Symposium 4-1

Environmental traffic noise triggers stress reactions, oxidative stress, inflammation and vascular dysfunction – comparison of studies in mice and men

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Noise exposure is associated with annoyance, stress, sleep disturbance, and impaired cognitive performance. Epidemiological studies have found that environmental noise is associated with an increased incidence of hypertension, myocardial infarction, heart failure, and stroke. Observational and translational studies in humans indicate that especially nighttime noise increases levels of stress hormones and vascular oxidative stress, which may lead to endothelial dysfunction and arterial hypertension. Novel experimental studies in mice found aircraft noise to be associated with oxidative stress–induced vascular damage, mediated by activation of the NADPH oxidase, uncoupling of endothelial nitric oxide synthase, and vascular infiltration with inflammatory cells. Transcriptome analysis of aortic tissues from animals exposed to aircraft noise revealed changes in the expression of genes responsible for the regulation of vascular function, vascular remodeling, and cell death. Importantly, adverse effects of around-the-clock noise on the vasculature and brain were mostly prevented by Nox2 deficiency. Around the clock aircraft noise exposure of the mice caused the most pronounced vascular effects and dysregulation of Foxo3/circadian clock as revealed by next generation sequencing (NGS), suggesting impaired sleep quality in exposed mice. Accordingly, sleep but not awake phase noise caused increased blood pressure, endothelial dysfunction, increased markers of vascular/systemic oxidative stress, and inflammation. Noise also caused cerebral oxidative stress and inflammation, endothelial and neuronal nitric oxide synthase (e/nNOS) uncoupling, nNOS mRNA and protein downregulation, and Nox2 activation. NGS revealed similarities in adverse gene regulation between around-the-clock and sleep phase noise. In patients with established coronary artery disease, night-time aircraft noise increased oxidative stress, and inflammation biomarkers in serum. Taken together the results of the present studies clearly indicate that transportation noise has to be considered as a cardiovascular risk factor and that noise levels have to be lowered as recommended by the WHO in order to avoid noise-induced cardiovascular and cerebral damage.

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Cutaneous and lung tissues as first targets of ozone induced tissue damage

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Lung and skin are two organs directly exposed to environmental pollution. Although they have different physiological functions, they share some similar tissue aspects. Indeed, in both organs the epithelium is protected, in the respiratory tract by a layer of mucus (RTLF), and in the skin, by the stratum corneum (SC), a multilayer of differentiated keratinocytes (corneocytes). Both RTLF and SC are equipped with enzymatic and non-enzymatic antioxidants molecules able to protect the tissues from oxidative challenges. Ozone is a toxic pollutant, able to induce antioxidant depletion as well as oxidation of lipids and proteins within the outermost skin layer (stratum corneum) and the lung respiratory tract lining fluids (RTLFs), without penetrating the tissue. To further define skin and lung responses to ozone exposure, SKH-1 hairless mice were exposed to either ozone or ambient air 6 h/day for 6 consecutive days. Ozone exposure resulted in the depletion of tissue alpha-tocopherol and induction of heme oxygenase 1, cyclooxygenase 2, and PCNA in both tissues. In addition, Scavenger receptor B1 (SR-B1) which is a trans-membrane protein involved several tissue physiological processes, such as bacteria and apoptotic cells recognition and regulation of intracellular tocopherol and carotenoids levels, was also investigated in response to ozone exposure. In vitro experiments in keratinocytes and lung epithelial cells showed that in both cells type ozone induced the loss of SRB1. This decline was driven by H$_2$O$_2$ as a consequence of NADPH oxidase activation, also confirmed by the use of specific inhibitors. Furthermore, ozone caused the formation of SRB1-4HNE adducts and its ubiquitination, a mechanism that could account for SRB1 protein loss. We conclude that in the models used in this study, ozone induced similar responses in both lung and skin, in terms of inflammation, SRB1 decrease and stress-related responses.

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Symposium 4-3

Exposure to PM$_{2.5}$ Air Pollution Disrupts Circadian Rhythm Through Alterations in Chromatin Dynamics

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Particulate matter ≤2.5μm (PM$_{2.5}$) air pollution is the leading global environmental risk factor contributing disproportionately to the global burden of non-communicable disease. There is an expanding evidence base linking PM$_{2.5}$ exposure to a variety of different NCD’s including cancer and metabolic disorders such as insulin resistance and Type 2 diabetes. Circadian disruption is increasingly implicated as a common denominator for a number of diseases associated with anthropogenic activity including light at night and air pollution. We compared impact of chronic exposure to PM$_{2.5}$ alone, or with light at night exposure (LL) on metabolism. PM$_{2.5}$ induced peripheral insulin resistance, circadian rhythm (CR) dysfunction, and metabolic and brown adipose tissue (BAT) dysfunction, akin to LL (with no additive interaction between PM$_{2.5}$ and LL). Transcriptomic analysis of liver and BAT revealed widespread but unique alterations in CR genes, with evidence for differentially accessible promoters and enhancers of CR genes in response to PM$_{2.5}$ by Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq). The histone deacetylases 2, 3 & 4, were downregulated with PM$_{2.5}$ exposure, with increased promoter occupancy by the histone acetyltransferase p300 as evidenced by chromatin immunoprecipitation (ChIP)-sequencing. These findings suggest a previously unrecognized role of PM$_{2.5}$ in promoting CR disruption and metabolic dysfunction through epigenetic regulation of circadian targets.

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Symposium 5-1

Caloric restriction reprograms fatty acid oxidation in tissue specific manner

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Caloric restriction (CR) is a dietary intervention with food intake reduced without malnutrition. CR has multiple metabolic benefits, delays aging and increases longevity across taxa. CR animals relay on fatty acid oxidation for energy production, but mechanisms of fat oxidation in CR are poorly understood. Recently we reported the effect of CR on fat metabolism in the liver. Mechanistically the expression of rate limiting enzymes in mitochondrial fatty acid oxidation such as CPTs and very long-chain and long-chain Acyl-CoA dehydrogenases (LCAD and VLCAD) were significantly up regulated in CR. The expression of other mitochondrial beta-oxidation oxidation enzymes and peroxisomal beta-oxidation enzymes was not affected in CR. CR induced reprogramming in fat metabolism is associated with increased level of liver short-chain acyl-CoAs, including acetyl-CoA, and cognate carnitines. In the current report we extended this observation to several metabolic and none-metabolic tissues: skeletal muscles, heart, lungs, brain and spleen. The expression of very-long, long, medium and short-chain Acyl-CoA dehydrogenases (ACADs) were investigated around the clock. We found that CR affected the expression of ACADs in time of the day and tissue specific manner and the pattern of CR induced changes in the enzyme expression was unique for each tissue. Circadian clock, an internal timekeeping mechanism, synchronizes processes in organism with environment. Circadian rhythms are significantly impacted by CR and circadian clock contributes to CR through optimization of energy metabolism under condition of limited resources. We propose here that circadian clock driven tissue specific reprogramming of fatty acid oxidation is essential for metabolic flexibility in CR.

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Symposium 5-2

NRF2/KEAP1 pathway is required to fine-tune circadian oscillations as part of the negative feedback loop of the molecular clock: implications for tissue homeostasis and therapeutic interventions

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Disruption of NRF2/KEAP1 antioxidant pathway is linked to many diseases in humans and animals models. We previously identified Nrf2 as a clock-controlled gene. This clock-dependent regulation of Nrf2 leads to daily patterns in NRF2 protein activation on target gene promoters, which is the basis for the rhythmic expression of antioxidant and xenobiotic enzymes. However, how NRF2 feeds back onto the molecular clock circuitry is currently not understood. We investigated the expression of core and output clock genes in a mouse model of Nrf2 DNA-binding loss over a 24hr day/night cycle in several peripheral tissues. Light-entrained Nrf2 KO mice show tissue-specific changes in clock gene expression, with alterations in several feedback loops. This was consistent with real-time bioluminescence imaging of peripheral tissues from Nrf2 KO/Per2::luc reporter mice which showed altered amplitude, phase and period of circadian oscillations. Cell-autonomous changes in clock gene rhythms were recapitulated in primary mouse embryonic and adult lung fibroblasts isolated from Nrf2 KO mice. Similarly, loss-of-function Nrf2 experiments in human cells revealed acute de-repression effects on clock gene expression. Moreover, pharmacological intervention using an Nrf2 activator led to time-of-day activation of antioxidant genes and a simultaneous repression of clock genes in vivo and in vitro. Mechanistically, NRF2 over-expression led to inhibition of positive clock complex CLOCK/BMAL1 on E-box containing clock gene promoters. Finally, we assessed Nrf2 KO mice for rhythmic wheel-running behavior in constant darkness, which demonstrated profound changes in periodicity of activity patterns as well as circadian oscillations in the brain's clock.

These novel results demonstrate that NRF2 is required to fine-tune circadian oscillations by providing a negative feedback to the molecular clock mechanism in a tissue-specific manner. NRF2 dysregulation may contribute to circadian clock desynchrony during ageing, inflammation and diseases such as chronic lung disease and cancer, which has important implications for therapeutic interventions.

Keywords: NRF2; circadian rhythms; antioxidant response; transcription; negative feedback.

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Symposium 5-3

Circadian Rhythms in the Innate Immune System – How the molecular clock shapes inflammation through redox control

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The transcription factor BMAL1 generates daily or circadian rhythms in physiological functions including the macrophage inflammatory response. Intracellular metabolic pathways direct the macrophage inflammatory response, in particular the production of the potent pro-inflammatory cytokine IL-1β. However whether the clock impacts on these pathways is unclear. We now describe that the macrophage molecular clock, through Bmal1, regulates the uptake of glucose, its flux through glycolysis and the Krebs cycle, including the production of the metabolite succinate and ROS to drive IL-1β transcription. We further demonstrate that BMAL1 modulates the level and localisation of the glycolytic enzyme PKM2, which in turn activates STAT3 to drive pro IL-1β production. Overall, this work demonstrates that BMAL1 is a key metabolic sensor in macrophages, and its deficiency leads to a metabolic shift of enhanced glycolysis, mitochondrial respiration, ROS and a heightened pro-inflammatory state. These data provide insight into the control of macrophage driven inflammation by the molecular clock, and the potential for time-based therapeutics against a range of chronic inflammatory diseases.

Keywords: macrophage inflammation; metabolism; ROS; molecular clock; pSTAT3; IL-1

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Redox biology mechanisms involved in muscle and neuronal functions: an overview

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Reactive oxygen species (ROS) comprise a group of chemical species produced by cells in response to intrinsic and extrinsic cues. They are deleterious for several cellular functions owing to their high oxidative properties that can modify the behavior of proteins, lipids, and nucleic acids. However, they also may serve physiological roles acting as signaling molecules that are linked to the control of important cellular features such as cell proliferation, differentiation, and responses to injury. It has been proposed that positive or negative effects induced by the generation of reactive oxygen species might be associated to a local and temporal production, the cellular/molecular source for ROS and the regulatory mechanisms that controls their production, maintenance, and management. It follows that a delicate and controlled balance for their production and functions is an essential component to ascertain their impact into cell biology. Post-mitotically differentiated cells would be highly sensitive to such tight regulation and in this symposium, we will discuss recent advances showing that abnormally high production of ROS supports the onset of muscle pathology such as Duchenne muscular dystrophy (DMD). During aging muscle tone is severely hampered owing to sarcopenia, that impacts the nerve cell component of the neuromuscular junction. The symposium will also address how ROS derived from mitochondria plays fundamental roles in this cell behavior. Finally, production of ROS in the nervous system will be discussed in the context of peripheral nerve regeneration and central nervous system functions. Contrary to the prevalent vision that ROS contribute to degenerative processes in the nervous system, the symposium will show evidence supporting that the production of hydrogen peroxide in neurons is essential to define neuronal morphology but also to support the regenerative capacity observed in axons after an injury.

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Role of NADPH oxidases in remodeling of dystrophic skeletal muscle

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Absence of dystrophin, as seen in Duchenne muscular dystrophy (DMD), makes skeletal muscle more fragile and prone to damage during activities performed by patients in everyday life (e.g. walking), leading to muscle deterioration and progressive muscle weakness. No cures exist and current treatments that slow muscle degeneration are largely ineffective. Reactive oxygen species (ROS) generation is a critical early event integrated with altered calcium homeostasis, inflammation, impaired autophagic flux and alterations in lysosomal biogenesis and function. Ongoing work by our group has identified NADPH oxidases as major ROS generating enzymes that lead to dystrophic skeletal muscle pathophysiology. ROS generated from Nox2, found in the sarcolemma and transvers tubules, activates a Src kinase dependent inhibition of autophagy and lysosome function, leading to muscle degeneration. Intriguingly, ROS generated from Nox4 induces calcium leak through the sarcoplasmic reticulum calcium release channel. The increased junctional space calcium concentration exacerbates Nox2 ROS; with the cumulative effect of disruption of downstream cellular processes that would ultimately contribute to reduced muscle or cellular performance.

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Symposium 6-3

NOX-dependent reactive oxygen species are essential regulators of axonal regeneration

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Understanding the fundamental biological mechanisms responsible for nerve injury-dependent signals controlling the regenerative programme is key to our ability to design strategies for the enhancement of regeneration and recovery after nerve damage. ROS, including superoxide and hydrogen peroxide, have long been recognized to cause cellular and tissue damage oxidizing both DNA and membranes via oxidative stress, eliciting cell dysfunction and death, contributing to cellular ageing, cancer and neurodegeneration. In contrast to this canonical thinking, more recently, a physiological role for ROS in cellular signalling has emerged. This includes the control of cell proliferation, differentiation and metabolism including regulating stem cell quiescence and cell fate commitment. ROS affect key intracellular signalling pathways, downstream trophic factors and inflammatory molecules such as PI3K, p38MAPK, and JNK dependent cascades. Direct reversible oxidation of proteins such as phosphatases and transcription factors are at the core of the signalling role for ROS. Here, we show that ROS are required for axonal regeneration and functional recovery after spinal injury. We found that ROS production in the injured sciatic nerve and dorsal root ganglia requires CX3CR1-dependent recruitment of inflammatory cells, which in turn, once recruited and activated on the lesion site, release exosomes that contain functional NADPH oxidase 2 (NOX2) complexes. These exosomes are then incorporated into injured axons via endocytosis. These multivesicular bodies mature into axonal endosomes, once in axonal endosomes, active NOX2 oxidase is retrogradely transported to the cell body via an importin-β1/dynein dependent mechanism. Endosomal NOX2 oxidizes PTEN, leading to its inactivation, thereby stimulating PI3K-pAkt signalling and regenerative outgrowth. Challenging the view that ROS are exclusively involved in nerve degeneration, we propose a novel role for ROS in axonal regeneration and recovery of function via a NOX2-PI3K-pAkt signalling pathway.

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Redox cross talk from motor nerves to skeletal muscle regulates muscle redox homeostasis

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Minor episodes of denervation of skeletal muscle fibres occur throughout life and are rapidly repaired. Substantial denervation of muscles occurs as a result of trauma and disease and can lead to severe atrophy and eventual loss of the muscle fibres. Age-related loss of skeletal muscle mass and function (sarcopenia) is associated with loss of innervation of groups of muscle fibres and loss of motor neurons. Ageing related changes in muscle are also associated with dysregulation in the generation and/or handling of reactive oxygen species (ROS) and we have investigated the role of denervation in controlling ROS activities within muscle. Data from experimental models in which ROS regulation is modified, such as mice lacking SOD1 (Deepa et al., Free Rad. Biol. Med. 132: 19-23, 2019) and those in which muscles undergo experimental denervation (Muller et al., Am. J. Physiol. 293: R1159-68, 2007) indicate that loss of motor neuron integrity leads to large increases in mitochondrial peroxide generation in the denervated muscle fibres. Furthermore, this increase occurs in mitochondria of neighbouring innervated fibres (Pollock et al., Free Rad. Biol. Med. 112: 84-92, 2017) indicating a propagation of the process within the muscle. Subsequent studies are examining the effect of this process on overall muscle redox homeostasis during ageing and the implications for prevention and treatment of sarcopenia will be discussed.

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Amplification of NET formation induces resolution of inflammation

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Although neutrophil granulocytes are usually regarded as archetypical pro-inflammatory cells, they also can exert anti-inflammatory and immunoregulatory functions. In previous studies, we showed that reactive oxygen species (ROS)-dependent formation of aggregated neutrophil extracellular traps (aggNETs) is crucial for resolution of inflammation of experimental arthritis and lupus. The underlying mechanism involves neutrophil serine proteases that degrade locally released cytokines and chemokines and thereby interrupt ongoing inflammatory processes. In this project we aimed to employ restoration and amplification of NET formation for inducing resolution of innate- and autoimmune-driven inflammation. To this end we made use of NADPH oxidase (NOX) 2-activating sulfonamides and aminoferrocene-based NOX2-independent ROS amplifiers. Preliminary data suggest that the NOX2-activator RE-02 induces (agg)NET formation and alleviates experimental lupus and that the aminoferrocene-based prodrug MIS43 triggers (agg)NET formation and prevents chronification of gouty arthritis in mice with non-functional NOX2. Treatment with MIS43 was associated with lower local levels of inflammatory cytokines/chemokines. Taken together, the studies suggest a therapeutic efficiency of ROS-induction in chronic inflammatory syndromes that occur in the context of defective NOX2.

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Platelet-derived extracellular vesicles express NADPH oxidase-1 (Nox-1), generate superoxide and modulate platelet function

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Background: Platelets release platelet-derived extracellular vesicles (PDEVs) upon activation – in a process that is regulated by generation of reactive oxygen species (ROS). Platelet NADPH oxidase-1 (Nox-1) contributes to ROS generation and thrombus formation downstream of the collagen receptor GPVI. Objectives: We aimed to investigate whether PDEVs contain Nox-1 and whether this is relevant for PDEV-induced platelet activation. Methods: PDEVs were isolated through serial centrifugation after platelet activation with thrombin receptor agonist TRAP-6 (activated PDEVs) or in the absence of agonist (resting PDEVs). The physical properties of PDEVs were analysed through nanoparticle tracking analysis. Nox-1 levels, fibrinogen binding and P-selectin exposure were measured using flow cytometry, and protein levels quantified by immunoblot analysis. ROS were quantified using DCF fluorescence and electron paramagnetic resonance. Results: Nox-1 was found to be increased on the platelet outer membrane upon activation and was found to be present in PDEVs. PDEVs induced platelet activation, while co-addition of GPVI agonist collagen-related peptide (CRP) did not potentiate this response. PDEVs were shown to be able to generate superoxide in a process at least partially mediated by Nox-1, while Nox-1 inhibition with ML171 (also known as 2-APT) did not influence PDEV production. Finally, inhibition of Nox-1 abrogated PDEV-mediated platelet activation. Conclusions: PDEVs are able to generate superoxide, bind to and activate platelets in a process mediated by Nox-1. These data provide novel mechanisms by which Nox-1 potentiates platelet responses, thus proposing Nox-1 inhibition as a feasible strategy to treat and prevent thrombotic diseases.

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Protein kinase Cα regulates the nucleocytoplasmic shuttling of KRIT1

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The KRIT1/CCM1 gene has been clearly associated with the pathogenesis of Cerebral Cavernous Malformation (CCM), a cerebrovascular disease of genetic origin affecting 0.5% of the population worldwide and characterized by abnormally enlarged and leaky capillaries that predispose to seizures, neurological deficits and fatal intracerebral hemorrhage. It encodes for KRIT1, a scaffolding protein whose loss-of-function causes pleiotropic effects, including altered cell-cell adhesion and actin cytoskeleton dynamics, defective autophagy, impaired redox homeostasis, and enhanced sensitivity to oxidative stress and inflammatory events. However, rather little is known about how KRIT1 is regulated. KRIT1 is found in both the cytoplasm and the nucleus, yet the upstream signaling proteins and mechanisms that regulate KRIT1 nucleocytoplasmic shuttling are not well understood. We identified a key role for protein kinase C (PKC) in this process. In particular, we found that PKC activation promotes the redox-dependent cytoplasmic localization of KRIT1, whereas inhibition of PKC or treatment with the antioxidant N-acetylcysteine leads to KRIT1 nuclear accumulation. Moreover, we demonstrated that the N-terminal region of KRIT1 is crucial for the ability of PKC to regulate KRIT1 nucleocytoplasmic shuttling, and may be a target for PKC-dependent regulatory phosphorylation events. Finally, we found that silencing of PKC inhibits phorbol 12-myristate 13-acetate (PMA)-induced cytoplasmic enrichment of KRIT1, suggesting a major role for PKC in regulating KRIT1 nucleocytoplasmic shuttling. Overall, our data point to PKC as a novel regulator of KRIT1 subcellular localization, with potential implications in the regulation of the functions that KRIT1 is known to play in the biology of endothelial cells, including its established key role in redox signaling and antioxidant defenses. This insight may provide a new means for pharmacological regulation of KRIT1 localization, which would be beneficial in studying its functions within specific subcellular compartments, as well as in the development of novel targeted therapeutic strategies for CCM disease.

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Oral Presentations

OP1

Thiol-mediated redox regulation of DICER-LIKE RNaseIII and small RNA metabolism

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Environmental constraints have major impacts on living organisms. Plants, as other species, develop various panels of mechanisms to cope with the effects of these stresses on growth and survival. Redox homeostasis is among the key players of cellular metabolism and cell responses to environmental constraints. It is sensitive to environmental changes and can signal these changes to response pathways, for example by modifying redox status of thiols residues in proteins. In eukaryotic cells, small RNAs (siRNA and miRNA) are major regulators for gene expression, involved in most developmental and stress response processes. The biogenesis of small RNAs is orchestrated by RNaseIII endonuclease enzymes called DICER-LIKE (DCL) and RNASE THREE-LIKE (RTL), which maturate almost all classes of double-stranded RNA precursors. We have showed that the RNaseIII activity of DCL and RTL family members in Arabidopsis thaliana depends on the oxidation state of specific cysteine thiols. Interestingly, the four DCL and the three RTL that carry dsRBD share a conserved cysteine (C230 in Arabidopsis RTL1) in their dsRBD. C230 is essential for RTL1 and DCL1 activities and is subjected to posttranscriptional modification. Indeed, under oxidizing conditions, glutathionylation of C230 inhibits RTL1 cleavage activity in a reversible manner involving glutaredoxins. Moreover, through whole-genome analyses, we showed that the repertoire of small RNA changes with the redox environment of the cell, suggesting that redox regulation of RNaseIII endonuclease enzymes might signal environmental changes to regulate small RNAs metabolism. We conclude that the redox state of the dsRBD ensures a fine-tune regulation of dsRNA processing by plant RNase III that might relay environmental signals to gene expression regulation.

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Regulation of metastasis suppressor NME1 by a key metabolic cofactor coenzyme A

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The metastasis suppressor protein NME1 is an evolutionarily conserved and multifunctional enzyme that plays an important role in suppressing the invasion and metastasis of tumour cells. The nucleoside diphosphate kinase (NDPK) activity of NME1 is well recognized in balancing the intracellular pools of nucleotide diphosphates and triphosphates to regulate cytoskeletal rearrangement and cell motility, endocytosis, intracellular trafficking, and metastasis. In addition, NME1 was found to function as a protein-histidine kinase, 3'-5' exonuclease and geranyl/farnesyl pyrophosphate kinase. These diverse cellular functions are regulated at the level of expression, post-translational modification, and regulatory interactions. The NDPK activity of NME1 has been shown to be inhibited in vitro and in vivo under oxidative stress, and the inhibitory effect mediated via redox-sensitive cysteine residues. In this study, affinity purification followed by mass spectrometric analysis revealed NME1 to be a major coenzyme A binding protein in cultured cells and rat tissues. NME1 is also found covalently modified by CoA (CoAlation) at Cys109 in the CoAlome analysis of HEK293/Pank1β cells treated with the disulfide-stress inducer, diamide. Further analysis showed that recombinant NME1 is efficiently CoAlated in vitro and in cellular response to oxidising agents and metabolic stress. In vitro CoAlation of recombinant wild type NME1, but not the C109A mutant, results in the inhibition of its NDPK activity. Moreover, CoA also functions as a competitive inhibitor of the NME1 NDPK activity by binding non-covalently to the nucleotide binding site. Taken together, our data reveal metastasis suppressor protein NME1 as a novel binding partner of the key metabolic regulator CoA, which inhibits its nucleoside diphosphate kinase activity via non-covalent and covalent interactions.

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Tryptophan and Cysteine residues mediate chain reactions and propagation of oxidative damage in concentrated casein solutions

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Milk is one of the most consumed liquid foods worldwide. The latter is a consequence of its well-accepted flavor, high nutritional value and low price. The co-existence of proteins, lipids and riboflavin (RF, vitamin B2; an endogenous photosensitizer found in milk), along with the harsh conditions encountered during milk processing (e.g. high temperatures, high pressures, light exposure) results in the formation of oxidants. Caseins represent 80% of the total protein content in milk, and due to their amphiphilic nature, they stabilize the oil-water interface; this results in exposure to both hydrophilic and lipophilic oxidants. We hypothesized that exposure of caseins (α-, β-, and κ-casein) to light in the presence of RF, or peroxyl radicals (ROO•) would trigger modifications to the amino acid side-chains of caseins, with downstream consequences for structure and function. We also predicted that the high casein concentrations in milk would facilitate chain reactions and damage propagation. Our results demonstrate that both ROO• and RF-mediated oxidation generates multiple different oxidation products (from Trp, Tyr, Met, His, Cys) and crosslinks including diTyr (from multiple caseins) and disulfide bonds (with κ-casein). At low casein concentrations (1 mg/mL) higher yields of crosslink products were detected by SDS-PAGE. However, at high protein concentrations (10–27 mg/mL) a great extent of total amino acid consumption and modification were detected, consistent with complex oxidation mechanisms. Thus, for example, an increase in the length of chain reactions from 2.21 to 10.52 was evidenced for oxidation of κ-casein at 1 mg/mL and 20 mg/mL, respectively. These results highlight the importance of understanding, at a molecular level, the processes occurring during oxidation of proteins in complex biological matrices such as foodstuffs, to enable the development of new strategies to improve industrial processing methods.

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Hypoxia of human endothelial artery wall cells affects arterial extracellular matrix remodelling and contributes to atherosclerosis development

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During the development of atherosclerosis, the major cause of cardiovascular disease, the vascular wall becomes thickened and remodelled as a result of the formation of atherosclerotic plaques. This results in a hypoxic environment as a result of a limited diffusion and increased demand for O₂. Increasing evidence suggests that hypoxia is a driver of the modifications of extracellular matrix (ECM) in the artery wall during atherosclerotic lesion development. These changes may result in mechanical instability and an increased risk of atherosclerotic lesion rupture, which ultimately results in myocardial infarction or stroke.

Aims: To investigate whether there is an altered composition of the ECM generated by human coronary artery endothelial cells (HCAEC) cultured under 1% compared to 20% O₂ and whether any ECM changes modulate HCAEC adhesion, proliferation, gene expression of inflammatory cytokines, and generation of reactive oxidants. Results: Changes in mRNA expression, and antibody recognition of ECM components, were observed in response to HCAEC exposure to 1% O₂ for 7 days. Marked increases were detected in the expression of the ECM proteoglycan versican. Decreased mRNA expression of cell adhesion molecule, ICAM-1, the inflammatory cytokines TNF-α and TGF-β, and increased oxidant generation were also detected. Consistent with decreased ICAM-1 expression, reduced adhesion of HCAECs to native ECM (generated under 1% O₂) was also observed. In contrast, cellular metabolic activity (as measured by the MTS assay) was increased. Conclusion: These data indicate that 1% when compared to 20% O₂ alters the ECM generated by endothelial cells. The increased production of versican may exacerbate the progression of atherosclerosis, as this proteoglycan is a well-established binding site for lipoproteins, and hence may contribute to lipoprotein retention in lesions. The concurrent increase in oxidant production may exacerbate lipoprotein modification, and the accumulation of lipid-laden cells in developing atherosclerotic lesions.

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The Biological Role of Redox Signalling by the Tumour Suppressor PTEN

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Phosphatase and tensin homologue (PTEN) is an antagonist of the AKT signaling pathway through its recruitment to the cell membrane as part of a multimeric complex and its phosphatase activity against the signalling lipid phosphatidylinositol-3,4,5-phosphate (PIP3). The AKT pathway modulates important numerous metabolic and cell survival processes. PTEN is redox sensitive, and is inactivated by oxidation which causes disulfide bond formation between the catalytic cysteine (C124) and the resolving cysteine (C71), for example by reactive oxygen species (ROS). Radical attack by ROS can also oxidise phospholipids resulting in lipid oxidation products (LOPs) such as acrolein and 4-hydroxyhexenal (4-HHE). This project is characterising the effect of LOPs on PTEN. Recombinant human PTEN-V5-His was overexpressed in Escherichia coli and purified by metal affinity chromatography. LC-MS/MS was used to confirm the integrity of the PTEN-V5-His, and an in vitro phosphatase assay using 3-O-methylfluorescein phosphate verified that the PTEN-V5-His was catalytically active. In vitro phosphatase assay and SDS-PAGE analysis demonstrated a dose-dependent inactivation and aggregation of the recombinant PTEN (PTEN-V5-His) after treatment with 10 µM to 1 mM acrolein (0.2:1 to 20:1 acrolein:PTEN). Significant inhibition of phosphatase activity was found after treatment with ≥100 µM acrolein. Analysis by LC-MS/MS showed a greater susceptibility of cysteine residues to modification, with modification of the resolving cysteine (C71) found even at the lowest acrolein treatments (≥10 µM). At higher acrolein treatment concentrations (≥0.5 mM) lysine modifications (K6, K223 and K313) were detected. As the acrolein concentrations increased the total modifications of PTEN also increased, with a further increase identified in aggregated PTEN. Immobilisation of native and LOP-modified PTEN in an affinity column followed by challenge with cell lysates and proteomic analysis of the captured proteins is being used to identify changes in the interactome of untreated and lipoxidised PTEN that may be responsible for observed cellular effects.

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Autophagy protects murine preputial glands against premature aging, and controls their sebum phospholipid and pheromone profile

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Preputial glands are large lipid and hormone secreting sebaceous organs of mice, and present a convenient model for the investigation of biological processes in sebocytes. Autophagy, an intracellular molecular recycling process, is active in murine skin, and its suppression results in a thickened stratum corneum, disturbed redox responses and enlarged sebaceous glands, secreting an altered lipid profile. We have now extended these studies to the male mouse preputial gland and find that autophagy significantly delays the onset of age-related ductal ectasia, influences lipid droplet morphology and contributes to the complete degradation of the sebocyte-like cells during holocrine secretion. scRNA sequencing of whole preputial glands identified many oxidative stress response and lipid metabolism associated genes that were down-regulated in both sebocyte-like and epithelial cells from the autophagy incompetent glands. Levels of all phospholipid classes, except choline plasmalogen, were decreased in the mutant glands, with a concomitant accumulation of diacyl glycerides. The decrease of the dominant phosphocholine species could be localized to the areas with maturing sebocytes by mass spectrometric imaging. In addition, we found a strong reduction in the amounts of the pheromone palmitoyl acetate. Thus autophagy in preputial gland SLC is not only important for an orderly breakdown of cells during holocrine secretion, but also regulates phospholipid and fatty acid metabolism, redox responses as well as pheromone production.

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Effect of mesenchymal stem cells-derived extracellular vesicles from young mice on senescent myoblasts

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INTRODUCTION: Cellular senescence is a permanent state of cell cycle arrest that contributes to the decline of regenerative potential, to inflammation and to tumorigenesis in aged tissues. Mesenchymal stem cells-derived extracellular vesicles (MSC-EVs) are vehicles of intercellular communication which have epigenetic effects in target cells. The aim of this study is to determine the effect of MSC-EVs from young mice on the senescence of C2C12 myoblasts, as well as to further investigate the underlying mechanisms. METHODS: MSCs were obtained from adipose tissue of young mice and cultured in a low-oxygen environment. MSC-EVs were isolated from the culture medium by differential ultracentrifugation and characterised by flow cytometry and transmission electron microscopy. Senescence was induced in C2C12 cell line myoblasts by treatment with cell cycle inhibitor Palbociclib (5 uM) for 48 hours. These cells were then co-treated with MSC-EVs (5 µg/mL) for 48 hours. Markers of senescence (β-Galactosidase activity), apoptosis (Annexin V) and cell death (DAPI) were determined by flow cytometry. RESULTS: Palbociclib treatment induced senescence in the C2C12 myoblast cultures. Treatment with MSC-EVs significantly decreased both senescence and apoptosis, increasing cell viability in the culture. We observed no differences in specific apoptosis of the senescent fraction of the culture. DISCUSSION: Our results show that MSC-EVs from young mice have a regulatory effect on palbociclib induced-cellular senescence. According to the observed decrease in apoptosis, the reduction in senescence induced by MSC-EVs is not explained by a senolytic mechanism. Alternative mechanisms to be examined in future research lines are oxidative stress, modulation of senescence-associated secretory phenotype and autophagy. In conclusion, this study suggests that MSC-EVs from young individuals could become a promising therapeutic strategy in the field of ageing.

Keywords: ageing; mesenchymal stem cells; extracellular vesicles; intercellular communication; senescence; apoptosis.

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Proteasome activation in C. elegans engages UPRmt

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Protein homeostasis is extensively regulated by a variety of mechanisms that are considered the guardians of the proteome and ensure correct protein synthesis, maintenance, function and elimination, and are called proteostatic mechanisms. These mechanisms tend to fail with ageing, contributing to loss of proteostasis, one of the ageing hallmarks. A growing body of evidence shows that proteostatic mechanisms engage in crosstalk with other cellular mechanisms and various organelles. Our group and others have previously shown that activation of the proteasome that is one of the main cellular proteolytic enzymatic complexes responsible for the degradation of normal and abnormal proteins, can prolong the lifespan of C. elegans and D. melanogaster. However, if and how other proteostatic mechanisms are involved in the phenomenon, remains unexplored. In this study we have focused on the effects of proteasome activation on other proteostatic mechanisms aiming to decipher the involved mechanisms through which proteasome activation confers lifespan extension in C. elegans. We have found that the mitochondrial unfolded protein response (UPRmt) is induced upon proteasomal activation. More specifically, the mitochondrial dynamics and membrane potential are affected in transgenic animals with activated proteasome, thus indicating a mitochondrial defect. Although further investigation is needed, it is possible that a mild mitochondrial defect can account for the lifespan extension found in the nematodes where proteasome activation occurs. Our future work will focus on determining the role that mitochondrial defects may have in the proteasome activation-mediated lifespan extension of nematodes.

Keywords: proteasome; C. elegans; proteostasis; mitochondria.

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Modulation of cerebrovascular dysfunction by dietary nitrate in a rodent model of vascular dementia

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The brain structural/functional integrity relies on a timely and dynamically regulated delivery of metabolic substrates to support the ongoing neural function. This process – neurovascular coupling (NVC) – involves a tight communication between active neurons and vascular cells and has been demonstrated to be critically regulated by nitric oxide (NO) via NMDAr-nNOS pathway. The dysfunction of NVC and global cerebral perfusion has been increasingly associated to neuronal dysfunction in several neurodegenerative conditions (e.g. Alzheimer’s Disease and Vascular Cognitive Impairment and Dementia – VCID), being recognized as relevant contributors to dysfunctional cascade leading neurodegeneration and cognitive decline. We hypothesize that the NVC deregulation may be instigated by oxidative stress conditions and consequent decline in ‘NO bioavailability. In this line, we suggest that dietary nitrate that under hypoxic conditions can act as ‘NO metabolic precursor via the nitrate-nitrite-NO, may be able to circumvent the ‘NO altered bioavailability and sustain the ‘NO-dependent mechanisms in conditions of limited ‘NO synthesis by the canonical pathways. We tested our hypothesis in a rodent model of VCID (2VO rats modeling a global cerebral hypoperfusion) submitted to an oral supplementation of sodium nitrate for 8 weeks. The animals were evaluated in terms of terms of memory performance (Barnes maze), functionally of the neurovascular coupling (laser Doppler flowmetry) and cerebrovascular structure (MRA/IHC). We have found that dietary nitrate was able to expressively attenuate the spatial learning and memory impairment observed in 2VO rats and modulate the neurovascular coupling and the cerebrovascular architecture. Specifically, dietary nitrate enlarged in the temporal profile of hemodynamic responses to glutamatergic activation and controlled the maladaptive vascular remodeling elicited by the global hypoperfusion. Overall data supports that dietary nitrate may improve cognitive function in VCID by modulating cerebral blood perfusion.

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Nrf2-dependent control of redox and metabolic profile in the skin of hibernating ground squirrel (*Spermophilus citellus*)

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Hibernators enter into an extreme state of prolonged torpor characterized by hypometabolism (over 95% metabolic suppression), significant suppression of vital functions, and hypothermia. Specific regulatory mechanisms control physiological processes and metabolic functions that allow hibernators to survive challenging environmental conditions. Skin is one of the most metabolically active organs with intense fluctuations in metabolic rates that are accompanied by adequate changes in the antioxidant status. However, there is little data concerning redox-metabolic-related changes in hibernators skin. This study aimed to investigate nuclear factor erythroid 2–related factor 2 (Nrf2)-dependent control of redox and metabolic responses in the skin (epidermis and dermis) of ground squirrel (*Spermophilus citellus*) in pre-hibernation, hibernation (torpor), and post-hibernation. To that end, we examined the protein expression of Nrf2, hypoxia-inducible factor 1 alpha (HIF-1α), and key antioxidant defense (AD) and metabolic enzymes. Our results showed no change in HIF-1α protein expression during circannual phases, indicating that skin is not hypoxic during hibernation. Moreover, Nrf2 was strongly elevated in hibernation and post-hibernation, accompanied by higher methionine sulfoxide reductase A protein expression in hibernation. Interestingly, in hibernation protein levels of most AD enzymes remain on pre-hibernation level, while post-hibernation was characterized by high expression of manganese superoxide dismutase and glutathione peroxidase, indicating a possible role of Nrf2 in preconditioning during hibernation. Indeed, such AD and Nrf2 increases correlated with upregulation of pyruvate dehydrogenase, citrate synthase, complex III of the mitochondrial electron transport chain, and fatty acid synthase in hibernation and post-hibernation, and with an additional increase in enzymes of mitochondrial and peroxisomal -oxidation in post-hibernation. The results suggest that Nrf2 is involved in the regulation of complex antioxidant response concerning changes in metabolic activity in the skin of ground squirrel in hibernation and post-hibernation to maintain optimal conditions for surviving.

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TRP14 deficiency markedly reduces the inflammatory response in acute pancreatitis through Nrf2 activation

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Acute pancreatitis (AP) is an inflammatory process of the pancreas that often leads to local and systemic complications. AP is currently one of the most common causes of hospital admission concerning gastrointestinal disorders. Two of the major players in the pathophysiology of AP are glutathione depletion and disulfide stress – a subtype of oxidative stress characterized by increased protein cysteinylation without changes in protein glutathionylation. Thioredoxin-related protein of 14 kDa (TRP14, Txndc17) is a cytosolic, ubiquitously-expressed protein, biochemically characterized as a disulfide reductase, and a candidate to regulate protein cysteinylation. Acute pancreatitis was induced in wild-type and TRP14-knockout C57BL/6 mice by seven intraperitoneal injections of cerulein (50 μg/kg) at 1 h intervals, and animals were sacrificed one hour after the last injection. AP induction led to increased cystine and protein cysteinylation levels in pancreas from TRP14-knockout mice, while protein cysteinylation levels dropped in wild-type mice with AP. Protein γ-glutamylcysteinylation increased as well in TRP14-knockout mice upon AP induction, with no changes in protein glutathionylation levels. Besides, both the transsulfuration pathway and GSH synthesis were induced upon TRP14 deficiency in AP. Interestingly, TRP14-knockout mice with AP exhibited increased mRNA expression of enzymes involved in the antioxidant defense (Trx1, TrxR1, Gr, Sod1, Sod2, Cat) as well as specific Nrf2 targets (Nqo-1, Gclc, Ho-1) upon AP induction when compared with wild-type mice with AP. Histological analyses showed decreased tissue edema and less inflammatory infiltrate in pancreas from TRP14-knockout mice with AP. Furthermore, myeloperoxidase activity in pancreatic tissue samples was lower in TRP14-knockout mice upon AP induction. Our results show that TRP14 deficiency triggers up-regulation of antioxidant enzymes and GSH synthesis in acute pancreatitis, at least in part through Nrf2 activation, leading to a milder form of the disease.

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Chlorination and nitration of extracellular matrix by inflammatory myeloperoxidase-derived oxidants in the presence of nitrite

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Oxidants are generated during many physiologic and pathological processes. Over-production is associated with host tissue damage, with this implicated in many human inflammatory diseases, including cardiovascular diseases, cystic fibrosis, asthma, kidney disease and degenerative neurological conditions. Unlike cells, the extracellular matrix (ECM) is poorly protected against oxidation, and evidence has been presented for significant ECM damage in atherosclerotic lesions. Activation of resident leukocytes results in O₂⁻ and H₂O₂ formation and the release of myeloperoxidase (MPO). MPO catalyzes conversion of H₂O₂ and Cl⁻ to the damaging oxidant HOCl but it can also oxidize Br⁻, I⁻, SCN⁻, NO₂⁻ and organic substrates. Although the effects of HOCl are established, modifications induced by the mixture of anions present in plasma is poorly understood. We hypothesized that these ions might modulate the damage induced by HOCl. We have quantified chlorination and nitration damage to both isolated human plasma fibronectin and cell-derived ECM (from human coronary artery smooth muscle cells) induced by a MPO-H₂O₂ system in the presence of Cl⁻, Br⁻, I⁻, SCN⁻, NO₂⁻ and organic substrates. Although the effects of HOCl are established, modifications induced by the mixture of anions present in plasma is poorly understood. We hypothesized that these ions might modulate the damage induced by HOCl. We have quantified chlorination and nitration damage to both isolated human plasma fibronectin and cell-derived ECM (from human coronary artery smooth muscle cells) induced by a MPO-H₂O₂ system in the presence of Cl⁻, Br⁻, I⁻, SCN⁻, NO₂⁻ and organic substrates. Although these data indicate that NO₂⁻ can inhibit chlorination induced by MPO-H₂O₂-Cl⁻. The extent of chlorination was also decreased by other anions (and combinations of these), with SCN⁻ inducing a marked decrease in the extent of damage. Overall, these data shown that both NO₂⁻ and SCN⁻ can modulate damage induced by the MPO system. These studies with physiologically-relevant anion levels better mimic the in vivo situation, and also suggest that elevation of both NO₂⁻ and SCN⁻, which can be readily achieved in humans, may modulate the extent of damage induced at sites of inflammation, including within the artery wall during atherosclerosis development.

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Interference with mitochondrial activity drives the on-set of cardiovascular disease following long-term treatment with SGAs

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The chronic intake of second generation antipsychotics (SGAs) has been related to increased risk of cardiovascular diseases. Taking into account the importance of redox balance in vascular function, we analysed the effect of Ari and Ola in the bioenergetics of primary bovine aortic endothelial vascular cells (BAEC). The results indicate that both Ola and Ari can interfere with mitochondrial function after 3h treatment, increasing the mitochondrial production of O2•−. After 24h, we observed a higher recovery capacity in Ola than of Ari treated cells, suggesting a higher mitochondrial toxicity or a blunted capacity to induce compensatory systems in Ari treated cells. The effects of these drugs on mitochondrial respiration were also measured in peripheral blood mononuclear cells of healthy volunteers treated with Ari or Ola. We found a stronger effect on respiration and increased proton leak, normally associated with increased ROS production and deficient ATP-linked respiration, with changes being more significant for Ari than for Ola. To analyse whether this SGAs can accumulate in mitochondria, we isolated liver mitochondria from mice ip injected with Ari or Ola and found that both Ari and Ola accumulated in the mitochondrial membranes, with Ola showing a trend for higher levels but also faster turnover. To evaluate the physiological effects of this inhibition we treated a mouse model of mitochondrial dysfunction (PGC-1α−/−) with Ari and Ola for 6 months and we observed a reduction in O2 consumption, cardiac fibrosis, left ventricular remodeling and exacerbated cardiac I/R Injury, with all parameters being more evident in Ari than in Ola treated mice. Remarkably, Ola treated mice showed increased mitochondrial content, suggesting that Ola allows a partial compensation by increasing mitochondrial mass. These results suggested that both Ari and Ola interfered with mitochondrial function, and thus lead to increased risk of cardiovascular disease.

Keywords: second generation antipsychotics; aripiprazole; olanzapine; mitochondrial dysfunction; cardiovascular diseases.

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Environmental aircraft noise aggravates oxidative DNA damage, granulocyte oxidative burst and nitrate resistance in Ogg1<sup>-/-</sup> mice

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Background: Large epidemiological studies point towards a link between the incidence of arterial hypertension, ischemic heart disease, metabolic disease and exposure to traffic noise, an independent cardiovascular risk factor. Here, we studied the impact of noise-induced oxidative DNA damage on vascular function in DNA-repair deficient 8-oxoguanine glycosylase knockout (Ogg1<sup>-/-</sup>) mice. Methods and Results: Noise exposure (peak sound levels of 85 and mean sound level of 72 dB (A) applied for 4d) caused oxidative DNA damage (8-oxoguanine) and enhanced NOX-2 expression in C57BL/6 mice with synergistic increases in Ogg1<sup>-/-</sup> mice (shown by immunohistochemistry). A similar pattern was found for oxidative burst of blood leukocytes and other markers of oxidative stress (4-hydroxynonenal, 3-nitrotyrosine) and inflammation (cyclooxygenase-2). We observed no additive effects of noise exposure and genetic Ogg1 deficiency on endothelium-dependent (acetylcholine) relaxation but endothelium-independent relaxation (nitroglycerin) showed a dramatic shift to the right only in the noise-exposed Ogg1<sup>-/-</sup> mice. Conclusions: The finding that chronic noise exposure causes oxidative DNA damage in mice is worrisome since these potential mutagenic lesions could contribute to cancer progression. Human field studies should investigate whether oxidative DNA damage is increased in urban populations with high levels of noise exposure (e.g. as in workers with high occupational noise exposure).

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Glutaredoxin-1 promotes pregnancy-induced vascular complications by altering placental angiogenesis

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Aims: Preeclampsia (PE) is a severe pregnancy complication characterised by increased oxidative stress and high levels of the anti-angiogenic factor sFlt-1 in the placenta and maternal circulation. Yet, antioxidant therapy has failed, in some cases worsening pregnancy outcomes. S-glutathionylation is a common oxidative post-translational modification (ox-PTM) reversed by Glutaredoxin (Glrx) and is emerging as an important redox-switch in cardiovascular diseases. Of significance, preeclamptic placenta are associated with higher levels of Glrx and S-glutathionylation removal during pregnancy promotes preeclampsia-like vascular complications and elevated sFlt-1 levels. Although S-glutathionylation is known to alter angiogenesis by modulating various targets in the VEGF pathway, its role has not been investigated in the context of PE. We aimed to identify the molecular basis for how S-glutathionylation removal may alter angiogenic signalling at the maternal-foetal interface and contribute to PE.

Methods: We combined bioinformatics proteomic analysis with \textit{in vitro} studies and Affymetrix exon-level microarray analyses to investigate the role of protein ox-PTM in angiogenic signalling and development of PE phenotype.

Results: Adenoviral Glrx overexpression disrupted EC angiogenic sprouting, inhibited trophoblast migration and fusion. Glrx mediated angiogenic imbalance by rising sFlt-1:PlGF ratio in EC, while opposite effects were detected in extra-villous trophoblasts. The sFlt-1 changes detected in EC were isoform-specific as the sFlt1-e15a splice variant was elevated while sFlt1-i13 levels remained unchanged. A genome-wide exon-level profiling of overexpressing Glrx transgenic vs littermate control mice placenta revealed a global alteration of alternative splicing events. Bioinformatic analysis identified redox-sensitive targets directly relevant to splicing and PE, and ox-PTM removal was found to disrupt the spliceosome machinery consequently affecting Flt-1 splicing to promote sFlt1-e15a expression.

Conclusions: Glrx-mediated removal of ox-PTMs disrupts placental angiogenic balance in a cell type-specific manner via the modulation of redox-sensitive targets in the spliceosome machinery, which may promote sFlt-e15a release from the placenta and the PE phenotype.

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Identification of a novel hydrogen sulfide-generating *Caenorhabditis elegans* protein, SEMO-1, that is orthologous to human selenium-binding protein 1 and modulates lifespan

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Selenium-binding protein 1 (SELENBP1) was recently identified as a methanethiol oxidase (MTO), catalyzing the conversion of methanethiol to hydrogen sulfide (H$_2$S), hydrogen peroxide (H$_2$O$_2$) and formaldehyde. *Caenorhabditis elegans* is frequently used as a model organism to study the impact of metabolism on aging processes. We previously demonstrated that the proposed *C. elegans* protein and SELENBP1 ortholog Y37A1B.5 is a pro-aging factor that confers selenite resistance (1). Here, we tested whether this protein also has MTO activity and therefore constitutes a novel potential source of hydrogen sulfide in *C. elegans*. First, we developed an MTO activity assay that can be used on isolated proteins and on cell and *C. elegans* lysates. The assay is based on in situ-generation of methanethiol from methionine as catalyzed by a bacterial recombinant L-methionine gamma-lyase (MGL), followed by detection of two of the three methanethiol oxidation products, H$_2$S and H$_2$O$_2$. We isolated recombinant Y37A1B.5 protein, followed by MTO activity analysis and found that it is active as a methanethiol oxidase, similar to recombinant human SELENBP1. We then sought to test for MTO activity of the protein in its natural environment and tested *C. elegans* lysates for MTO activity. To assess the overall contribution of Y37A1B.5 to any *C. elegans* MTO activity, we also used a newly generated Y37A1B.5-deficient *C. elegans* mutant strain. Whereas MTO activity was easily detected in wildtype *C. elegans* lysates, no MTO activity was detected in the mutant strain, suggesting that the Y37A1B.5 protein is the major *C. elegans* MTO. Moreover, life span analysis revealed that the mutant strain had an extended lifespan, similar to the previously reported wild-type worms exposed to Y37A1B.5-specific RNAi (1). In summary, the Y37A1B.5 protein is a novel methanethiol oxidase and therefore a novel potential source of hydrogen sulfide in *C. elegans*. It is also a factor apparently shortening lifespan. Based on its homology to human selenium-binding protein 1, we renamed it SEMO-1 (SELENBP1 ortholog with MTO activity).

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Differentiating rheumatoid arthritis and osteoarthritis by nanodiamond magnetometry

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Arthritis is a common disease which is characterized by a decline of cartilage in joints. It can lead to disabilities and diminished quality of life. There are multiple causes for arthritis which require different treatment and prevention strategies. The two most common types of arthritis are osteoarthritis (OA) where cartilage damage occurs in degenerative diseases and rheumatoid arthritis (RA) where the decline occurs during chronic inflammation of joints. These two types of arthritis are characterized by certain level of oxidative stress that is caused by a variety of molecules produced by synovial fluid cells. Among them, the most reactive free radicals are difficult to detect due to their low concentration and short lifetime. Diamond magnetometry is a new method which offers a solution for some of these issues. The method is based on a fluorescent defect in diamond, which regulates its optical properties based on the magnetic surrounding. Since optical signals can be read out more sensitively, the technique allows nanoscale magnetic resonance measurements. In this study we made use of a specific type of diamond magnetometry called relaxometry. This method is sensitive to spin noise and thus particularly suited for sensing radicals. In this study, we have applied this method for the first time to detect free radicals in samples from arthritis patients. We have found significant difference in level of free radicals in RA and OA synovial fluids and derived cells. The proof-of-concept experiment has also shown that nanodiamond magnetometry enables to monitor efficiency of anti-inflammatory therapeutics in real-time.

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OP18

Mass spectrometry method to profile isoprostanes and neuroprostanes in brain tissue: a study in Alzheimer's Disease

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Alzheimer’s disease (AD) is a neurodegenerative disease with complex aetiology. Due to its high content of polyunsaturated fatty acids (PUFA), the brain is highly susceptible to free radical-mediated oxidative damage. As a result, isoprostanes (IsoP) and neuroprostanes (NeuroP) are commonly observed lipid peroxidation products in brain. However, due to their low abundance, these metabolites are difficult to measure using traditional analytical tools. The aim of this work is to develop a highly sensitive and robust multiple reaction monitoring based LC-MS/MS method for the quantification of 24 different non-enzymatic F-IsoP and F4-NeuroP and to apply this method to analyse post-mortem brain samples from patients with AD. We analysed brain tissues from ten patients with AD and matched control brain tissue samples (0.1 mg) received from Brains for Dementia, UK. Samples were homogenised in 100% methanol and spiked with internal standards (d4-4(RS)-4-F4t-NeuroP, d4-10-F4t-NeuroP and d4-10-epi-10-F4t-NeuroP). Metabolites were enriched using two-step solid phase extraction (SPE) with a polymeric SPE column (HLB PRIME, Waters) and further separation was achieved by LC-MS/MS. Our assay has a linear dynamic range (R2 > 0.93) between 0.04ng/ml-20ng/ml for the 24 F-IsoP and F4-NeuroP. High intra- and inter-day precision (CV < 11%) was observed from the QC samples. Overall, F-IsoP and F4-NeuroP were present at higher levels in AD patient brain tissue compared to healthy subjects. Adrenic acid-derived dihomo-isofurans and docosahexaenoic acid-derived F4NeuroP were significantly higher in AD patients compared to healthy subjects (P < 0.01). This data demonstrate the potential to analyse of different classes of F-IsoP and F4-NeuroP providing new opportunities to study lipid peroxidation in neurodegenerative diseases such as AD.

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Receptor for advanced glycation end products and glyoxalase-1 in the total circulating extracellular vesicles from mild cognitive impairment and Alzheimer’s disease patients

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Background: Growing evidence supports that receptor for advanced glycation end products (RAGE) and glyoxalase-1 (GLO-1) are implicated in the pathophysiology of Alzheimer’s disease (AD). Extracellular vesicles (EVs) are nanovesicles secreted by almost all cell types, contribute to cellular communication, and are implicated in AD pathology. Recently, EVs are considered as promising tools to identify reliable biomarkers in AD. Objective: The aim of our study was to determine the levels of RAGE and GLO-1 in circulating EVs from MCI (mild cognitive impairment) and AD patients and to analyze their correlation with the clinical MMSE and MoCA scores. We have studied the possibility that neuronal cells could release and transfer GLO-1 through EVs. Methods: RAGE and GLO-1 levels were measured in circulating EVs by Luminex assay and Western blot. Released-EVs from SK-N-SH neuronal cells were isolated and GLO-1 levels were determined by Western blot. Results: Our data showed higher levels of RAGE in EVs from late AD patients while GLO-1 levels in EVs from early AD were lower as compared to control and MCI patients. Interestingly, levels of RAGE and GLO-1 in EVs were correlated with the cognitive scores regardless of age. For the first time, we demonstrated that GLO-1 was released from neuronal cells through EVs. Conclusion: Although more samples will be needed, our preliminary results highlighted the potential use of GLO-1 and RAGE levels in peripheral EVs as a clinical signature for AD progression.

Keywords: alzheimer’s disease; mild cognitive impairment; extracellular vesicles; receptor for advanced glycation end products (RAGE); glyoxalase-1.

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OP20
Mitochondrial dynamics and quality control pathways impairment in Rett Syndrome and Autism Spectrum Disorder

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Mitochondrial dynamics and quality control pathways such as fusion, fission and mitophagy are essential for proper functioning of mitochondria. Any dysfunction of these critical processes is linked to deleterious changes in cell and tissue homeostasis and recognized as a pathological contributing factor in a very wide range of disorders, many of which present neurologic and psychiatric symptoms. Here, we investigated mitochondrial dynamics and mitophagy in Rett syndrome (RTT) and Autism Spectrum Disorder (ASD), two neurodevelopmental conditions that share similar symptoms including impaired social interaction and communication, as well as common pathophysiological mechanisms such as the oxinflammation phenomenon.

In RTT, cells obtained from affected patients have networks of hyperfused mitochondria with morphological abnormalities and increased mitochondrial volume. Challenging RTT cells with FCCP did not trigger a proper fusion of mitophagosome with lysosome as assessed by a fluorescence imaging assay. Moreover, analysis of mitophagic flux by immunoblotting revealed an impaired PINK1/Parkin-mediated mitochondrial removal associated with increase of mitochondrial fusion proteins Mitofusins 1 and 2 (MFN1 and 2) and decrease of fission mediators Dynamin related protein 1 (DRP1) and Mitochondrial fission 1 protein (FIS1). Similarly, we found atypical mitochondrial morphology, abnormal bioenergetics profile, and increased mitochondrial superoxide production in ASD cells. Moreover, by evaluating expression of factors regulating mitochondrial fusion/fission dynamics and quality control pathways, we detected a low protein expression of DRP1 along with an increased fusion activity, as indicated by upregulated MFN1 protein expression. In addition, decreased levels of Parkin coupled with enhanced PINK1 expression could be indicative of a possible mitophagy impairment that could lead to an altered clearance of damaged and hyperactive mitochondria in ASD cells. Taken together, our findings confirm the pathological relevance of mitochondrial dysfunction in RTT and ASD, providing the first evidence of similar impairment in mitochondrial dynamics and mitophagy in these close pervasive developmental disorders.

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Sildenafil improves the redox homeostasis and pro-inflammatory activation in systemic sclerosis fibroblasts exposed to reactive oxygen species

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Oxidative stress plays a key role in systemic sclerosis (SSc) pathogenesis and an altered redox-state could be responsible for abnormal inflammatory status, fibrosis and tissue damage. Emerging evidences highlight the beneficial effects of sildenafil, a phosphodiesterase type 5 inhibitor (PDE5i), in protecting different cell lines from cell damage induced by reactive oxygen species (ROS). The purpose of this study was to evaluate the sensitivity of SSc dermal fibroblasts to an oxidative insult and the ability for sildenafil to influence redox homeostasis, cytotoxicity, as well as cell proliferation and cell cycle progression due to ROS action. Additionally, we evaluated its effect on the activation of pro-inflammatory response, fundamental in the perpetuation of oxidative stress-related inflammation in different autoimmune diseases. We demonstrated that SSc fibroblasts have an increased sensitivity to a pro-oxidant environment in comparison to healthy controls. The sildenafil treatment counteracted the negative effects of ROS on cell viability and proliferation, promoting the activity of specific enzymes involved in redox homeostasis maintenance (i.e., GSH/GSSG and MnSOD). Moreover, this PDE5i exerts an inhibitory effect on gene expression and release into the culture medium of selected cytokines (IL6 and IL8) and chemokines (CXCL9, CXCL10, and CXCL11), by interfering with the activation of upstream pro-inflammatory pathways, such as STAT1, STAT3, JNK, ERK, PKB/AKT and p38MAPK, involved in their expression and release. This in vitro study demonstrates, for the first time, that sildenafil administration prevents ROS-induced instability in human dermal fibroblasts isolated by SSc patients. These results support clinical studies to consider the efficacy of sildenafil in the preventing tissue damage and fibrosis in SSc, by targeting central biomarkers of disease progression, vascular injuries and fibrosis and reducing the pro-inflammatory activation induced by oxidative stress.

Keywords: systemic sclerosis; oxidative stress; inflammation; PDE5 inhibitors; chemokines.

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Antiglioma effect of ascorbic acid and menadione combination in U251 glioblastoma cell line is mediated by ROS-dependent downregulation of Akt

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Glioblastoma multiforme (GBM) represents the most common and aggressive brain tumor that still lacks effective treatment options. Tumorigenesis and progression of GBM is tightly connected with over-activation of PI3K/Akt pathway, as well as with perturbed reactive oxygen species (ROS) generation in tumor cells and microenvironment. Breaking the redox balance within the tumor cells by enhancing ROS production is one of the proposed strategies for the treatment of malignancies. The aim of this study was to investigate potential antiglioma effect of ascorbic acid (AA) and menadione (MD) combination (AA+MD), the well-known oxidative stress inducer, and determine the interplay between Akt kinase activity and ROS generation in AA+MD-treated human U251 glioblastoma cells. To this end, U251 cells were treated with AA, MD and AA+MD, in the presence or absence of antioxidant N-acetylcysteine (NAC) or selective Akt inhibitor 10-DEBC hydrochloride (DEBC). Cell viability was assessed using crystal violet and MTT assays, ROS production was evaluated by flow cytometry of dihydrorhodamine-labeled cells, while Akt activity was determined using immunoblot. In contrast to AA and MD alone, combined treatment significantly decreased viability of U251 cells. The prominent toxicity of AA+MD was accompanied by an increase in ROS generation and Akt inhibition. ROS scavenger NAC diminished both Akt inhibition and cytotoxic effect of AA+MD, suggesting that Akt inactivation and cell death induced by AA+MD are ROS-dependent. Additionally, specific Akt inhibitor DEBC further enhanced death of U251 cells and elevated AA+MD-induced ROS production. Collectively, these results suggest that PI3K/Akt serves as pro-survival pathway, and its abolishing due to excessive ROS accumulation leads to glioblastoma cell death. Further, a pro-survival role of PI3K/Akt might encompass ROS removal. In conclusion, treatment with AA and MD, particularly in combination with Akt-targeted therapy, has great potential in combating GBM which is worthy of further investigation.

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Nitroxide radical-containing redox nanoparticles protect neuroblastoma SH-SY5Y cells against 6-hydroxydopamine toxicity

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Parkinson’s disease (PD) patients can benefit from antioxidant supplementation, and new efficient antioxidants are needed. The aim of this study was to evaluate the protective effect of selected nitroxide-containing redox nanoparticles (NRNPs) in a cellular model of PD (human neuroblastoma SH-SY5Y cells treated with 6-hydroxydopamine (6-OHDA)). NRNPs, TEMPO, and 4-amino-TEMPO (25-150 μM) protected SH-SY5Y cells from 6-OHDA-induced viability loss; the protection was much higher for NRNPs than for free nitroxides. NRNPs were better antioxidants in vitro and permeated better the model BBB formed using hCMEC/D3 cells than free nitroxides. Exposure to 6-OHDA decreased the GSH level after 1 h and increased it considerably after 24 h (apparently a compensatory overresponse); NRNPs and free nitroxides prevented this increase. NRNPs and free nitroxides prevented the decrease in ATP level after 1 h and increased it after 24 h. 6-OHDA increased the intracellular ROS level estimated with dihydroethidine 123 and mitochondrial superoxide level assayed with Fluorimetric Mitochondrial Superoxide Activity Assay Kit. All antioxidants studied mostly decreased ROS and superoxide levels. 6-OHDA decreased the mitochondrial potential evaluated with JC-1 and mitochondrial mass assayed with 10-nonyl-Acridine Orange; both effects were prevented by NRNPs and nitroxides. These results suggest that the mitochondria are the main site of 6-OHDA-induced cellular damage and demonstrate a protective effect of NRNPs in a cellular model of PD.

This study was performed within the project “Nanomolecular antioxidants: biological basis of targeted therapy of neurodegenerative diseases” (number of the application 2016/22/E/NZ7/00641) financed by the National Science Centre, Poland.

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OP24
Hydrogen sulphide reduces TNF-α-mediated endothelial dysfunction by improving mitochondrial function

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The development of endothelial dysfunction is initiated, at least in part, by mitochondrial reactive oxygen species production in endothelial cells. Chronic inflammatory insults such as tumour necrosis factor-alpha (TNF-α) affect cellular redox state and mitochondrial superoxide production associated with mitochondrial dysfunction. Exogenous Hydrogen sulphide (H₂S) has been reported to rescue mitochondrial dysfunction via its antioxidant properties. However, full understanding mechanisms involved in the effects of H₂S in the preservation of endothelial and mitochondrial function have not been elucidated. This study aims to understand how slow-releasing H₂S donor GYY4137 affect mitochondrial function in endothelial cells. Human vein endothelial cells (HUVECs) were maintained in complete endothelial growth media. Cells were treated with one ng/mL TNF-α for 3 hours, followed by washing and 21 hours post-treatment with 100 µM GYY4137. Cellular hydrogen peroxide and mitochondrial superoxide levels were measured as CM-DCFDA and MitoSox oxidation, respectively. RT-PCR measured the expression of antioxidant genes, and mitochondrial membrane potential (Δψm) was analysed by immunofluorescence using the JC-1 probe. Mitochondrial bioenergetics were measured using an XF24 Seahorse Analyser. GYY4137 post-treatment successfully attenuated intracellular hydrogen peroxide and mitochondrial superoxide levels in HUVECs exposed to TNF-α. GYY4137 treatment enhanced thioredoxin and heme oxygenase-1 gene expression. The Δψm was re-established upon GYY4137 treatment. Exogenous H₂S rescued TNF-α induced mitochondrial proton leak in HUVECs. Cellular oxygen consumption linked to ATP increased in the presence of GYY4137 post-treatment. Interestingly, extracellular acidification rates (ECAR) parameters are increased in TNF-α-treated HUVECs, potentially as a compensatory mechanism to sustain cellular energetics demands. Following GYY4137 post-treatment for 21 hours, ECAR parameters remained elevated in TNF-α-treated HUVECs. The results demonstrate the protective roles of exogenous H₂S against TNF-α induced endothelial dysfunction by restoring redox state and cellular bioenergetic functions. These findings support a role for H₂S as a promising therapeutic molecule for endothelial dysfunction.

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Microglial signaling mediates oxidative and inflammatory response to aircraft noise via lysozyme m+ cells

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Background: Epidemiological data support a significant impact of traffic noise exposure on cardiovascular health. Animal studies identified noise-induced immune cell activation and infiltration into vascular tissues with subsequent oxidative damage and impairment of vascular function as key mechanisms. Methods: Using the LysMCre iDTR mouse model, we conducted selective ablation of LysM+ cells with diptheria toxin and confirmed our ablation via FACS in blood and aorta and to probe changes in immune cell populations in brain. We used western blot, dot blot, immunohistochemistry and rtPCR to assess protein and gene expression. Isometric tension and video microscopy yielded insight to macro- and micro-vessel reactivity. To measure oxidative stress, we used HPLC and DHE staining. Results: LysM+ cell ablation normalized endothelial function and ROS levels in the aorta. Increases in NOX2, VCAM-1, and eNOS mRNA and other oxidative and inflammatory parameters in the aorta were also prevented by LysM+ ablation. However, interestingly, levels of corticosterone, adrenaline, noradrenaline, IL6 and IL1-β in plasma were elevated in noise, DTX, and DTX Noise groups. We also found partial normalization of ROS levels and endothelial function in retinas of ablated noise-exposed mice, indicating another source of inflammatory signaling and oxidative stress. We discovered significant microglial activation in the brain following noise exposure that was not attenuated by DTX treatment, as demonstrated by CD68+, CD80+ and MHC-II+ cell count and considerable infiltration by peripheral immune cells into the brain. Additionally, parameters of the NFκB-NLRP3 pathway were upregulated. Conclusion: Our data indicate a robust microglial response to noise-induced stress and imply a signaling mechanism to peripheral cells across the blood-brain-barrier. LysM+ cells play an important role in the hypertension and damage arising from noise exposure and appear to be important mediators of the stress response from the brain to the periphery.

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Effects of changes in ambient oxygen levels and hypoxia-reoxygenation on intracellular zinc levels in human coronary artery endothelial cells

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Zinc deficiency is a significant risk factor for coronary heart disease and zinc supplementation has been reported to afford protection against ischemia-reperfusion injury. Notably, zinc and other key metals play important roles in cellular responses to changes in oxygen tension. We have investigated the effects of adapting (5 days) human coronary artery endothelial cells (HCAEC) to standard culture conditions (18 kPa O2, hyperoxia), physiological normoxia (5 kPa) and hypoxia (1 kPa O2). HIF-1α was not stabilised in cells adapted to 5 kPa O2, although induction of Nrf2-target genes was attenuated and the expression of metal transporters and binding proteins were significantly altered. When inductively Coupled Plasma Mass Spectrometry (ICP-MS) was employed to determine whether changes in ambient O2 affect total metal content, total zinc levels (ng/µg protein) were 0.345 (18 kPa O2), 0.267 (5 kPa O2) and 0.117 (1 kPa O2), respectively. Moreover, subjecting HCAEC hypoxia for 1 h and reoxygenation did not significantly alter total zinc levels. Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) of cultured HCAEC monolayers enabled us for the first time to monitor changes in the spatial distribution of zinc and other metals, confirming our findings using ICP-MS analysis of cell lysates. To confirm that hypoxia-reoxygenation induces redox stress, we examined mobilisation of labile iron using the fluorescence probe FeRhoNox-1, and noted a significant increase in Fe2+ on reoxygenation. These findings provide novel insights into the role of metal homeostasis in vascular cell culture under defined ambient oxygen levels and may thereby inform therapeutic interventions ischemia-reperfusion injury.

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Lipid peroxidation induced by UV-A and UV-B irradiation of protoporphyrin IX in MLV liposomes. TBA-MDA test

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Metal-free porphyrin - protoporphyrin IX (PPIX) has great potential as a photosensitizer in various applications. Liposomes are phospholipidic submicroscopic vesicles which can be used as the drug-delivery systems for PPIX in controlled transport systems. Lipid peroxidation (LP) is a following phenomenon involved in the oxidative damage to cell structures. Main goals of this study were production of multilamellar vesicles (MLV) incorporated with PPIX and detection of LP level after their irradiation by UV-A and UV-B light due to PPIX photosensitizing role. PPIX-loaded MLV liposomes were prepared using dry PPIX-lipid-film method. Lipid peroxidation was initiated by photosensitization reaction of PPIX-MLV with UV-A and UV-B irradiation. Formation of the LP product malon-dialdehyde (MDA) with thiobarbituric acid (TBA) was measured spectrophotometrically by using TBA-MDA test. The irradiation treatments were performed in photochemical reactor with emission maximums at 350 and 300 nm (UV-A and UV-B, emission flux at 12.9 Wm⁻² and 15 Wm⁻², respectively). Irradiation of PPIX-MLV system resulted in LP process detectable as continuous increase in absorbance band at 530 nm due to production of TBA-MDA, in both cases. Lipid peroxidation obeys first-order kinetics with the calculated rate constants 0.0109 and 0.0178 min⁻¹ for UV-A and UV-B irradiation, respectively. In the samples without photosensitizer, or samples with photosensitizer kept in dark, LP wasn't detected. The irradiation induces LP in PPIX-MLV system and UV-B is faster in comparison to UV-A induced LP, due to energetic differences.

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The antioxidant activity of ethanolic extracts from black pepper (*Piper nigrum* L.) fruits obtained by different extraction techniques

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Black pepper (*Piper nigrum* L.) is a flowering plant, originating from the evergreen forests of Southwestern India and Southeast Asia. Black pepper is mainly used as a spice, but has also been explored for its biological properties and its bioactive compounds. This study aimed to determine the antioxidant potential of black pepper fruits ethanolic extracts (BPFEE) obtained by different extraction techniques (maceration-M, reflux extraction-RE, ultrasonic extraction-UE, and Soxhlet extraction-SE), using several antioxidant assays (DPPH, ABTS, FIC, FRAP, and Ferricyanide method). The total phenols and total flavonoids content was determined spectrophotometrically by the method of Folin-Ciocalteu and AlCl$_3$, respectively. The BPFEE obtained by UE at a room temperature resulted in the highest phenolic content (85.64 mgGAE/g d.e.), while the highest flavonoid content was observed for BPFEE obtained by RE at a boiling temperature (97.56 mgRE/g d.e.). BPFEE obtained by RE at a boiling temperature was found to possess the highest antioxidant activity including the scavenging of DPPH and ABTS radicals (EC$_{50}$ values determined by DPPH and ABTS tests were $0.112 \pm 0.0012$ mg/ml and $1.010 \pm 0.002$ mg/ml, respectively). The same extract was reached the maximum iron ions chelating ability determined by the FIC test (EC$_{50}$ value was $1.146 \pm 0.0155$ mg/ml), and the highest reducing effect (FRAP value was $67.82 \pm 0.08$ mgEFe$_{2+}$/g d.e.). The best ability to reduce the ferric-ferricyanide (Fe$_{3+}$) complex has shown BPFEE obtained by UE at room temperature with a value of $31.2 \pm 0.22$ mgGAE/g of dry extract. A significant correlation was found between the antioxidant activity of extracts and their total phenolic and flavonoid content. The presented results show that there is space for further investigations into the isolation and identification of antioxidant components and their mechanism of action to better understand their ability to be used as a safer alternative antioxidant agents.

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The uptake and metabolism of α-tocomonoenol in HepG2 cells is more similar to that of α-tocopherol than that of α-tocotrienol

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Introduction: α-Tocomonoenol (T1) is a minor derivative of vitamin E and has been reported for different food items. No information regarding the cellular uptake and metabolism of αT1 is currently available. Since the chemical structure of α-tocomonoenol resembles that of α-tocopherol more closely than that of α-tocotrienol, we hypothesized that its uptake and metabolism will be more similar to the tocopherol.

Methodology: The uptake of α-tocomonoenol, α-tocopherol and α-tocotrienol and their conversion into metabolites (α-CMBHC and α-CEHC) was investigated in HepG2 cells incubated for 48 and 72 h.

Results: The uptake of α-tocomonoenol into HepG2 cells was similar to that of α-tocopherol for both incubation periods and significantly lower than that of α-tocotrienol at 72 h. Fewer metabolites were produced from α-tocomonoenol than from α-tocotrienol, while no metabolites were produced from α-tocopherol. α-CMBHC, not α-CEHC, was the main metabolite produced.

Conclusion: The uptake and metabolism of α-tocomonoenol in HepG2 cells is more similar to that of α-tocopherol than that of α-tocotrienol, which may have implications regarding the biological activities of this congener.

Keywords: α-Tocomonoenol; HepG2; short chain metabolites; vitamin E.

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Human antioxidant A1M protects renal tubule epithelial cells from heme-induced damage in vitro

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Acute kidney injury and chronic kidney disease progression are associated with oxidative stress, which is defined as an increase in reactive oxygen species and/or decrease in antioxidant defense. Since there are limited treatment options for these disorders it has been suggested to use antioxidants, for example $\alpha_1$-microglobulin (A1M). A1M is a human reductase, radical scavenger and heme-binding protein which can be internalized in cells and protect mitochondrial function. Previously, A1M was shown to ameliorate renal damage in animal models of preeclampsia and radiotherapy. Therefore, we studied the protective effects of recombinant A1M (rA1M) in two kidney cell lines, HK-2 and RPTEC, exposed to heme-induced oxidative stress. Both cell lines demonstrated increased viability after co-incubation with rA1M during addition of heme. Stress-related genes, analyzed with both qPCR and the in-situ hybridization technique RNAscope, displayed lower up-regulation in presence of rA1M. The Seahorse extracellular flux analyzer was used to examine parameters of the mitochondrial function. In response to heme, mitochondrial function was affected in the HK-2 cells but were normalized to control levels when rA1M had been added. In conclusion, our data suggest a protective role by A1M, which is attributable to preserving mitochondrial respiration in addition to reducing heme-associated oxidative stress.

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Necrostatin-1 enhances menadione/ascorbic acid–induced oxidative stress and their cytotoxic potential in human glioblastoma U251 cell line

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The aim of this study was to investigate the role of necroptosis inhibitor necrostatin-1 (Nec-1) in death of human glioblastoma U251 cells exposed to ascorbic acid (AA), menadione (MD), and their combination, in vitro. Nec-1 augmented cytotoxicity of AA+MD, and slightly increased death of MD-treated U251 cells, as assessed by crystal violet (CV) assay. In line with previous, flow cytometric analysis of annexin/propidium iodide-stained cells showed that Nec-1 triggered cell death in MD and significantly enhanced ability of AA+MD to increase number of necrotic cells, substantiating necrosis as the mechanism of U251 cell death induced by combined treatments – AA+MD, Nec-1+MD, and Nec-1+AA+MD. Further, Nec-1 elevated mitochondrial and cellular reactive oxygen species (ROS) generated by both MD and AA+MD co-treatment, as assessed by flow cytometry analysis of MitoSOX- and DHR-stained cells, respectively. N-acetyl cysteine (NAC), a well-known antioxidant, opposed U251 cell death induced by AA+MD, Nec-1+MD, and Nec-1+AA+MD, indicating crucial role of oxidative stress in Nec-1-potentiated cytotoxicity of MD and AA+MD. Also, Nec-1 activated AMP-activated protein kinase (AMPK), and its effector molecule ULK1 (Ser317) over the level induced by MD and AA+MD, as showed by immunoblot. AMPK, highly conserved serine/threonine protein kinase, is activated under the conditions of oxidative stress probably as a consequence of depleted cellular ATP and elevated AMP levels. This result implies important role of AMPK in necrosis detected in AA+MD-, Nec-1+MD-, and Nec-1+AA+MD-treated U251 cells. Therefore, it can be concluded that ability of Nec-1 to enhance cytotoxic potential of AA+MD co-treatment and trigger cytotoxicity of MD is associated with its capacity to amplify cellular and mitochondrial ROS production, leading to necrosis-like cell death of U251 cells. Obtained results reveal potential use of Nec-1 as anti-glioblastoma agent, especially in combination with AA+MD.

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Pyrazolo[3,4-d]pyrimidine derivatives, Si306 and pro-Si306, induce oxidative stress and cell death in patient-derived glioblastoma cells

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Glioblastoma is the most frequent and aggressive brain tumor in adults. The main characteristics of glioblastoma include high proliferation rate, infiltrating nature, and resistance to chemotherapy and radiation. Glioblastoma cells highly express Src tyrosine kinase which has a key role in regulating survival, proliferation, angiogenesis and invasiveness of tumor cells. This tyrosine kinase is also an important regulator of reactive oxygen species production and cellular homeostasis. Anticancer properties of two pyrazolo[3,4-d]pyrimidine derivatives and Src tyrosine kinase inhibitors, Si306 and its prodrug pro-Si306, were investigated in human glioblastoma cell line U87, its multidrug-resistant (MDR) counterpart U87-TxR, and patient-derived glioblastoma cell culture. Si306 and pro-Si306 triggered reactive oxygen species and reactive nitrogen species production in sensitive and MDR glioblastoma cell lines and primary glioblastoma cells as evidenced by elevated levels of superoxide anion, hydrogen peroxide and peroxynitrite anion. Additionally, western blot analysis revealed elevated expression of superoxide dismutase 1, superoxide dismutase 2, and thioredoxin reductase 1 in glioblastoma cells after Si306 and pro-Si306 treatments. The levels of phosphorylated histone H2A.X increased in all glioblastoma cells after treatments with these inhibitors, demonstrating DNA damage. Both compounds also induced significant cell death in primary glioblastoma culture. In addition, the Src tyrosine kinase inhibitors prompted primary glioblastoma cells to enter senescence. The presence of the MDR phenotype did not reduce the activity of the compounds. Overall, the investigated pyrazolo[3,4-d]pyrimidines displayed significant anti-glioblastoma effects making them good candidates for further development as anticancer agents.

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Molecular mechanisms of antitumor activity of 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin in human colon cancer HCT-116 cells

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The aim of this study is to investigate molecular mechanisms of antitumor effects of 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin on human colon cancer HCT-116 cells. The cells were treated with increasing concentrations of compound (0.01 μM up to 100 μM) during 24 h (short-term) and 72 h (long-term) to evaluate the cytotoxic effect by determining lactate dehydrogenase release and by MTT assays. The concentrations with optimal cell inhibiting effects (1 μM and 10 μM) were selected for further examination of this compound to cell cycle, ERK signaling and activation of caspases. After 72 h treatment the expression of anti-apoptotic and pro-apoptotic proteins was detected by Western blot analysis. Both time treatments in tested concentrations led to an increase in the number of cells in the G0/G1 phase of the cell cycle compared to non-treated cells, with the most significant antiproliferative effect in 72 h treatment at concentration of 1 μM, where more than 40% of cells were arrested in G0/G1 phase. At both tested concentrations, 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin stimulated caspase activation, detected by an increasing of Apostat fluorescent signal. The treatments also decreased expression of the ERK1/2 kinases, as one of the most common signaling targets of various antitumor therapeutics. The obtained results of immunoblot analysis show significant decrease in the phospho/total ratio of ERK1/2 in HCT-116 cells after treatment with both applied concentrations, compared to the values in non-treated cells. This result certainly indicates that the treatment with the tested 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin significantly attenuates the signaling pathway mediated by ERKs. Both total ERK1/2 and its phosphorylated form levels were elevated in HCT-116 cells compared to non-malignant cell lines, while in our treatment the total and phosphorylated ERK1/2 pools were reduced compared to non-treated cells, indicating that the antitumor effects recorded in this study were, at least to a certain extent, achieved through interference with the ERK signaling pathway.

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Spin-labeled hydrogels for cell viability assessment by EPR

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Cancer is the second leading cause of death worldwide, and enormous efforts are put into discovering natural, or synthesizing new anticancer drugs. Testing their effectiveness is time-consuming, as it requires a multitude of assays specific for different molecular targets that play key roles in cancer cell destruction. The aim of this work was to develop a prototype hydrogel that would quickly and accurately assess the cytotoxicity of anticancer drugs in preclinical studies, by designing a biocompatible protein serum albumin hydrogel that would chemically respond to its redox environment. The experimental approach is based on the fact that in vivo reduction kinetics of electron paramagnetic resonance (EPR) active spin probes are different in healthy and hypoxic tumor tissues. If a potential drug leads to cancer cell destruction, it would change the probe redox environment, resulting in tissue-specific reduction kinetics. The presented results describe the initial phase in the hydrogel prototype design, in which its suitability for cell redox state assessment is tested in the yeast (Saccharomyces cerevisiae) model system. The results showed that 3-carbamoyl-PROXYL-labeled hydrogel is sensitive to cell viability, and most importantly that this can be visualized by EPR imaging. Moreover, it was determined that the EPR signal decrease is proportional to the number of live yeast cells, whereas the temperature-inactivated yeast did not cause signal loss. This is consistent with the fact that spin probes convert to their EPR-invisible hydroxylamine anion/oxoammonium cation forms in living organisms, due to redox reactions with biological compounds, enzymes, and transition metal ions. The applicability of the described prototype, which represents the “diagnostics” part of the hydrogel, will subsequently be tested in cancer cell lines. Finally, taking advantage of albumin’s extraordinary drug-binding capacity, the prototype will further be developed into an anticancer drug depot-anchoring hydrogel intended for molecular imaging of drug treatment response.

Keywords: protein hydrogel; EPR imaging; cell viability; redox state; cancer.

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Antioxidant activity of *Saccharomyces boulardii* in human serum

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Recent studies have shown antioxidant activity of *Saccharomyces boulardii* (SB) in several digestive system-related diseases. Our aim was investigating potential antioxidant activity of SB in human serum pool (SP) as an indicator of strain’s activity in biological environment. Standard strain of *Saccharomyces cerevisiae* var. *boulardii* (ATCC® MYA-796) was prepared as saline solution (SS) in concentrations: 5, 2.5 and 1.25 million. Two groups of samples were formed: lyophilised SB and SB cultivated in Sabouraud dextrose agar with chloramphenicol, incubated 2 h or 24 h at 37 °C. SP/Trolox and SP/TBH were used as internal controls. All SB samples were prepared in SP and with TBH or Trolox additions in order to confront strong prooxidant and strong antioxidant with SB, respectively. Two antioxidative (total sulphydril groups, total antioxidative capacity) and two prooxidative parameters (total prooxidative potency, prooxidative-antioxidative balance) were measured and Oxydative Scores (OS) calculated by using Z score statistics. OS was derived from the difference between Prooxidative Scores and Antioxidative Scores calculated as mean Z scores from above-mentioned parameters. Statistical analysis was performed by using Friedman’s test with subsequent Wilcoxon’s nonparametric paired test in SPSS. Native SB samples showed mild prooxidative effects similar to SP/TBH combination. After 2 h and 24 h incubation all SB concentrations in SP showed significant OS suppression, regarding OS values. The best OS diminishing results showed 2.5 M samples: -0.8928 (-2.713 – 1.4012) vs. 1.25 M: 0.2454 (-0.6212 – 6.5526) and 5 M: 0.8834 (-0.1698 – 3.2401), p < 0.001. Results were more pronounced after longer incubation time. SB showed clear antioxidative effect in biological environment, which was dose-dependent in reverse U-shaped manner. Serum, specimen consisted from different biomolecules is used here in order to estimate probiotic activity in biological medium. The natural milieu of this strain after oral ingestion is digestive system, so the future research based on genuine conditions for probiotic activity is warranted.

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PP10

AMPK and Nrf2 drive redox-metabolic reprogramming of cancer-associated adipose tissue in breast cancer

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Breast cancer behaves as a complex pseudo-organ where the adaptive behavior of cancer cells is deeply context-dependent, reflecting various local and systemic influences. Recently, cancer-associated adipocytes emerged as critical players in cancer progression, adapting to metabolic demands of proliferating cancer cells and responding through the supply of diverse energy substrates. To decipher underlying mechanisms that enable such plastic response, we cross-examined biopsies of cancer-associated adipose tissue from normal-weight and overweight women with invasive ductal carcinoma compared to mammary adipose tissue of weight-matched women with benign fibroadenoma. To that end, we analyzed mitochondrial copy number and master regulators of energy and redox homeostasis (AMP-activated protein kinase – AMPK and nuclear factor erythroid 2-related factor 2 – Nrf2), followed by key enzymes in their downstream pathways such as glycolysis, pentose phosphate pathway, fatty acid oxidation, and antioxidant defense. Compared to mammary adipose tissue, cancer-associated adipose tissue showed concomitantly higher AMPK and Nrf2 protein expression followed by overexpression of hexokinase 2, glucose-6-phosphate dehydrogenase, peroxisomal acyl-coenzyme A oxidase 1, first-line antioxidant defense enzymes (CuZn- and Mn- superoxide dismutase and catalase), as well as higher mitochondrial copy number. In contrast, in cancer-associated adipose tissue of obese women, a sole increase in AMPK protein expression without Nrf2 was followed by increased protein expression of analyzed metabolic enzymes but not antioxidant defense enzymes or mitochondrial copy number. The results indicate that simultaneous activation of AMPK and Nrf2 signaling promotes a specific metabolic phenotype of cancer-associated adipose tissue, resembling the Warburg effect with high mitochondrial content and increased redox homeostasis threshold. Moreover, context-dependent disruption of the AMPK–Nrf2 axis could prevent the establishment of such phenotype in obesity.

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Metformin treatment reduces ROS levels, improves mitochondrial function and dynamics in leukocytes from type 2 diabetic patients

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Metformin is a first-line hypoglycemic treatment for type 2 diabetes. Lately, it has been proposed that the beneficial effects of metformin could be mitochondria-mediated. In the present study, our aim was to describe the effect of metformin in the mitochondrial function and dynamics in leukocytes from type 2 diabetic patients. For this end, 80 type 2 diabetic patients and 80 healthy volunteers were recruited and peripheral blood was extracted. Of all type 2 diabetic patients, 40 were under 1700 mg/day metformin treatment for at least 1 year. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood samples following a ficoll density and employed to evaluate the subsequent parameters: The analysis of mitochondrial mass, mitochondrial membrane potential and ROS content were studied with specific fluorophores by flow cytometry; the protein analysis was performed by SDS-PAGE western blot of these PBMC samples; and gene expression analysis was performed following RT-PCR and relativized to Actin signal following the $2^{-ΔΔCt}$ method. We observed that type 2 diabetes causes a drop in mitochondrial mass and in mitochondrial membrane potential, and a rise in ROS levels. Regarding protein levels of fusion and fission proteins, type 2 diabetic leukocytes displayed lower levels of fusion proteins and genes (MFN1, MFN2 and OPA-1) and a rise in fission protein and genes levels (DRP-1 and FIS-1) compared to those of healthy subjects. Metformin had beneficial effect by restoring all the parameters to control levels, an increase in fusion markers and a decrease in fission parameters in type 2 diabetic patients. In conclusion, metformin is an useful treatment that can improve mitochondrial function and dynamics in type 2 diabetes.


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Interaction of 1,2,4-triazole and imidazole nitro derivatives with carbon- and oxygen-centered radicals: a steady-state radiolysis study

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In the present work, a series of 1,2,4-triazole and imidazole nitro and dinitro derivatives have been synthesized. The interaction of the obtained compounds with carbon- and oxygen-centered radicals formed during the radiolysis of deaerated or oxygen saturated ethanol solutions was studied by the steady-state radiolysis method. It has been established that 1,2,4-triazole and imidazole nitro derivatives quantitatively oxidize α-hydroxyethyl carbon-centered radicals formed during radiolysis of ethanol, while 1,2,4-triazole and imidazole slightly change the ratio of the radiation-chemical yields of radiolysis products in favor of acetaldehyde. It was shown that the introduction of the second nitro group into the 1,2,4-triazole cycle does not result in substantial changes in the efficiency of the interaction of the tested substances with α-hydroxyethyl radicals. Using the radiolysis of a 1 M aqueous solution of ethanol, it was demonstrated that 5-nitroimidazoles quantitatively oxidize α-hydroxyethyl radicals; this manifested itself in the absence of 2,3 butanediol among the radiolysis products and a ~20-fold increase in the yield of acetaldehyde, as compared to that in a control experiment. It was found that studied compounds insignificantly decrease or do not affect radiation-chemical yields of oxygenated ethanol radiolysis products. The experimental data obtained indicate that the nitro derivatives of 1,2,4-triazole and imidazole cannot compete with oxygen for α-hydroxyethyl radicals, and they do not interact with oxygen-centered radicals formed in the system. The reaction rate constant of the oxidation of α-hydroxyethyl radicals by the nitro derivatives of 1,2,4-triazole was found to be $k \leq 4.6 \times 10^9$ L mol$^{-1}$ s$^{-1}$ using the competing reactions calculation method. The results obtained in this study are important for understanding the molecular mechanisms of the formation of radiation damage during radiation therapy of cancer diseases using the radiation sensitizers based on nitro derivatives of 1,2,4-triazole and imidazole.

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PP13

The human skin bacteria *Staphylococcus epidermidis* ameliorates UVB-induced free radicals through reduction of labile iron

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UVB-induced skin damage results in various inflammatory disorders through the induced generation of reactive oxygen species that quickly inundate tissue antioxidants. To investigate efficacies of human skin commensal bacteria *S. epidermidis* with glycerol which on fermentation produces electrons. *In vivo* affirmation on mice has confirmed the antioxidative role of topically applied live bacteria with glycerol against UVB irradiation and maintained sufficient expression of 4-hydroxynonenal and cyclobutane pyrimidine dimer, a major biomarker for oxidative stress. Upon UVB irradiation in keratinocyte cell lines treated with glycerol mediated bacteria fermentation product shows the reduced intracellular oxidative stress. Glycerol or bacteria alone in *in vivo* topical application in mice skin and in vitro analysis in keratinocytes does not influence the level of oxidative stress. Furthermore, electrochemical behavior of glycerol mediated bacterial fermented medium found to produce electron this result suggests that electrogenic and antioxidant property of skin bacteria. The electrons produced by bacteria fermentation product initiate reduction of free radicals by converting toxic Fe$^{3+}$ back to non-toxic Fe$^{2+}$; thereby it terminates Fenton's reaction and maintains iron hemostasis.

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Effect of prolonged hemodialysis on SOD activity and relative telomere length in chronic kidney disease patients – potential improving of cardiovascular disease outcomes

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Introduction: The most common outcome of chronic kidney disease (CKD) is cardiovascular disease (CVD), resulting in extremely high mortality and morbidity rates in this patients. Since oxidative stress and short telomere length is considered to be included in pathogenesis of CVD, we tested whether relative telomere length (rTL) or some of redox status parameters might be a marker for these diseases. The Aim: The main objective of this study was to investigate whether the prolonged hemodialysis treatment have impact on redox status and telomere length in CKD patients, and improvement of heart function. Material and Methods: The study included 79 CKD patients of which 39 patients were on hemodialysis treatment for 4 hours during the three months, as conventional treatment group (cHD), while 40 patients were on prolonged hemodialysis treatment (plHD group) for 5 hours for the same period of time. Superoxide dismutase (SOD) activity was measured in plasma, while paraoxonase 1 (PON1) activity, total antioxidant status (TAS), and total oxidant status (TOS) were measured in serum. All parameters of redox status were determined by appropriate spectrophotometric methods. rTL was measured from peripheral blood leukocytes using the qPCR method. Cardiac function was evaluated by echocardiography. Results: SOD activity was increased in plHD group compared to cHD patients (p = 0.043). There were no significant differences in PON1 activity, TAS and TOS between these two groups. We have observed trend toward longer telomere in plHD patients, but the difference was not statistically significant (p = 0.154). End-diastolic interventricular septal thickness (IVS) and left ventricular ejection fraction (EF) were significantly improved in plHD patients (p = 0.030). Conclusion: Prolonged hemodialysis sessions over three months improved antioxidant defense system due to increasing of SOD activity, which can contribute to rTL elongation and thus cardiomyocytes preservation and finally better CVD outcomes in CKD patients.

Keywords: SOD; CKD; prolonged hemodialysis; rTL.

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PP15

Thioredoxin 1 is a stress granule component important for cell survival upon arsenic exposure

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Arsenic is a major water pollutant and health hazard, associated with numerous diseases including cancer development. Arsenic exerts its pronounced cellular toxicity by oxidizing proteins, preferentially at vicinal dithiol groups. When exposed to As(III), cells mount a protective stress response to the oxidative damage by reducing the rate of protein synthesis. This response requires activation of the heme regulated inhibitor (HRI) kinase and consecutive phosphorylation of the translation initiation factor eIF2\textalpha. Upon translation suppression stalled pre-initiation complexes accumulate and condense together with a distinct set of RNA-binding proteins into cytoplasmic structures stress granules (SGs). Besides RNA-binding proteins and translation factors, SGs also contain other proteins that are recruited to SGs via protein-protein interactions. The biological role of SGs is diverse: they are important for cell recovery, regulation of cell signaling and cell survival under stress conditions. In this study, we identified Thioredoxin 1 (Trx1) as a novel component of SGs that are induced upon exposure to arsenic. In addition, we found that Trx1 is required for the formation of arsenic-induced SGs, but not for SGs that are induced by other stresses. Finally, we could confirm that Trx1, regardless of translation inhibition, is important for cell survival upon acute arsenic exposure.

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Potentiation of polyunsaturated fatty acids anti-inflammatory action through redox signaling in fructose-treated endothelial cells

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Fructose intake is associated with low-grade inflammation and increased oxidative stress. Among long chain polyunsaturated fatty acids (LC-PUFAs), Ω-6 are recognized as a contributing factor to inflammation, while Ω-3 LC-PUFAs are considered as functional foods with beneficial effects, including inhibition of pro-inflammatory pathways. The aim of this study was to analyze combined effects of physiologically relevant LC-PUFAs and fructose on inflammation and antioxidant enzymes in the in vitro model of vascular endothelial cells. We examined the effects of 0.5 mM fructose, alone and in combination with Ω-6 (arachidonic (AA) and linoleic (LA)) and Ω-3 (eicosapentaenoic (EPA) and docosahexaenoic acid (DHA)) LC-PUFAs on expression of pro-inflammatory cytokines (tumor necrosis factor α (TNFα) and interleukin 6 (IL6)) in EA.hy926 cells. The protein levels of nuclear factor-κB (NF-κB) and IκB, as well as its phosphorylation, together with superoxide dismutase (SOD) 1 and 2, catalase and glutathione reductase (GR) were also analyzed. Total ROS amounts were determined using flow cytometric analysis of cells stained with redox sensitive dihydrorhodamine 123 dye. The results showed that treatment of cells with fructose increased TNFα and decreased IL6 mRNA levels. Additional treatment with LA, DHA and EPA reduced TNFα and led to further decrease of IL6 expression. The observed changes were not associated with NFβ activation. All examined enzymes were unchanged after fructose treatment, while GR was increased by LC-PUFA addition. SOD2 was reduced in cells treated with AA, LA and EPA, while increased ROS amounts were observed with AA, DHA and EPA. This was also evident in combined treatment with fructose. These preliminary results suggest that LC-PUFAs, besides effect on pro-inflammatory cytokines, reduce SOD2 levels and increase ROS. The increased levels of ROS could stimulate production of PUFA-derived peroxides, which in GSH-enriched environment might be converted into anti-inflammatory derivatives, additionally suppressing inflammation in fructose treated endothelial cells.

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Acute NCLX inhibition prevents HIF-1α stabilization in hypoxia

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Reactive oxygen species (ROS) generated by mitochondria are a well-known example of mitochondria-derived signals that can drive cell adaptations or trigger cell damage. This is the case of the increase in ROS during the first minutes of hypoxia. Recently, we have discerned the molecular pathway leading to mitochondrial ROS production upon acute hypoxia, triggered by the mitochondrial sodium/calcium exchanger (NCLX) activation, which is used in specialized cells to trigger acute responses to hypoxia. However, the long-term adaptation to hypoxia relies on hypoxia-inducible factors (HIFs). During normoxia, the α subunit of HIFs is hydroxylated by the prolyl-hydroxylases (PHDs) which target it to ubiquitination and degradation by the proteasome. In hypoxia, however, PHDs are inactivated and the α subunit stabilizes, what allows initiation of the hypoxic transcriptional programme. Although PHDs depend on O2 concentration to perform the hydroxylation reaction, HIF-α stabilization may also depend on ROS production by mitochondria. We show that NCLX inhibition, which blocks hypoxic ROS production by mitochondria without affecting overall mitochondrial respiration, is able to inhibit hypoxic HIF 1α stabilization and the expression of HIF targets. Interestingly, the prevention of HIF-1α stabilization during hypoxia occurred only after acute inhibition of NCLX, either pharmacologically or genetically. In contrast, under chronic NCLX knock-down, HIF-1α stabilization in response to hypoxia was maintained. Our results point out to NCLX as a key regulator of mitochondrial redox signalling, linking acute and long-term responses to hypoxia.

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Short-term endurance training in untrained individuals: Comparing the features of PBMCs and plasma extracellular vesicles in oxidative stress-related biomarkers

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Tissue crosstalk, mediated by extracellular vesicles (EVs), is emerging as a main mechanism for systemic adaptations induced by physical exercise. EVs are membrane-bound vesicles which carry bioactive molecules, such as various RNAs and proteins, which are important in cross-tissue communication. In the circulatory system, peripheral blood mononuclear cells (PBMCs) largely contribute to the release of EVs. Furthermore, a large body of evidence highlights the adaptive response of PBMCs to exercise-induced stress. This study investigated the possible link between EVs and PBMCs adaptation to short-term aerobic exercise, focusing on redox homeostasis and heat shock protein 70 (HSP70). Seven healthy male volunteers (26.6 ± 3.1 years), with medium fitness levels (36 < VO_{2} max < 47 mL/kg/min), were subjected to 5 consecutive days of endurance training (treadmill, 70% HRmax x 30' /each). Blood samples were collected before and after the 5-day exercise intervention. To monitor physiological adaptation, lactate and HR were measured during each training session. Both EV and PBMC proteins were analyzed for their content in antioxidant enzymes (SOD1, SOD2, Catalase, TrxR1), HSP70, and oxidative stress biomarkers [lipid peroxidation (LP), protein carbonylation (PCO)]. After the training intervention, PCO and LP values were significantly lower in both PBMCs (p < 0.002) and EVs (p < 0.05). In PBMCs, only TrxR1 protein was downregulated by training (p < 0.05), while, in EVs both SOD2 and Catalase content was significantly downregulated (p < 0.05). No significant modulation by the 5-day training was observed for HSP70, neither in PBMCs nor in EVs. Our results demonstrated that regular, short-term aerobic training was effective in decreasing protein and lipid oxidation in both PBMCs and EV cargo. Indeed, the training period modulated, although differently, the content of antioxidant enzymes in PBMCs and EVs, which may suggest an early adaptation to physical exercise with regards to redox homeostasis.

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Liquid-liquid phase separation (LLPS) is the process in which macromolecules arrange themselves into liquid compartments without any delineating membranes or scaffolds. Proteins that are known to undergo LLPS contain an intrinsically disordered region (IDR) – regions of low sequence complexity, leading to the lack of stable secondary or tertiary protein structure. With respect to the concept of LLPS striving to define new ways of “membrane-less” compartmentalisation, most membrane bound organelles are largely affected by the dynamics of contact sites between each other – the areas where two membranes come into close proximity. It is emerging that these areas of contact serve a functional purpose for mediating the transport of essential lipids or calcium ions to regulate calcium homeostasis which in turn links to Oxidative Phosphorylation. Nowhere is this more important than at the synapse where calcium-triggered exocytosis occurs with ms precision. PDZD8, an ER-resident protein, was recently shown to tether the ER to mitochondria. Its absence disrupts calcium clearance at the synapse, but the precise mechanism that underlies PDZD8-driven buffering of calcium during neurotransmission is largely unknown. Here, using minimal reconstitution systems and LLPS assays, we show that PDZD8 can undergo phase separation. PDZD8 contains an intrinsically disordered region, which is sufficient to trigger LLPS at concentrations as low as 5 µM in the presence of crowding reagents. This is even accelerated in the presence of the dimerization motif and scaffold regions of PDZD8. Moreover, LLPS of PDZD8 responds to changes in salt concentration suggesting its ability to act as a charge sensor. Together, our data suggests that in addition to its tethering role, PDZD8 changes its self-organisation properties with respect to variations in ion concentrations. This is particularly important in the context of dynamic calcium fluxes that occur at synapses upon activation.

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Microglial heme-oxigenase I (HO-1) implication during aging and inflammatory-related neurodegenerative diseases

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Neuroinflammation and oxidative stress are being recognized as characteristic hallmarks in many neurodegenerative diseases, being microglial cells responsible for the brain’s innate immune response. Heme-oxygenase 1 (HO-1) is an inducible enzyme known for its anti-inflammatory, antioxidant and neuroprotective effects. However, increased expression of HO-1 during aging and age-related neurodegenerative diseases has been associated to neurotoxic ferric iron deposits. The aim of this study was to elucidate HO-1 brain expression pattern with aging and to understand the role of microglial HO-1 under inflammatory conditions in aged mice. For this purpose, aged wild type (WT) and LysMCreHmox1ΔΔ (HMOX1M-KO) mice that lack HO-1 in microglial cells, were used. The results showed an increased brain HO-1 expression with aging in different brain regions. Furthermore, this increase was even higher when exposed to an inflammatory stimulus (LPS via i.p.) and was accompanied by alterations in different iron-related metabolism proteins, resulting in an increase of iron, oxidative stress, ferroptosis, cognitive decline and inflammation. Interestingly, all these alterations were prevented in aged HMOX1M-KO mice and WT mice treated with the iron chelator deferoxamine (DFX). In conclusion, this study highlights how during aging after an inflammatory insult, microglial HO-1 overexpression contributes to neurotoxic iron accumulation providing deleterious effects.

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The opposite effects of trehalose on 6-hydroxydopamine and 1-methyl-4-phenylpyridinium induced oxidative stress in human neuroblastoma SH-SY5Y cells

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6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium (MPP+) are the most common neurotoxins used to induce experimental model of Parkinson’s disease both in vivo and in vitro. Neurotoxic action of 6-OHDA and MPP+ is mediated by oxidative stress, mitochondrial damage and induction of apoptotic cell death. Natural disaccharide trehalose exhibits antioxidative properties and stimulates removal of damaged proteins, and thus exhibits powerful neuroprotective effect in certain brain injury models. We investigated the effects of trehalose in 6-OHDA and MPP+- induced oxidative stress and neurotoxicity in human neuroblastoma SH-SY5Y cells. The effects of trehalose on the cell viability and death were assessed by MTT, crystal violet, lactate dehydrogenase assay and AnnexinV-FITC/propidium iodide staining. The production of reactive oxygen species was analyzed by flow cytometry using redox-sensitive dyes dihydrorhodamine 123 (DHR) and MitoSOX Red. Further, activation of stress-related MAP kinases, p38 and JNK were investigated by immunoblot analysis. Our study demonstrated that trehalose pretreatment significantly improved cell viability and reduced neurotoxic effect of 6-OHDA, while slightly decreased cell viability and increased neurotoxic effect of MPP+. Trehalose decreased the number of 6-OHDA-induced apoptotic cells (shown by the reduced % of Annexin V+ and AnnexinV' PI' cells) whereas it increased apoptosis in MPP+ treated cells. Flow cytometric analysis of DHR and MitoSOX stained cells demonstrated that trehalose pretreatment significantly reduced 6-OHDA-triggered ROS and superoxide anion radical generation. However, in MPP+-treated neurons trehalose augmented oxidative stress and production of superoxide anion. Immunoblot analysis showed that trehalose significantly decreased p38 and JNK activation only in 6-OHDA treated cells. These results indicate that trehalose has different effects on oxidative stress induced by two different neurotoxins, 6-OHDA and MPP+, and suggests further exploration of the mechanism of its antioxidative action.

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Utilization of dietary extracellular vesicles as new carrier of functional molecules: modulation of cellular stress response in cultured Ea.hy926 cells and transfer genetic cargo in C57BL/6 mice

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Extracellular vesicles (EVs) have been recognized as important vehicles of intercellular communication, both in prokaryotes and eukaryotes. EVs do not only originate from endogenous synthesis but may also be obtained from dietary source and utilized as a vehicle of bioactive cargo and as a possible strategy of a cross-species and cross-kingdom transfer of functional molecules (proteins, lipids and nucleic acids, including miRNAs) to target cells. The present work aims to assess 1) the cellular uptake of food-derived EVs and their ability to modulate oxidative stress and transcriptomic profile in Ea.hy926 challenged with TNF-α; 2) the bioavailability and biodistribution of food-derived EVs and their cargo in mouse tissues. Methods: 1) cells culture study: Human stabilized endothelial cells line (Ea.hy926) was used to assess cellular uptake of fluorophore-labeled EVs. The transcriptomic profile after the administration of EVs was assessed using a TaqMan Array Human Inflammation Panel. Selected modulated genes were validated by qRT-PCR. 2) animal study: Fluorophore-labeled EVs were administered by oral gavage in male and female C57BL/6 mice. After sacrifice, tissue distribution was observed using Odyssey Clx (LI-COR). Results: 1) Ea.hy 926 cells internalize EVs in a dose dependent manner. Pretreatment with EVs prevented reactive oxygen species (ROS) formation and countered TNF-α-induced ICAM-1 and IL-6 genes expression. Pathway analysis allowed the identification of specific cell response significantly affected by the exposure to EVs. 2) Specific fluorophore-labeled miRNAs transfected into food-derived EVs demonstrated unique distribution profiles, the presence of distinct species of microRNAs and the accumulation in intestinal mucosa, spleen, liver, heart or brain. Conclusions: Dietary miRNAs are bioavailable and affect gene expression in cell culture and in mouse models, supporting the hypothesis that food-derived EVs act as vector of bioactive compounds possibly contributing to modulate the response to different stimuli.

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PP23

High molecular weight uremic retention solutes stimulate GSTP protein expression and oxidative damage of peripheral blood leukocytes

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Chronic kidney disease, CKD, is a condition of immune dysfunction associated with oxidative stress and premature death of peripheral blood mononuclear leukocytes (PBL). High molecular weight uremic retention solutes (HMW) have been assumed to play an underlying role in these cellular disturbances. In this study we investigate the effect of HMW on the PBL expression and activity of glutathione S-transferase P (GSTP), a GSH-dependent protein with key role in the enzymatic detoxification of cellular electrophiles and redox modulation of signaling proteins of stress response and death pathways. The results demonstrate that both the spontaneous and HMW-induced premature death of uremic PBL are associated with: 1) increased generation of cellular electrophiles, especially H2O2, 2) higher expression and oxidative damage of GSTP protein, 3) increased levels of cellular GSH and lowered protein glutathionylation, 4) JNK/c-Jun pathway activation, 5) complete absence of protein expression, but markedly increased mRNA levels of Nrf2 transcription factor. These effects of uremic HMW were also recapitulated in healthy control’s PBL and in different mononuclear cell lines. Simulating the GSTP overexpressing phenotype of uremic PBL in THP1-derived macrophages by human GSTP1 transfection, and GSTP ablation in mouse embryonic fibroblasts demonstrated the important role of GSTP protein expression in the control of H2O2 metabolism, protein glutathionylation and apoptotic signaling by the treatment with uremic HMW. Proteomics data confirmed the presence of oxidation and glycoxidation epitopes in several proteins of the HMW fraction of the uremic, but not of control, plasma. In conclusion, uremic HMW stimulate GSTP protein expression and damage, which are characteristic molecular events of the altered redox and stress response of uremic PBL. These effects on GSTP are expected to influence the death program of PBL by the activation of JNK/c-Jun pathway and Nrf2 repression which are potential underlying molecular events and biomarkers of immune dysfunction in CKD.

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The influence of uremic serum and GSTM1 knockdown on redox homeostasis in HUVECs

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Introduction: Deletion polymorphism of glutathione S-transferase M1 (GSTM1), a phase II detoxification and antioxidant enzyme, increases susceptibility to cardiovascular diseases (CVD) among end stage renal disease (ESRD) patients and leads to their shorter cardiovascular survival. The mechanisms by which GSTM1 downregulation contributes to oxidative stress in endothelial cells in uremic conditions have not been investigated so far. Aim: To determine the effect of GSTM1 knockdown on the oxidative stress parameters in human umbilical vein endothelial cells (HUVECs) exposed to uremic serum. Method: GSTM1 knock-down was performed using 100 nM GSTM1 siRNA. 96 h post transfection, diminished GSTM1 expression was confirmed by immunoblotting which showed around ~90% reduction in GSTM1 protein levels in HUVECs treated with GSTM1 siRNA (GSTM1+/−) compared to the control (GSTM1+/+). GSTM1+/+ and GSTM1+/− cells were treated with 30% control/uremic serum for 6h. Oxidative stress parameters were analyzed as follows: total reactive oxygen species (ROS) by flow cytometer, malondialdehyde (MDA) by ELISA and superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity spectrophotometrically. Results: Uremic serum treatment led to a significant decrease in the SOD and GPX activity (p < 0.05), as well as increase in MDA levels (p < 0.05) in GSTM1+/+ HUVECs, compared to their counterparts incubated in control serum. Silencing of GSTM1 gene did not affect assessed oxidative stress parameters, but a trend towards increased MDA levels was observed in GSTM1+/− HUVECs compared to GSTM1+/+ HUVECs in control serum (p = 0.053). Conclusion: Incubation of HUVECs in uremic serum induces redox imbalance characterized by enhanced lipid peroxidation and decreased antioxidant enzyme activities, independently of the GSTM1 knockdown.

Keywords: end stage renal disease; endothelial dysfunction; GSTM1; oxidative stress.

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Biological effects of sub-chronic low-dose exposure of human endothelial cell line EA.hy926 to dibutyl phthalate

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Vascular endothelium is exposed to a variety of chemicals from the environment, which can cause damage and endothelial dysfunction. Dibutyl phthalate (DBP) is widely used in the production of various industrial and consumer products. Although epidemiological studies suggest a possible association between DBP exposure and cardiovascular diseases, very few mechanistic and functional in vitro studies that mimic "real-life" exposure scenario have been conducted to date. Our goal was to examine changes associated with endothelial dysfunction after sub-chronic exposure of human endothelial cell line EA.hy926 to low concentrations of DBP (10^{-9}, 10^{-8}, and 10^{-7} M) during 4 weeks. Increased adhesion to extracellular matrix and decreased cell migration were observed after 2 and 4 weeks of exposure, followed by increased mRNA expression of several genes encoding integrin alpha and beta subunits: ITGA2, ITGA5, ITGAV, ITGB1, and ITGB3. Sub-chronic DBP exposure had no effect on endothelial permeability, as observed by measuring the passage of fluorescently-labeled dextran through the EA.hy926 cell monolayer using the transwell system. This result is in accordance with no detectable changes in the expression of two tight junction proteins: occludin and ZO-1. At the same time, no difference in the adherence of monocytes to the EA.hy926 monolayer was detected, concurrent with no significant changes in mRNA expression of the genes encoding three cellular adhesion molecules: ICAM1, VCAM1, and SELE. After 4 weeks, we detected lower production of nitric oxide with 10^{-7} M DBP, followed by diminished activation of endothelial nitric oxide synthase. We also detected dose-dependent reduction in reactive oxygen species levels. In summary, obtained results indicate that sub-chronic exposure of EA.hy926 cells to low concentrations of DBP alters certain functional characteristics of these cells, which can be associated with endothelial dysfunction, warranting further comprehensive investigation aimed at uncovering the exact mechanisms and the effects of DBP on vascular cells.

Keywords: dibuthyl phthalate; cardiovascular diseases; endothelial cells; endothelial dysfunction; nitric oxide.

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Oxidative damage on proteins is enhanced in crowded environments

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Biological milieus are highly complex and crowded environments where biomolecules are in close proximity to each other. Limited data have demonstrated that these crowded systems – largely dominated by interfacial physical chemistry – modulate the biochemical reactions of proteins which are the most abundant macromolecules inside cells. Despite evidence demonstrating that small neutral oxidants of moderate reactivity (e.g. \textsuperscript{1}O\textsubscript{2}), can diffuse freely in packed systems such as lipid bilayers and cells, slower and anomalous diffusion is expected for larger oxidants, such as peroxyl radicals (ROO\textsuperscript{•}) generated during lipid or protein peroxidation. This, along with the close proximity of proteins in biological systems, may affect the propagation of oxidative damage at an inter- as well as intra-molecular level. Therefore, we hypothesized that crowding might modulate the rate and extent of protein oxidation. To test this hypothesis, we have examined model \textit{in vitro} systems containing free amino acids (Trp, Tyr and Cys), and small proteins that lack, or have low numbers of these residues and measured the rate of consumption induced by ROO\textsuperscript{•} in the absence and the presence of inert crowding agents. Kinetic data and mass spectrometry analyses indicate that the rate and extent of consumption of the amino acids is enhanced under macromolecular crowding conditions. Thus, for example, the rate of Trp oxidation was increased from 15.0 ± 2.1 \textmu M/min in PBS to 30.5 ± 3.3 \textmu M/min in the presence of dextran (60 mg/mL). These data imply an increase in the length of chain reactions, and therefore propagation of oxidative damage, from 1.9 in diluted systems to 3.8 under macromolecular crowding conditions. A better understanding of these processes may allow the development of strategies to prevent amino acid and protein oxidation in crowded environments including protein-based medicines and vaccines where concentrations of up to 100 mg protein/mL are encountered.

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A novel role for an antioxidant transcription factor Nrf2 as a transcriptional repressor of the circadian molecular clock

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NRF2 is a master transcriptional mediator of antioxidant responses in many tissues and cells. Disruption of NRF2 pathway is linked to ageing and many diseases, from cancers to neurodegenerative disorders. We previously identified Nrf2 as a clock-controlled target leading to daily oscillations in transcriptional activation of antioxidant gene promoters. Circadian clock is a universal timing mechanism inherent to most cells relying on transcriptional/translational feedback loops to generate ~24 h rhythms in physiology and metabolism. We show that the loss of Nrf2 in vivo leads to de-repression of clock genes in the positive, stabilising and negative feedback loops of the molecular circadian clock within several peripheral tissues and isolated primary cells. Loss-/gain-of-function experiments in human cells recapitulated this transcriptional clock gene deregulation. Real-time bioluminescence imaging of primary cells harbouring a Per2::Luc luciferase reporter show that Nrf2 KO cells display altered amplitude of clock gene oscillations associated with significant changes in both the periodicity length and phase of oscillations. These new findings reveal a novel role for NRF2 as a transcriptional repressor of clock genes in vivo and suggest that abnormal NRF2 expression may contribute to the clock de-synchrony which occurs with ageing.

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Neuronal differentiation disrupts the Nrf2-Notch1 axis in SH-SY5Y neuroblastoma cells

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Nrf2 targets hundreds of genes, not all involved in redox homeostasis. Indeed, a novel role for Nrf2 in stem cell regulation has been revealed through its direct upregulation of key stemness factors including Notch1. As yet, no mechanistic explanation has been described for how Nrf2 governs the behaviour of neural stem/precursor cells and neurogenesis, though it may involve this Nrf2-Notch1 axis. Here we report for the first-time evidence that Nrf2 regulates the Notch1 gene and induces Notch signalling within neural precursor-like cells capable of neurogenesis, SH-SY5Y cells. In undifferentiated SH-SY5Y cells, we show forced expression of Nrf2 and its endogenous activation with diethylmaleate (DEM) dose-dependently drives Notch1 transactivation using reporter gene assays. Downstream of this, Nrf2 enhanced Notch signalling events at its target DNA motif site CBF-1 and transactivation of Notch1 target genes, Hes1 and Hey1. While we confirm this heightened Notch signalling was both dependent on Nrf2 and occurred via the canonical Notch pathway, DEM was found to further enhance Notch signalling by an additional Nrf2-independent pathway through a possible interaction with the Notch intracellular domain. Interestingly, when SH-SY5Y cells are induced to undergo neuronal differentiation, Nrf2 remains able to regulate the Notch1 gene but no longer induces Notch signalling. We find that the differentiation programme blocks Notch signalling itself thereby preventing any enhancement by Nrf2. Our findings highlight the existence of a Nrf2-Notch1 axis in neural precursor-like SH-SY5Y cells and, notably its loss during neuronal differentiation, may be indicative of a role for the Nrf2-Notch1 axis in this process.

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PP29

A new algae-rosemary formulation as a novel technological approach to prevent pollution induced skin damage

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Growing literature has now demonstrated that air pollution can cause severe damage to the human skin, triggering and/or exacerbating disorders such as inflammatory reactions, allergies, skin aging and cancer. Nowadays there is an increasing consumer demand for natural cosmeceuticals, a hybrid concept applied to products originated from green, renewable sources with cosmetic and pharmaceutical attributes that enhance both skin beauty and health, and help protect skin from environmental insults. Rosemary (Rosmarinus officinalis) is a species traditionally used in herbal medicine for antioxidant, antimicrobial and anti-inflammatory benefits, due to its potent phytoactive contents (carnosol, carnosic acid, and rosemarinic acid). However, these health-protective phytochemicals are susceptible to thermal and oxidative degradation, if not encapsulated with a protective carrier. Marine algae such as spirulina and chlorella are not only a rich source of protective proteins, but also contain valuable metabolites known for their skin-protective properties. In this study, we complexed rosemary extract with proteins derived from algae in a gel formulation as a way to deliver natural phytoactive compounds in a stable matrix with preserved potency and enhanced attributes. To evaluate the ability of the algae-rosemary formulation to mitigate pollution-induced skin structural alteration and oxiinflammation, human skin explants were first topically treated with the aforementioned formulation and then exposed to diesel engine exhaust (DEE). We observed that DEE exposure induced a significant increase in the levels of the oxidative markers 4-hydroxynonenal and matrix-metallopeptidase-9 (MMP-9), and a loss of the cutaneous-barrier-associated protein filaggrin. However, topically applied algae-rosemary formulation was able to prevent the harmful cutaneous effects of DEE. In conclusion, this study suggests that this technological formulation of algae-rosemary can be an adjuvant strategy to prevent the DEE-induced oxiinflammatory damage and delay premature skin aging.

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Overexpressing G6PD potentiates adaptations to physical training in old mice

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Introduction: Physical exercise improves frailty-related parameters, such as cardiorespiratory fitness, muscle mass, strength and bone health, as well as induces an increase in antioxidant agents in the body. Glucose-6-phosphate dehydrogenase (G6PD) regulates the NADPH levels, protecting the cell against oxidative damage. Mice that overexpress G6PD have higher levels of NADPH, lower levels of oxidative damage, and better protection from age-associated functional decline. Therefore, our primary purpose was to study the effect of combining physical training and G6PD overexpression in old mice. Methods: Twelve male C57BL/6J mice and 17 male G6PD-Tg mice (20-months-old) followed a multicomponent high-intensity interval training for 10 weeks. The animals were trained five days a week of motor coordination, resistance and endurance exercises. All mice were evaluated before and after the program for different functional parameters. After completing the intervention, blood plasma samples were obtained to study oxidative damage. Independent and paired t-tests were conducted to compare means between and within groups before and after the intervention. All data were expressed as mean (standard error of mean). Results: Post-intervention, compared to the C57BL/6J mice, G6PD-Tg mice showed an improvement in: (i) grip strength [4.76 (0.26) vs 5.7 (0.2) g of force / g of body weight, p < 0.05]; (ii) maximal carrying load (ladder-climbing test) [170.80 (0.09) vs 206.9 (4.8)% of body weight, p < 0.05]; and (iii) endurance (continuous-treadmill test) [1561 (306.3) vs 2679 (279.7) m, p < 0.05]. Moreover, the “Valencia Score” for frailty showed a decrease in the percentage of frail mice in the G6PD-Tg group when compared to the C57BL/6J one (18.82 vs 27.69%, p < 0.05). By last, no differences were found in lipid peroxidation nor protein carbonylation levels between C57BL/6J and G6PD-Tg mice. Conclusion: Compared to C57BL/6J mice, overexpressing G6PD potentiates physical function adaptations and increases protection against frailty.

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Enzymatic debridement by proteolytic enzymes: *in vivo* and *in vitro* studies


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Wound necrotic tissue removal, known as debridement, promotes wound healing and amplifies the formation of granulation tissue. The most common debridement is the surgical. However the most promising is the enzymatic. Collagenase, Dispase, Accutase, Serrapeptase and Lysozyme were tested for their suitability for enzymatic debridement. The enzymes were tested for their toxicity and oxidative stress in UV exposed 3T3 fibroblasts. Their debridement efficiency was evaluated to SKH-hr1 and SKH-hr2 hairless mice induced wounds. The enzymes were topically applied after formation of necrotic tissue. The assessment was realized after clinical, histopathological and biophysical evaluation. Lysozyme, Dispase, Accutase, increased *in vitro* cell viability and decreased oxidative stress. *In vivo* Dispase and Accutase removed efficiently the necrotic tissue and promoted wound healing. Dispase and Accutase could be potential candidates for enzymatic debridement.

Keywords: proteolytic enzymes; debridement; dispase; accutase; necrotic tissue.

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PP32

Exploiting redox vulnerabilities to induce ovarian carcinoma cell death: a novel role for tocotrienols

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Ovarian cancer is one of the major causes of cancer-related death among women worldwide. Currently, its treatment is based on cytoreductive surgery followed by chemotherapy, which is unfortunately accompanied by severe toxicity and drug resistance development. Thereby, more effective and better-tolerated therapeutic approaches are urgently needed. Tocotrienols (TTs) have recently shown great potential in ovarian cancer management. However, the molecular mechanisms underlying this potent antitumor activity are not clear. Here, we investigated the anticancer effects of δ-TT on IGROV-1 and SKOV-3 cells. We demonstrated that it could trigger cell cycle block at G1-S phase and mitochondrial apoptosis. In particular, we observed that the cytotoxic activity of δ-TT was associated with mitochondrial ROS generation and subsequent JNK and p38 phosphorylation. Interestingly, the compound could also synergize with cisplatin in inducing ovarian cancer cell death and was able to selectively eradicate platinum-resistant ovarian cancer cell populations. In conclusion, we found that δ-TT induces ROS/MAPK-related cytotoxicity in ovarian cancer cells and sensitize them to chemotherapy, thus representing a promising option for the treatment of this tumor.

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PP33

Nrf2-regulated redox signaling in human coronary smooth artery smooth muscle cells under hyperoxia, physiological normoxia and hypoxia

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The concept of cell culture under defined ambient oxygen levels that recapitulate pO₂ experienced in vivo has recently acquired renewed research focus. The majority of high throughput drug screening is conducted with cells adapted to hyperoxia (~18 kPa O₂) in standard incubators, known to reduce the lifespan of cells, alter adaptive antioxidant defenses and limit translation of findings from bench to bedside (see Keeley & Mann, Physiol. Rev. 2019;99:161-234). In this study, human coronary artery smooth muscle cells (HCASMC) were adapted for 5 days to hyperoxia (18 kPa O₂), physiological normoxia (5 kPa O₂) or hypoxia (1 kPa O₂), and basal and/or sulforaphane (SFN, 2.5 µM, 16 h) stimulated antioxidant protein expression assessed by immunoblotting. Expression of calponin-1, a key marker of smooth muscle contractile phenotype, was increased in cells under 5 kPa and 1 kPa O₂ vs 18 kPa O₂ and associated with increased cell proliferation (n = 6, P < 0.05). Notably, stabilization of HIF-1α was only observed in cells adapted to 1 kPa O₂. Although protein levels of catalase, MnSOD and CuZnSOD were not significantly different under 18 kPa vs 5 kPa O₂, protein levels were decreased in cells adapted to 1 kPa O₂ (n = 6, P < 0.05). In contrast to our previous studies in human venous and brain microvascular endothelial cells, SFN induced activation of Nrf2-HO-1 signaling was not attenuated in cells under 5 kPa O₂. When HCASMC were loaded with a luminescence probe L-012 and exposed to hypoxia (1 h) and reoxygenation, superoxide generation (PEG-SOD inhibitable) was only detected on reoxygenation. Our findings provide important insights into the effects of ambient O₂ levels on the redox phenotype of coronary artery smooth cells and aim to inform researchers involved in drug screening for protection against ischemia-reperfusion injury.

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Preventive effect of Lactobacillus plantarum YS1 on TNBS-induced colitis in BALB/c mice

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Lactobacillus plantarum YS1 (LP-YS1) is a new strain isolated and identified by our research group. The preventive effect of LP-YS1 on colitis was determined by the TNBS induced colitis mice model. After determination of serum and colon tissue of mice, the results showed that LP-YS1 could reduce the disease activity index (DAI) in colitis mice, meanwhile LP-YS1 could also raise the colon length and the colon weight/colon length ratio in colitis mice. The serum determination results showed that LP-YS1 reduced MPO, NO, MDA levels and increased GSH in colitis mice. The serum cytokine level of IL-2 in colitis mice was lowest and the IL-10 level was highest, LP-YS1 could increase the IL-2 level and decrease the IL-10 level in colitis mice. By the qPCR and western blot assays, LP-YS1 upregulated the mRNA and protein expression of the c-Kit, SCF and downregulated the expression of the IL-8, CXCR2 in the colon tissue of colitis mice. From the results, LP-YS1 has a good preventive effect on colitis, and the effect is positively correlated with the concentration. LP-YS1 is a strain with probiotic potential.

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Insight into Keap1/Nrf2 pathway activation by new milk-derived bioactive peptides

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Milk-derived bioactive peptides have been identified as potential ingredients of functional foods acting as antihypertensive, antimicrobial and antioxidant factors. This study is focused on the evaluation of the antioxidant mechanism of action of new milk-derived bioactive peptides in Caco-2 cells. From fermented milk, antioxidant peptide enriched fractions were extracted, purified and identified through mass spectrometry. Twenty-three peptides were chosen, synthesized and analysed in vitro and in a cellular model for their antioxidant properties. Four peptides, N-15-M, E-11-F, Q-14-R and A-17-E, were selected for their protective effects against oxidative stress induced by TbOOH, both as rescue of the viability and inhibition of ROS production. The molecular mechanism of the peptides was evaluated analyzing their capability of modulating Keap1/Nrf2 response, which is the main pathway involved in the protection from oxidative stress. The action of N-15-M, E-11-F, Q-14-R and A-17-E on Keap1/Nrf2 pathway in Caco-2 cells was assessed. N-15-M, Q-14-R and A-17-E were shown to be able to determine the Nrf2 translocation to the nucleus, suggesting that these peptides can modulate this pathway leading to the transcription of antioxidant and phase II enzymes. For this reason, the gene expression of TrxR1, GR, NQO1 and SOD1 was determined by RT-PCR and confirmed by Western blot analysis in cells treated for 24 h with N-15-M, E-11-F, Q-14-R and A-17-E. In particular, N-15-M, Q-14-R and A-17-E, activate Keap1/Nrf2 pathway determining the overexpression of the studied enzymes in Caco-2 cells. Moreover, the peptides, although to a different extent, were able to increase the TrxR1 and GR activities in treated cells. Finally, molecular docking analysis confirmed the interaction of N-15-M, Q-14-R and A-17-E with the residues of the Keap1 pocket involved in the binding with Nrf2, suggesting that these antioxidant bioactive peptides can act as disruptor of Keap1/Nrf2 interaction.

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Additive effect of combined pollutants exposure to UV-induced skin OxInflammation damage

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The skin, due to its location and composition, has been identified as one of the main target tissues for environmental pollutants. Ozone (O₃), particulate matter (PM), and ultraviolet radiation (UV) have all been shown to trigger inflammatory and oxidative stress reactions within the skin, resulting in the so-called “OxInflammation” condition, altering skin functionality and homeostasis. However, few studies have explored whether these stressors can act synergistically in affecting the cutaneous tissue homeostasis. In the present work, we evaluated whether O₃, PM, and UV, the most common environmental skin insults, act synergistically in inducing skin damage. Human skin explants were sequentially exposed to 200 mJ UV light, 0.25 ppm O₃ for 2 h, and 30 min. of diesel engine exhaust (DEE), alone or in combination for 4 days (time point D1 and D4). We observed a clear additive effect of O₃ and DEE in combination with UV in increasing levels of several oxidative (4HNE, HO-1), inflammatory (COX2, NF-κB, Ahr) markers and loss of barrier-associated proteins, such as keratin 10, filaggrin and involucrin. Moreover, we also determined a loss of skin integrity and functionality, represented by the depletion of tight junction’s proteins claudin-1 and desmocollin 1 (DSC1) and the protein channel aquaporin 3 (AQP3), involved in the cutaneous bidirectional water flux in the cells. In addition, we also observed the enhanced activation of the inflammasome pathway NLRP1 in response to the synergistic pollutants exposure, suggesting a possible involvement of this multiprotein complex in mediating the pollutants-induced ox-inflammatory and structural skin damage. In conclusion, this study is the first to demonstrate that the concomitant exposure to environmental pollutants can induce a cascade of pathways able to interact to each other (OxInflammation) and potentiate together the skin damage induced by the single stressors.

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Adapting bEnd.3 brain microvascular endothelial cells to defined ambient oxygen levels has direct consequences for hypoxia-reoxygenation injury

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Treatments available for ischemic stroke remain limited due to failures in clinical translation and to improve this, physiological oxygen levels encountered in vivo need to be considered in the design of cell culture experiments. As cells in vivo experience O_{2} levels ranging from ~13 kPa to ~1 kPa, cells cultured under room air (18 kPa O_{2}) are exposed to hyperoxia and oxidative stress. Using an O_{2} sensitive probe (MitoXpress-INTRA, Agilent), we identified that long-term culture under 5 kPa O_{2} is required to recapitulate reported intracellular O_{2} levels in the brain. Long-term culture under 5 kPa O_{2} further highlights a distinct redox phenotype different to cells cultured under 18 kPa O_{2}, as evidenced by downregulation of specific Nrf2 target antioxidant genes. Superoxide production measured using luminescent L-012 and mitochondrial-specific superoxide indicator MitoSOX™ corroborated findings that long-term culture under 5 kPa O_{2} markedly attenuated superoxide production associated with hypoxia-reoxygenation injury. Similarly, real-time labile Fe^{2+} measurements revealed less Fe^{2+} release following reoxygenation of bEnd.3 cells adapted to 5 kPa O_{2}. Exaggerated superoxide production in cultures exposed to an hyperoxic environment may create misleading insights for high throughput screening of drugs to combat hypoxia-reoxygenation injury. The present study provides evidence that adapting cells to physiological normoxia has direct consequences for hypoxia-reoxygenation injury. Future in vitro studies should consider a paradigm shift by conducting cell culture studies under physiological O_{2} levels encountered in vivo to enhance translation to the clinic.

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Analysis of chemiluminescence from organisms using an artificial neural network approach

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It is generally accepted that most of the organisms endogenously can emit a small amount of light, which is produced during the oxidation-reduction reactions \cite{1}. A brilliant hypothesis is that this light can be used in means of a non-invasive tool to investigate the underlying processes. However, the certain molecules and pathways responsible for that are still unknown. Moreover, reactive oxygen species which play a critical role as redox modulators and signaling molecules in organisms can be a good candidate for this light. Therefore, a qualitative study and analysis of these species can lead to useful information about the organism. Since molecular oxygen itself is actually a free biradical, it has a central role in the production of reactive oxygen species. With this motivation, we implemented an artificial neural network method to obtain a deeper understanding of the level of singlet oxygen in organisms, especially in mitochondria as potential emitters. Our finding confirmed that there is a meaningful correlation exists between the amount of singlet oxygen and stress in plant mitochondria.


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The protective role of bioactive quinones in stress-induced senescence phenotype of endothelial cells exposed to cigarette smoke extract

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Endothelial dysfunction represents the initial stage in atherosclerotic lesion development which occurs physiologically during aging, but external factors like diet, sedentary lifestyle, smoking accelerate it. Since cigarette smoking promotes oxidative stress and cell damage, we developed an in vitro model of endothelial dysfunction using vascular cells exposed to chemicals contained in cigarette smoke, to help elucidate the protective effects of anti-inflammatory and antioxidant agents, such as ubiquinol (QH) and vitamin K, that play a fundamental role in vascular health. Treatment of young Human Umbilical Vein Endothelial Cells (HUVECs) for 24h with cigarette smoke extract (CSE) decreased cellular viability in dose-dependent manner, induced apoptosis via reactive oxygen species (ROS) imbalance and mitochondrial dysfunction and promoted an inflammatory response. Moreover, the senescence marker SA-β-galactosidase was observed in both young CSE-exposed and in senescent HUVECs suggesting that CSE exposure accelerates aging process in endothelial cells. Supplementation with 10 μM ubiquinol and menaquinone-7 (MK7) counteracted oxidative stress and inflammation, resulting in improved viability, decreased apoptosis and reduced SA-β-galactosidase, but were ineffective against CSE-induced mitochondrial permeability transition pore opening (mPTP). Other K vitamins tested like menaquinone-4 (MK4) and phylloquinone-1 (K1) were less protective showing only a slight improvement in viability and cytosolic ROS content. In addition, MK4 amplified the CSE-induced increase of O$_2^-$ and consequently increase the mPTP opening, causing the amplification in mitochondrial dysfunction. In conclusion, CSE exposure was able to promote a stress-induced senescent phenotype in young endothelial cells likely contributing to endothelial dysfunction in vivo. Furthermore, the molecular changes encountered could be offset by ubiquinol and menaquinone-7 supplementation, the latter resulting in the most bioactive K vitamin in counteracting CSE-induced damage.

Keywords: endothelial dysfunction; cigarette smoke; aging; vitamin K; menaquinone; ubiquinol; oxidative stress; mitochondrial dysfunction.

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The role of RNA oxidation in islet dysfunction in Type 2 diabetes

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Oxidative stress and reactive oxygen species (ROS) play a key role in the development of insulin resistance, β-cell dysfunction, and impaired glucose tolerance in type 2 diabetes (T2D). ROS initiate reactions that promote the modification of biological molecules and aberrant signalling to result in cellular dysfunction and death. RNA could be a key target for ROS due to its localization within the cells and lack of RNA repair mechanisms. The nucleobase guanosine, is highly sensitive to oxidation, which forms 8-oxoguanosine (8-oxoGuo), a product strongly associated with T2D morbidity. However, the pathway by which 8-oxoGuo is formed in patients with T2D and whether 8-oxoGuo has a causal role in disease progression and islet dysfunction remains unknown. In this study, we examined the reactivity of 8-oxoGuo and related modified nucleoside with INS-1 cells, as β-cell model, and assessed the pathways responsible for ROS formation on exposure of β-cells to pro-inflammatory cytokines or glucolipotoxicity (GLT). Initial studies focused on the effect of the treatments on intracellular thiol concentration and the formation of hydrogen peroxide (H$_2$O$_2$). Exposure of INS-1 cells to TNFα, IL-1β or GLT conditions (25 mM glucose / 100 mM palmitic acid) resulted in a significant time-dependent loss in thiols. Exposure to TNFα and/or IL-1β, but not GLT, resulted in an increase in H$_2$O$_2$ formation. Experiments were also performed with INS-1E cells treated with 8-oxoGuo, 8-oxodeoxyguanosine (8-oxodG), 8-oxoGTP, 8-chloroguanosine (8ClG), and 8-chlorodeoxyguanosine (8CldG). This resulted in a non-significant loss in thiols, but an elevation in the production of H$_2$O$_2$, particularly with 8ClG after 4 and 24 h exposure times. In both sets of experiments, evidence was obtained for the alteration of 8-oxoGuo and 8-oxodG within the cellular RNA and DNA respectively. Overall, this project provides new data regarding oxidative pathways in different T2D models of β-cell dysfunction.

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Anthocyanin-rich bilberry extract exerts a complex nutrigenomic effect in hippocampus of ApoE\(^{-/-}\) mice

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Epidemiological, clinical and pre-clinical studies suggest that consumption of anthocyanins, natural antioxidants from plant foods, may slow cognitive decline and improve cognitive performance but also exert neuroprotective effects against neurodegenerative disorders, including Alzheimer’s and Parkinson’s diseases. However, underlying mechanisms of action of dietary anthocyanins, known to exert complex genomic modifications, are not fully understood. This study aimed to examine the effects of anthocyanin-rich bilberry extract supplementation on the global gene expression in the hippocampus of ApoE\(^{-/-}\) mice to help the understanding of molecular mechanisms related to the reported neuroprotective effects of anthocyanin-rich food consumption. Male ApoE\(^{-/-}\) mice were fed for 12 weeks either a control diet or a control diet supplemented with 0.02% anthocyanin-rich extract. Hippocampi were collected, RNA extracted, and global gene expression was accessed using pangenomic microarrays. Profound bioinformatics analysis was performed to identify functions affected by these bioactives. Gene expression analysis revealed 1,698 differently expressed genes, with 1,087 genes upregulated and 611 down-regulated. Bioinformatics analyses showed that these genes are involved in regulating different biological processes, including neurogenesis, inflammation, oxidative stress, metabolism, cell to cell adhesion, as well as Alzheimer’s and Parkinson’s disease pathology. Bioinformatics analyses also identified potential microRNAs (e.g., mir-124-3p, mir-181a-5p, mir-329-3p, mir-9-5p) and transcription factors (e.g., sp1, Nf-\(\kappa\)B, p53, jun) involved in the observed nutrigenomic effects, and molecular docking suggested that anthocyanins could bind to these transcription factors and affect their activity and gene expression regulation. In conclusion, integrated bioinformatics revealed a multi-target mode of action of anthocyanins in the hippocampus underlying their neuroprotective properties.

Keywords: anthocyanins; bilberry extract; nutrigenomics; bioinformatics; hippocampus; ApoE\(^{-/-}\) mice.

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Failure of the brain glucagon-like peptide-1 receptor-mediated control of intestinal redox homeostasis in a rat model of sporadic Alzheimer’s disease

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Objectives: The gastrointestinal (GI) system dyshomeostasis and oxidative stress (OS) may be involved in the etiopathogenesis of the insulin-resistant brain state (IRBS) and Alzheimer’s disease (AD). Our preliminary evidence suggests the failure of the brain glucagon-like peptide-1 receptor (GLP-1R) signaling in a rat model of AD induced by intracerebroventricular streptozotocin (STZ-icv). We aimed to explore whether inhibition of brain GLP-1R induces intestinal OS, and whether OS is present in the GI system of the STZ-icv animals. Methods: Male Wistar rats (n = 39) were treated with STZ-icv (3 mg/kg) or vehicle and either 85 μg/kg exendin-3(9-39)amide (Ex-icv) or saline after one month. 1,2,3-trihydroxybenzene autooxidation (THB), thiobarbituric acid reactive substances (TBARS), and nitrocellulose redox permanganometry (NRP) were measured in plasma, and SOD, TBARS, NRP, low molecular weight thiols (LMWT), protein sulfhydryls (SH) and catalase (CAT) were analyzed in duodenum and ileum. Results: Our results show for the first time that STZ-icv affects the GI system by inducing OS in the duodenum, but not in the ileum. STZ-icv increases duodenal TBARS and CAT and decreases LMWT, SH, and SOD. Inhibition of the brain GLP-1R acutely induces duodenal OS in the control animals but fails to potentiate redox dyshomeostasis in the gut of the STZ-icv rats. Conclusions: Increased levels of OS are present in the duodenum of a rat model of sporadic AD and may be involved in the development of systemic and neuro-inflammation. Brain GLP-1Rs are involved in the regulation of duodenal redox homeostasis, and resistance of the brain-gut GLP-1R signaling may be involved in the development of pathophysiological changes in the GI tract in the STZ-icv animals.

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The effect of new natural long-chain fatty acid esters from millipede defensive secretion on reactive oxygen species (ROS) production in a cell-free model system

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During millions of years of evolution, arthropods “acquired and developed” a spectrum of chemicals for prey capture and/or for defense against predators or pathogenic microorganisms. Recent studies showed that, among arthropods, members of the millipede order Julida (Myriapoda: Diplopoda) are a group that is endowed with diverse chemical defense in the form of secretions from defense glands (DSs). It is known that julidan DSs are blends of quinones, phenolics, alcohols, esters, anthranilate derivatives, aldehydes, and ketones. Such chemodiversity is related to findings that these DSs exhibit different (antibacterial, antifungal, antioxidative, and antineurodegenerative) biological activities. Also, previous data highlight that julidan DSs have prominent toxic potential in in vitro and in vivo model systems. As julidan DSs are usually complex mixtures of at least two classes of chemicals and contain several dozens of compounds, it is of interest to determine which chemical class(es) and compound(s) are responsible for the observed toxic potential. To explore this question, we tested the ability of two new natural long-chain fatty acid esters from chemically complex (mixture of esters, quinones, ketones, aldehydes and alcohols) DS of Megaphyllum bosniense (Verhoeff, 1897), 3-phenylpropyl heptadecanoate and 3-phenylpropyl nonadecanoate, to produce reactive oxygen species (ROS) in cell-free DCF (2,7-dichlorodihydrofluorescein) assay. Both esters were synthesized by N,N-dicyclohexylcarbodiimide-facilitated coupling of the commercially available 3-phenyl-1-propanol and the corresponding acids. The yields of the esters obtained after dry-flash chromatography on SiO2 were in both cases above 95%. Our data show that both esters exhibit the ability, albeit mild, to induce the production of ROS in the tested system. Ongoing and further tests will show whether these and other esters from M. bosniense DS can induce ROS production in different in vitro and in vivo model systems.

Keywords: oxidative stress; esters; defensive secretions; diplopoda.

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Antinociceptive effect of ethanolic peel extracts from three varieties of Mexican pomegranate (*Punica granatum* L.) in the formalin test

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Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”. Pain treatment involves a wide variety of drugs with many adverse effects, some of them with a high impact on health. On the other hand, pomegranate (*Punica granatum* L.) is an ancestral fruit used in traditional medicine for its beneficial health properties. Pomegranate peel, rich in antioxidants, is known to have analgesic and gastroprotective effects. However, no studies have been found comparing the antinociceptive effect of different pomegranate varieties, in this case: bittersweet, green, and red, in a model of nociceptive and inflammatory pain (formalin test). The goal was to evaluate and compare the antinociceptive effect of ethanolic extracts in three Mexican varieties of pomegranate (ExG1, ExG2, and ExG3). Male Wistar rats weighing 180-200 g were used. The formalin test (2 %) was carried out, with an intragastric pre-treatment of 316 mg/k of ExG1, ExG2, and ExG3 (vehicle 1% tween 80). Also, 100 mg/kg of acetylsalicylic acid (ASA) dissolved in carboxymethylcellulose at 0.5 % as a reference drug. The results indicate that the three varieties had a statistically significant antinociceptive effect against the vehicle (p < 0.05), mainly in the second phase of the test. The overall antinociceptive effect was 48, 57, and 52%, respectively, ASA was 48%. Ethanolic extracts of pomegranate peel have an antinociceptive effect in both phases (nociceptive and inflammatory) of the formalin test, probably due their high antioxidant content, such as free organic acids like ellagic acid, polyphenols (punicalagin), and flavonoids. In this context, we consider that pomegranate peel is a natural option that helps to alleviate pain.

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The effect of spermidine on the expression of selected antioxidant genes in honey bees (Apis mellifera Linnaeus, 1758)

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The honey bee (Apis mellifera L.) is one of the most important pollinators worldwide. However, with the widespread misuse of chemical agents in agriculture, which are known to cause oxidative stress in honey bees, their numbers have steadily declined over the past few decades. Oxidative stress and the consequent pathophysiological state characterized by damage in cells and tissues constitute one of the possible pathways leading to aging. Spermidine is a polyamine which can be found ubiquitously in all living organisms. In recent surveys, model organisms, such as fruit flies and roundworms, exhibited a significant increase in average lifespan after supplementing their diet with spermidine. Therefore, the aim of our study was to investigate the short- and long-term effects of spermidine-supplemented diet on the expression of antioxidant genes: catalase (Cat), superoxide dismutase 1 and 2 (Sod1, Sod2) and transcription factor Nrf2 in honey bees. We detected a significant decrease in the expression of Cat and Sod2 after short-term exposure (48 hours) to spermidine at a concentration of 0.1 mM and a significant increase in Sod1 expression followed by long-term exposure (10 days) to spermidine at a concentration of 0.01 mM. The obtained results indicate that spermidine can alter the expression of antioxidant genes and thus affect the antioxidant defense mechanism of honey bees. The change in individual gene expression can be interpreted in multiple ways, as the exact mechanism by which spermidine acts in the honey bee remains to be determined. For this reason, future studies should be directed toward elucidating the precise change in signaling mechanisms caused by spermidine treatment.

Keywords: oxidative stress; catalase; superoxide dismutase; Nrf2.

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Radiation induced one-electron oxidation of 2-thiouracil in aqueous solutions

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In 1988 George H. Hitchings and Gertrude B. Elion were awarded Nobel Prize in Physiology and Medicine for their groundbreaking work which laid the foundations for development of new drugs against a variety of diseases. Their research in the 1950s on the therapeutic properties of sulfur-substituted nucleobases (thiobases) resulted in new chemotherapeutics. The aforementioned studies were devoted mostly to purine based thiobases, but thiopyrimidines also have their place on the wide spectrum of biologically active compounds. First, they naturally occur in bacterial tRNA, up to date 11 different thiopyrimidine based compounds have been identified in bacteria, where they play important role in cellular metabolism. There is also some evidence that thiobases play important role in metabolism of higher organism, e.g. lack of certain sulfur-substituted nucleobases in mitochondria has been proven to lead to development of diabetes in mice. In this work we utilized pulse radiolysis to unveil mechanisms of radical reactions of 2-thiouracil (2-TU). Oxidative damage to 2-TU by hydroxyl and azide radicals produces various primary reactive intermediates. Their optical absorption spectra and kinetic characteristics were studied by pulse radiolysis with UV-vis spectrophotometric and conductivity detection and by TD-DFT method. We discovered that the main route of thiobases reactions with OH involves reactions with sulfur atom. This reaction route is different from reactions occurring in unmodified nucleic bases in which OH radicals reacts mainly via addition to double bonds and hydrogen atom abstraction. Such striking differences in reaction mechanism lead to completely different transients and final products. Hydroxyl radical attack on sulfur atom results in thiyl radical formation, which is stabilized by three-electron bond with parent 2-TU molecule. Obtained results can help understand mechanisms of thiobases-based drugs transformations in living organisms in the oxidative stress conditions.

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The role of thioredoxin / thioredoxin reductase system in the redox homeostasis of the endoplasmic reticulum

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Introduction: The endoplasmic reticulum (ER) is the major site of protein thiol oxidation during post-translational modification. Due to the dominance of oxidized proteins, the lumen of the ER is usually considered as an oxidative environment, although some processes which require reducing agents, such as NADPH, are also found here. The parallel occurrence of oxidized thiol-disulfides and reduced pyridine nucleotides may indicate that the ER lumen lacks components which connect the two systems. Our aim was to investigate the luminal presence of the thioredoxin (Trx)/thioredoxin reductase (TrxR) proteins, capable to link the protein thiol and pyridine nucleotide systems.

Methods: Protein expression of Trx/TrxR isoforms was examined on subcellular fractions by Western blot analysis. TrxR activity in each organelle was measured using a colorimetric kit. The intracellular distribution of Trx/TrxR isoforms was also examined by immunofluorescent microscopy. An in silico analysis was performed to analyze the predicted localization of each isoform.

Results: We showed that the specific activity of TrxR in the ER is around zero (0.02 U/mg ± 0.01), while we measured higher activities in the cytoplasm (1.26 U/mg ± 0.11) and mitochondria (1.57 U/mg ± 0.19). Analysis of rat liver subcellular fractions revealed that the two isoforms of Trx, and the three isoforms of TrxR are not expressed in the ER. Immunofluorescent analysis confirmed that Trx and TrxR isoforms did not show colocalization with ER-specific marker Grp94. In silico prediction analysis also predicted a very low probability of luminal localization for each isoform (0 – 5%).

Conclusions: Our results show that none of the components of the Trx/TrxR system is expressed in the ER lumen. The absence of this electron transfer chain may explain the uncoupling of redox systems in the lumen, allowing the parallel presence of a reduced pyridine nucleotide pool and oxidized proteins.

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PP48

S-Nitrosation and denitrosation of proteins by variation of superoxide/nitric oxide ratio–implications for prevention of sulfoxidation-dependent enzyme inactivation during ischemia/reperfusion

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Oxidative stress plays a key role for the development of cardiovascular, metabolic and neurodegenerative disease. This concept was never widely proven by the therapeutic use of classical antioxidants in large scale clinical trials. However, we know from numerous (pre)clinical studies that the formation of reactive oxygen and nitrogen species (RONS) as well as stable oxidative stress markers are a hallmark of most forms of diseases and higher oxidative stress markers are frequently associated with higher disease risk and mortality. Besides the detrimental role of RONS, they are also involved in cellular functions via redox signalling. We have previously identified an efficient mechanism of S-nitrosation by low levels of nitric oxide and superoxide (3:1 ratio) with potential formation of N2O2 [Daiber, Schildknecht et al. and Ullrich, FRBM 2009]. Here we, elucidated whether S-nitrosation in the presence of higher nitric oxide than superoxide concentrations (as observed under hypoxic conditions) could prevent sulfoxidation and thereby oxidative inactivation of enzymes in the presence of higher superoxide than nitric oxide concentrations (as observed during reoxygenation). We found that increasing concentrations of xanthine oxidase/hypoxanthine caused conversion of S-nitrosoglutathione (GSNO) to reduced glutathione (GSH) up to a certain concentration of xanthine oxidase, indicating that superoxide can induce denitrosation of GSNO. This finding was unexpected since in the presence of excess superoxide one would not expect regeneration of reduced GSH from GSNO. This was even more surprising since we observed substantial dihydrorhodamine oxidation that was prevented by uric acid and tyrosine nitration of albumin during denitrosation of GSNO by superoxide, all of which points to intermediary formation of peroxynitrite. In summary, we propose that S-nitrosation of (mitochondrial) proteins during ischemia represents a protective mechanism to prevent irreversible overoxidation of thiols during the reperfusion phase and to re-establish reduced thiol state in (mitochondrial) key enzymes of energy metabolism and cell survival. Wide-spread mitochondrial protein S-nitrosation may represent a central feature of the protective preconditioning effects of nitric oxide.

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Keywords: S-nitrosylation; denitrosation; superoxide and nitric oxide; ischemia/reperfusion.

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Targeting oxidative stress to restore ER-mitochondria communication in Friedreich’s Ataxia

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Friedreich ataxia (FRDA) is a neurodegenerative disorder characterized by neuromuscular and neurological manifestations. It is caused by mutations in gene FXN, which results in loss of the mitochondrial protein frataxin and leads to mitochondrial dysfunction associated with redox imbalance and reduced mitochondrial energy production. Calcium (Ca²⁺) homeostasis is crucial for numerous cellular mechanisms and both the mitochondria and the endoplasmic reticulum (ER) regulate this process through specific inter-organelle contact sites termed endoplasmic reticulum-mitochondria associated membranes (MAMs). The interaction between these compartments is mainly mediated by the voltage-dependent anionic channel 1 (VDAC1) located in the mitochondrial outer membrane, the inositol-1,4,5-trisphosphate receptor (IP3R) in the ER membrane and the chaperone glucose-regulated protein 75 (GRP75). MAMs are involved in a wide variety of cellular processes, including lipid metabolism, autophagy, mitochondrial morphology, which are usually altered in several neurodegenerative disorders, like FRDA. But little is known about the relationship between MAMs and FRDA, so the aim of our study was to assess MAMs architecture and integrity in our FRDA cell model. Our findings indicate that MAMs communication is disrupted in our FXN-silenced cells, displaying structural alterations through the reduction of the interactions between VDAC- IP3R and VDAC-GRP75. Also, cells with frataxin deficiency show altered mitochondrial Ca²⁺ influx and elevated lipid peroxidation levels. The addition of different compounds, including antioxidants, increased the interaction between the two compartments, restoring the Ca²⁺ flux into the mitochondria and decreasing lipid peroxidation levels. These results suggest the stabilization of the ER-mitochondria contacts by means of the reduction of the redox environment in the MAMs’ domain. Remarkably, we found frataxin as a member of the protein network of MAMs, where it interacts with IP3R and GRP75. These results suggest a new role of frataxin and highlight MAMs as novel therapeutic candidates to improve patient’s conditions.

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Role of histone modification by hypochlorous acid on vascular cell function

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Neutrophil extracellular traps (NETs) consist of spindles of DNA together with histones and granule proteins including myeloperoxidase (MPO). They are released from neutrophils as an innate immune defense, but are increasingly implicated in thrombosis and the development of atherosclerosis and other chronic inflammatory pathologies. MPO present on the NETs produces the potent oxidant hypochlorous acid (HOCl), which reacts rapidly with proteins. In this study, we investigated the reactivity of HOCl with histones, which are the most abundant protein in the NETs, and assessed whether these modifications altered the effects of histones on vascular cell models. Experiments were performed with a preparation of histones containing histone H1, H2A, H2B, H3 and H4. Treatment of this histone preparation with reagent HOCl or the MPO/H2O2/Cl- system, resulted in the modification of Lys residues and the formation of unstable chloramines, which decomposed over 24 h. Evidence was also obtained for a dose- and time-dependent decrease in the concentration of Arg and Tyr residues, which was accompanied by the formation of stable oxidation products, including 3-chloro-Tyr and protein carbonyls. Exposure of human coronary artery endothelial cells (HCAEC) or human coronary artery smooth muscle cells (HCASMC) to non-modified histones resulted in a dose-dependent loss of viability, consistent with the known toxicity of histones when present in the extracellular environment. However, this loss in viability was attenuated on pre-treatment of the histones with HOCl. The ability of HOCl to decrease histone-induced cell death was dependent on the extent of oxidative protein modification. Given the close association of MPO with histones in NETs, these data provide new insight into potential pathways by which NETs could influence cellular function during chronic inflammation. This may be particularly relevant in atherosclerosis, where NETs are associated with lesion development and vascular dysfunction.

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PP51

Age-related microRNA overexpression in Lafora disease: at the crossroads between inflammation and oxidative stress

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MicroRNA-mediated regulation of gene expression lies at the center of an increasing number of molecular networks critical for the maintenance of cell homeostasis. As such, alterations in microRNA expression can be found in many pathological conditions, to the point that microRNA profiles are being proposed as biomarkers of disease onset, progression and treatment monitoring. Several specific microRNAs have been directly related to regulation of the antioxidant response, and at the same time, oxidative stress has proven to affect the expression of certain microRNAs. In the present work, we provide data on the age-related altered expression of two specific microRNAs (inflamma-miRs), miR146a and miR155, in two different mouse models of the rare progressive myoclonus epilepsy called Lafora disease (LD). These animal models have previously shown important alterations in gene expression mainly related to inflammatory pathways, as well as an increase in oxidative stress parameters and a defective antioxidant response. Our results show an association between these two microRNAs and the expression of antioxidant, pro- and anti-inflammatory genes, which can also be observed in cellular models of the disease, as well as in other cellular models subjected to oxidative stress or inflammatory stimuli. These data pave the road for the use of these microRNAs as biomarkers for LD and, at the same time, provide further insights on their role at the crossroads between inflammation, oxidative stress and LD pathophysiology.

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PEGylated liposomes encapsulating hydrogen sulfide donor ADTOH prolongs in vivo circulation time and improves cellular uptake

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Hydrogen sulfide (H2S) is involved in the regulation of several physiological and pathophysiological processes and promotes cardioprotective properties in irreversible ischemia/reperfusion injury whilst also exerting a vasorelaxant effect in other vascular tissues. Moreover, H2S donors have been shown to promote angiogenesis and cellular bioenergetics by enhancing the mitochondrial oxygen consumption. The objective of this study was to determine the profile of ADTOH, a H2S donor, when delivered using liposome-based carriers in cultured endothelial cells and in vivo. PEGylated liposomes encapsulating ADTOH were formulated, and pharmacokinetic parameters were determined. The ability of liposome encapsulated ADTOH to promote angiogenesis and enhance mitochondrial bioenergetics was also investigated. ADTOH (0.01% w/v) loaded liposomes were formulated from phosphatidylcholine, DSPE-PEG(2000) and cholesterol. Liposomes or ADTOH in PBS were intravenously injected in C57BL/6 mice and blood samples undertaken at defined time points over 72 hours to determine drug concentration in the circulation quantified using HPLC with UV analysis. Tube formation assays were performed as indicative of angiogenic potential and mitochondrial oxygen consumption was evaluated in cultured endothelial cells. Liposomes doubled in vivo circulation time with the half-life increasing to 22.9 hours resulting in a greater area under the curve, correlating to drug exposure for liposomal ADTOH, at 0.91 mg/ml x hour. Clearance was halved from 0.02 ml/hour to 0.01 ml/hour. Application of formulation onto HUVEC’s observed that ADTOH (0.1 mg/mL) enhanced tubular network formation and the basal oxygen consumption rates after 30 min of treatment in HUVECs. Liposomal delivery of ADTOH may provide a promising approach in the treatment of various cardiovascular conditions. Using liposomes as a controlled release formulation may also be useful to provide long term low levels of H2S in order to reduce toxicity and off targets effects as well as reduce the need for repeated dosing.

Keywords: hydrogen sulfide donors; controlled release; liposomes.

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Noise-induced vascular dysfunction, oxidative stress and inflammation are improved by pharmacological heme oxygenase-1 induction

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Vascular oxidative stress, inflammation and subsequent endothelial dysfunction are consequences of traditional cardiovascular risk factors and triggers of cardiovascular disease. Emerging environmental stressors, such as traffic noise exposure may facilitate the development of cardiovascular and metabolic diseases. In our previous studies, we investigated the influence of aircraft noise exposure on molecular mechanisms identifying oxidative stress and inflammation as central players in mediating vascular dysfunction. In our previous studies, we investigated the influence of aircraft noise exposure on molecular mechanisms identifying oxidative stress and inflammation as central players in mediating vascular dysfunction. Peak sound levels of 85 and mean sound level of 72 dB(A) applied for 1, 2 and 4 days caused an increase in systolic blood pressure, stress hormones and induced endothelial dysfunction, oxidative stress and inflammation. The role of heme oxygenase-1 (HO-1) as an antioxidant response is currently being investigated. C57BL/6J mice were treated with the HO-1 activator hemin (25 mg/kg i.p.) and the Nrf2 inducer dimethyl fumarate (DMF, 20 mg/kg d.o The initial data suggested that exposure to aircraft noise during 4 days causes vascular dysfunction primarily due to the stimulatory effects on vascular ROS production. In addition, there was a trend of normalization of noise-triggered oxidative stress in hemin infusion suggesting a protective effect. Within the ongoing studies, we will address the impact of HO-1 induction in noise-exposed mice on expression of genes and proteins involved in pathways of oxidative stress and systemic inflammation. The present study aims at identifying new targets for mitigation strategies against adverse health effects of environmental noise exposure of the general population. Since HO-1 and Nrf2 induction can be achieved by natural dietary constituents, these pathways represent promising targets for preventive measures.

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Functional and structural characterization of Hypocrates, a genetically encoded biosensor for hypochlorite

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Hypochlorous acid (HClO), an aggressive oxidant, is important in immune defense against pathogens. The current lack of tools to monitor the dynamics of hypochlorous acid in live cells and tissue hinders a better understanding of inflammatory processes. Recent studies have identified two HClO-sensing transcription factors: HypR¹ and NemR², which use different mechanisms to sense HClO. In this study, we compared the rates of these two transcription factors towards HClO, and engineered a genetically encoded biosensor, Hypocrates, for the visualization of hypochlorous acid³. Hypocrates consists of a circularly permuted yellow fluorescent protein (cpYFP) integrated into the structure of a single cysteine variant of the transcription factor NemR from E. coli. We determined the sensitivity, selectivity, and the reaction rates of this ratiometric redox biosensor towards HClO, and solved its X-ray crystal structure³. The response of Hypocrates to varying hypochlorite concentrations was monitored in HeLa Kyoto cells.


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PP55

Different levels of exploring the antioxidant and antitumor potential of *Salvia officinalis* and *Salvia rosmarinus*

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*Salvia officinalis* (sage) and *Salvia rosmarinus* (previously *Rosmarinus officinalis*, rosemary) are commonly used medicinal plants from the Lamiaceae family, with strong antioxidant abilities. This study was carried out in several stages to comprehensively and gradually explore the antioxidant and antitumor mechanisms of their ethanolic extracts using different experimental approaches. Their \textit{in vitro} antioxidant activity was assessed using ABTS assay. Plasmid pUC19 E. coli XL1-Blue was used to evaluate their genoprotective activity, while SOS/umuC assay on Salmonella typhimurium TA1535/pSK1002 and Comet assay on human lung fibroblast cell line (MRC-5) were employed to examine their antigenotoxic potential. Furthermore, their antitumor mechanisms were analyzed on the colorectal cancer cell line (HCT-116) by testing their impact on the cells’ viability (MTT assay), as well as the production of reactive oxygen species (ROS) (NBT test) and nitric oxide (NO) (Griess assay). The results showed that sage extract had slightly higher ABTS-scavenging and antigenotoxic activities in the bacterial model when compared to the rosemary extract, however, opposite results were obtained for the acellular model. Moreover, both extracts protected the MRC-5 cells from the induced genomic damages. As for tumor cells, sage extract reduced their viability by approximately 10%. Additionally, both extracts significantly reduced the ROS production, while also increasing the NO production, especially the rosemary extract. These results delivered novel information about the nature of antioxidant effects of sage and rosemary ethanolic extracts. They provided substantial protection of the acellular, bacterial and healthy human cellular models by employing radical-scavenging and antigenotoxic mechanisms. At the same time, both extracts exhibited antitumor activity towards colorectal cancer cells \textit{in vitro} that could be mediated by inducing an increase in NO production. With that being said, sage and rosemary ethanolic extracts represent potent genoprotective, antioxidant and antitumor agents. However, their exact mechanisms of action remain to be further explored and clarified.

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Antioxidative potential of *Viscum album* L. extract in imidacloprid-induced hepatotoxicity

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Imidacloprid (IMD) belongs to a novel class of insecticides, neonicotinoids. Beside its well established generally low toxicity towards mammalian tissues, certain detrimental effects of IMD in various organs were also detected, including damages of liver. *Viscum album* is a hemiparasitic plant which has been used for decades in traditional medicine due to its plentiful bioactive compounds, including flavonoids. Therefore, the present study was carried out to evaluate the toxic effects of IMD on metabolic and histological parameters of liver tissue damage, and to investigate the potential protective role of *Viscum album* aqueous extract (VAE) from a pear tree. Thirty male Wistar rats were divided into: I group of animals served as control; II, IV and V group received IMD (70 mg/kg, via intragastric tube); III and IV groups received higher dose of VAE (350 mg/kg i.p) and V group received lower dose of VAE (175 mg/kg i.p). The results displayed that IMD administration increased total cholesterol, triglycerides and aminotransferases (AST and ALT), reduced albumins in serum and induced hepatic histopathological damage. IMD also increased LPO concentration and the activity of antioxidant system parameters (SOD, CAT, GSH-Px, GR and GST) in the liver, while AChE was inhibited. The treatment with VAE overcome toxic effects of IMD by reducing ALT, AST and lipids, and also by alleviating histological changes of liver tissue. The attenuation of oxidative stress was evidenced in reduced LPO levels and antioxidative enzyme activities (SOD, CAT and GSH-Px). These findings revealed the efficacy of VAE against liver induced IMD toxicity. These antitoxic and antioxidative effects could be ascribed to polyphenols present in the extract in high amount.

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Kinetic data for the reactions of alpha,beta-unsaturated aldehydes shed light on their molecular targets and biological effects

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A wide range of alpha, beta-unsaturated carbonyls (ABuCs) are encountered in everyday life. The toxic molecule acrolein is the smallest ABuC family member, which is present in cigarette smoke, fried foods or automobile exhausts. Peroxidation of polyunsaturated fatty acids also yields acrolein among other ABuCs such as crotonaldehyde, 4-hydroxynonenal and 4-hydroxypentenal. The latter ABuCs have also been proposed to have a signaling action rather than a toxic one. ABuCs have also been utilized as treatments for diseases, with dimethylfumarate used to treat multiple sclerosis and psoriasis, and itaconate showing promise as an anti-inflammatory agent. ABuCs act as soft electrophiles and react with biological nucleophiles via Michael addition. Cysteine residues are major targets since they are relatively abundant and kinetically-favored over other targets (e.g. DNA bases, lysine and histidine residues on proteins). However, the kinetics and selectivity of ABuCs–cysteine reactions are incompletely understood. We therefore aimed to determine kinetic data (rate constants) for these reactions and their downstream effects. The rate constants for addition of GSH to acrolein, crotonaldehyde, dimethylfumarate, cyclohex-1-en-2-one and cyclopent-1-en-2-one are shown to vary by a factor of 350 (rate constants, \(k\), 0.5 – 186 M\(^{-1}\) s\(^{-1}\)) indicating that the ABuC structure is a determining factor for reactivity, with acrolein being the most reactive. We also show that the microenvironment of the cysteine residue, and its pKa value, have an impact on the reactivity. Protein incorporated cysteine react up to 30 times faster than free cysteine and GSH. The toxic species acrolein is highly reactive, and reacts in an unspecific manner. Dimethylfumarate on the other hand reacts more slowly and is much more specific. Enzymatic assays show that these Michael addition reactions can inhibit enzyme activity, highlighting the functional consequences of ABuC reactivity. These data can help explain why acrolein is toxic, while dimethylfumarate has beneficial biological effects.

Keywords: alpha,beta-unsaturated carbonyls; acrolein; dimethylfumarate; protein modification; michael reaction.

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PP58

Frailty index of aging female rats changes under different dietary protocols

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The continuous increase of a lifespan imposes need to prolong the healthspan - period of life spent free of age-related disabilities. Some interventions such as dietary restriction (DR) can retard aging process, improve quality of life and increase healthspan. However, recent studies have re-evaluated generality of DR's beneficial effect, as different outcomes of DR have been reported in dependence of onset and duration of DR. In order to test the impact of various dietary restricted regimens in female rats during aging, we used frailty assessment as one of the best indicators of healthspan. Female Wistar rats of various age (young adult, middle-age and aged) were exposed to DR (60% of AL daily intake) feeding regimens. We determined and compared frailty index (FI) during aging in ad libitum fed (AL) and in rats exposed to DR. Frailty index was constructed using 22 parameters. FI was increased in both 18- and 24- month old AL animals in comparison to young control counterparts. Life-long type of restricted diet decreased FI in 18- month old group while shorter duration of DR regimens failed to lower frailty index in 18- and 24- month old animals. Our results indicated that life-long DR can alter animal's frailty during aging, while shorter duration and later onset DR have no effect on FI in female rats.

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Association of the intake and status of essential microelements on cardiometabolic parameters in dislipidemic subjects

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Objectives: Lipid disorders are identified as one of the key risk factors in the etiopathogenesis of cardiovascular disease. The aim of this study was to examine the association of dietary intake and biomarkers of zinc (Zn), copper (Cu) and iron (Fe) status with cardiometabolic parameters, lipid profile, plasma phospholipid fatty acids, desaturase enzyme activity and linoleic and dichomo-gamma-linolenic acid ratio LA/DGLA) in 27 dyslipidemic subjects aged 39-72 years, mean body mass index (ITT) 28.2 kg/m². Methods: Dietary intake assessment was based on repeated 24 h recalls and Serbian national food composition database. The concentration of microelements in plasma was determined by atomic absorption spectrophotometry, and the fatty acid profile by gas chromatography. Results: Elevated values of total (Cho) and LDL cholesterol (LDL-c) were found in 88% of subjects, while 30% had HDL-c values below the recommended threshold. Hypertriglyceridemia, in addition to hypercholesterolemia, low HDL-c and elevated LDL-c was found in 18.5% of the sample. Dietary Zn intake was below the recommended level in 65% of subjects, with a mean estimated value of 7.42 ± 1.82 mg/day. An inverse correlation was found between Zn intake and LA/DGLA ratio (r = −0.38, p = 0.05). Low plasma Zn concentration (0.75 ± 0.10 mg/L) and increased Cu/Zn ratio (1.30 ± 0.28) were noted, which indicates Zn deficiency and inflammatory condition. Additionally, an inverse correlation was observed between Cu/Zn ratio and BMI (r = −0.488, p = 0.02), as well as lipid profile parameters LDL-c/HDL-c and Cho/HDL-c (r = −0.518, p = 0.011r = −0.541, p = 0.008, respectively). Conclusion: Dietary interventions targeting optimization of the intake and status of essential trace elements can play a significant role in the prevention and treatment of dyslipidemia.

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The role of interleukin-6 in redox homeostasis and migration capacity in human trophoblast cells JEG-3 under condition of chemically induced hypoxia

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Highly regulated processes of proliferation, invasion and survival of extrovillous trophoblasts on endometrium and spiral arteries of uterus are crucial elements for successful establishment of placenta. Elevated plasma levels of interleukin-6 (IL-6), the main inflammatory cytokine, during certain stages of gestation has been implicated as a potential risk factor in the development of some pathological forms of pregnancy. The aim of the study was to investigate the possible contribution of increasing elevated concentrations of interleukin-6 in chemically induced hypoxia on physiological dysfunctioning of trophoblasts, and in elucidation of its pathological potential in placental disorders associated with inadequate trophoblastic invasion and survival. Throughout the study, cell viability, migration capacity and the parameters of oxidative metabolism (such as superoxide anion radical and nitrites), were evaluated. IL-6 slightly elevated trophoblast migration capacity in hypoxic condition, while provoking an excessive migration boost in normoxia. The results show that treatment with interleukin-6 maintained cell proliferation ratio, compared to non-treated cells. IL-6 treatment in hypoxic conditions has not induced statistically significant changes in production of superoxide anion radical, in contrast to the reduced values in normoxic cells, implying its potential significant contribution in maintaining of optimal levels of oxidative metabolism in conditions of reduced oxygen availability. The reduction of nitrite levels may also be of physiological significance in reducing of excessive mobility of trophoblasts and inadequate colonization of the endometrium. In condition of chemically induced hypoxia, IL-6 exerted beneficial role on maintenance of cell viability, redox metabolism and migration potential, in contrast to the effects obtained in normoxia where it may affect trophoblast migration and redox balance. Our results suggest that in normoxic, later stages of pregnancy, the elevated levels of IL-6 may have disturbing effects on redox balance and trophoblast migration capacity, making it a possible risk factor in the pathogenesis of certain placental disorders.

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Novel anti-cancer compound – inhibitor of TrxR distress GSH system in glioma cells

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Glioblastoma is the most frequent malignant brain tumor, with limited therapy options due to aggressive invasiveness and resistance to therapy. Elevated expression of redox system components, thioredoxin (Trx) and thioredoxin reductase (TrxR), is a common feature of cancer cells and correlates with cancer progression and poor prognosis. Therefore, Trx and TrxR are attractive targets for chemotherapy development. Inhibition of Trx system may also affect another important redox system – the glutathione (GSH) system. Here, we investigated whether GSH system can compensate Trx system inhibition by novel TrxR1 inhibitor (DVD-444) in human and rat drug resistant glioma cell lines and their sensitive counterparts. RT-qPCR analysis showed that DVD-444 decreased GSH peroxidase 1 (GPX1) and 4 (GPX4) mRNA expression in all glioma cell lines. Decrease in GPX expression indicates suppression of another antioxidant defense system, besides Trx system, leaving cells vulnerable to oxidative stress. Tested compound caused an increase in expression of GSH reductase (GR) and GSH-S transferase π (GSTπ). The observed increase in GR could be the consequence of oxidative stress imposed by treatment with TrxR1 inhibitor, while elevated GSTπ implies that GSTπ is highly involved in detoxification of the applied compound. Furthermore, colorimetric GSH assay showed that DVD-444 increased GSH cell content in glioma cell lines. AV/PI staining following treatment with TrxR1 inhibitor demonstrated significant level of cell death in rat glioma cell lines. Based on CFSE staining DVD-444 showed antiproliferative effect in human glioma cells. In conclusion, TrxR1 inhibitor caused changes in components of GSH system. However, the changes in GSH system did not prevent inhibition of cell proliferation and cell death evasion after TrxR1 inhibition, making DVD-444 perspective candidate for glioblastoma treatment strategy.

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Validating personalized redox biology: the effect of targeted and non-targeted antioxidant supplementation on exercise performance

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We have shown that vitamin C and N-acetylcysteine (NAC) supplementation improves performance in vitamin C and glutathione deficient individuals, respectively. In the present study, we aimed to validate the idea of personalized redox supplementation by subjecting individuals to targeted and non-targeted antioxidant supplementation schemes. Seventy-three volunteers were screened for plasma vitamin C and erythrocyte glutathione levels. Three groups were formed: i) the “low vitamin C” group (L-VitC; n = 12), ii) the “low glutathione” group (L-GSH; n = 12) and iii) a control group (CON; n = 12 individuals with normal antioxidant levels). The three groups received orally 1 g of vitamin C or 1.2 g of NAC daily for 30 days in a crossover design with a wash-out period of 30 days. Prior to and at the end of each treatment the participants underwent a series of performance tests evaluating aerobic and anaerobic capacity as well as muscle function and resistance to fatigue. Both antioxidant treatments reduced the increased baseline systemic oxidative stress levels, assessed via F₂-isoprostanes, in the L-VitC and L-GSH groups (P < .05). A significant group × time interaction (P < .05) was found for VO2max (aerobic capacity) and isometric peak torque after both treatments, with the L-VitC and L-GSH groups exhibiting improved performance only after the targeted treatment. CON group exhibited similar or impaired performance after supplementation. A significant group × time interaction (P < .05) was also found for fatigue index after NAC treatment, but not after vitamin C treatment. No interaction was found for the Wingate test (anaerobic capacity). Collectively, most of the evidence presented herein verifies the idea that antioxidant supplementation increases performance when a particular deficiency is reversed. This indicates that the presence of oxidative stress per se does not rationalize the use of antioxidants and emphasizes that the successful delivery of a redox treatment may depend on our ability to identify “responsive” phenotypes.

Keywords: antioxidants; exercise performance; falsification; personalized nutrition.

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Neuronal NOX4 knockdown alleviates pathological tau-related alterations in a humanized mouse model of tauopathy

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Approximately 44 million people worldwide live with Alzheimer’s disease (AD) or a related form of dementia. Aggregates of the microtubule-associated protein tau are a common denominator in several neurodegenerative diseases (NDDs) collectively known as tauopathies, and constitutes a defining feature of AD. Given that pathological tau is one of the main drivers of neuronal toxicity in several NDDs and the lack of efficacy to date of tau therapies, new targets to halt tau progression is becoming a matter of clinical urgency. In this study, we have demonstrated that among NADPH oxidases (NOX), the isoform 4 (NOX4) is upregulated in Frontotemporal dementia (FTD), AD patients and in a humanized mouse model of tauopathy. Interestingly, NOX4 genetic suppression and more specifically, neuronal-driven NOX4 downregulation in vivo, diminish the accumulation of pathological hyperphosphorylated tau, once tauopathy is established, by a mechanism that implicates the modulation of the macroautophagy-lysosomal system. In this context, NOX4 inhibition translated in neuroprotection and prevention to cognitive decline, suggesting a disease-modifying effect in tau pathology. Thus, NOX4 is a previously unrecognized causal, mechanism-based target in tauopathies and blood-brain barrier permeable specific NOX4 inhibitors could have therapeutic potential even in established disease.

Keywords: Alzheimer’s disease; tauopathy; autophagy; NADPH oxidases; NOX4.

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3. 5-DCPBC: A magic bullet for melanoma therapy

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Drugs with a high potency and selectivity toward multiple biological targets represent a novel and efficient drug discovery paradigm. Signal transduction in cancer cells is a sophisticated process that involves receptor tyrosine kinases (RTKs) that eventually trigger multiple cytoplasmic kinases, which are often serine/threonine kinases. In clinical trials, highly selective or specific blocking of only one of the kinases involved in these signaling pathways has been associated with limited or sporadic responses. Improved understanding of the complexity of signal transduction processes and their roles in cancer has suggested that simultaneous inhibition of several key kinases at the level of receptors and/or downstream serine/threonine kinases may help to optimize the overall therapeutic benefit associated with molecularly targeted anticancer agents. Immunotherapy with CTLA-4 and PD-1 antibodies has emerged as recent breakthrough in the therapy of metastatic melanoma, that is considered as one of the most deadliest oxidative stress induced malignancy. However limited response rate, severe life-threatening or fatal side effects and resistant nature of malignant melanoma further fuels the urgent quest for new strategies in the battle against metastatic melanoma. A newly emerging concept for the treatment of oxidative stress induced advanced malignant melanoma is based on developing new synthetic compounds targeting multiple signaling pathways and their corresponding genes. We have discovered a novel multi targeted molecule belonging to the class of bis-coumarin, as a potential anti-melanoma and anti-metastatic drug candidate and have suggested it for further preclinical and clinical trials based on its ability of selectively killing melanoma cells and inhibiting their migration via targeting multiple phosphokinases.

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Redox status in the steatotic liver graft cold storage using PEG-35

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Considering the increasing incidence of obesity, metabolic disorders and consequent NAFLD, there is a need to use steatotic livers for transplantation. However, suboptimal organs are more vulnerable to ischemic injury and organ failure. Therefore, finding the optimal composition of preservation solutions is crucial for the well-being of the organ by avoiding ischemic damage. We have previously demonstrated that the addition of Polyethylene glycol 35 (PEG-35) as oncotic agent provides mitochondrial protection. The aim of the present study is to assess whether the addition of PEG-35 protects fatty livers against oxidative damage. Three different preservations solutions have been used: IGL-0 (without PEG-35), IGL-1 (PEG-35 at 1 g/L) and IGL-2 (PEG-35 at 5 g/L). Zucker rats, a model of genetic obesity, were used. Fatty livers were isolated and preserved at 4 °C for a 24 h. After the cold storage period, livers were flushed with Ringer Lactate solution and homogenized for subsequent biochemical analysis. As oxidation biomarkers, advanced oxidation protein products (AOPP), lipid peroxides (TBARS) and 4-hydroxynonenal protein adducts (4-HNE) were measured. As antioxidants, we measured reduced (GSH) and oxidized (GSSG) glutathione, and the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione s-transferase (GST). The expression of nuclear factors erythroid 2-like 2 (Nrf2) and kappa activated B cells (NFκB), the Uncoupling Protein 2 (UCP2), inflammasome NLRP3 and ATP levels were also analyzed. Our results indicate that PEG-35 improves the antioxidant response, via Nrf2, and increases the expression of UCP2, which can help maintain ATP levels. PEG-35 provides an advantage by limiting mitochondrial ROS production and inhibiting the NFκB pathway and inflammation. The present findings show that the oncotic agent PEG-35 modulates the redox state, for which its clinical application would be recommended.

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Urate oxidase is modulated by NO-derived post-translational modifications during the ripening of sweet pepper fruit

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In plants, urate oxidase (UO; EC 1.7.3.3), also called uricase, is an important enzyme involved in the nitrogen cycle through the ureides’ metabolism. This enzyme catalyzes the oxidation of urate to allantoin and is localized in unspecialized peroxisomes, where it plays an important role in nodules from leguminous species. In addition, UO has also been detected in other plant organs, such as leaves from several species, although at a very low level. Thus far there are no reports on either the presence of urate oxidase in fleshy fruits or how this enzyme is modulated by nitric oxide (NO) and other reactive nitrogen species (RNS) in higher plants. In this work, comparative and complementary RNA-seq transcriptomic, gene expression through RT-PCR, and iTRAQ proteomic analyses of green and red sweet pepper (Capsicum annuum L.) fruits were carried out. UO activity in peroxisomes isolated from pepper fruits, and the presence of uric acid and allantoin by HPLC-MS in this reproductive organ was also studied. The results show that UO from pepper fruits contains 307 amino acids, with a molecular weight of 34.82 kDa and pl 6.31. Although UO activity remains unchanged, the gene expression is down regulated during ripening, a physiological process where NO and RNS play relevant roles. Besides, it was found by in vitro assays that UO is modulated by nitration and S-nitrosation events. By coupling a biotin-switch method and mass spectrometry, a cysteine susceptible to be S-nitrosated was identified in the enzyme purified from the yeast Candida utilis. Overall, the results suggest that UO could be involved in the ripening process of pepper fruits, where the enzyme might regulate the levels of both, uric acid and peroxynitrite (ONOO⁻), two molecules that interact and scavenge one to another.

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PP67

Upregulation of toll-like receptors 4 in peripheral mononuclear cells of chronic renal disease patients on hemodialysis is associated with poor cardiac function and increased mortality

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Introduction: Chronic renal disease (CKD) treated with hemodialysis increases the risk of heart failure development. So far, inflammation and oxidative stress were underlined as possible mechanisms that contribute to the aggravation of heart function and the development of a failing heart. Previous studies have suggested that increase activation of TLR4 signalling causes impairment of mitochondrial function and promotion of oxidative stress leading to dysfunction of cardiomyocytes. The Aim: This study aimed to investigate if TLR4 mRNA levels were associated with the extent of heart failure according to New York Heart Association (NYHA) Functional Classification, as well as with mortality. Material and Methods: The study included 75 patients (49 men and 26 women) with CKD on hemodialysis. According to the NYHA classification our patients were classified into three stages of heart failure: 18 patients in class I (without symptoms and physical activity limitations, e.g. palpitation, dyspnea, anginal pain etc.), 45 patients in class II (mild symptoms and slight physical activity limitations), 12 patients in class III (marked physical activity limitations). TLR4 messenger ribonucleic acid (mRNA) levels were measured by quantitative polymerase chain reaction and normalized to GAPDH mRNA as a reference gene. Results: Upregulation of TLR4 mRNA levels were observed in the patients belonging to NYHA classes II and III compared to the patients in class I (p = 0.043). Besides, during the study 11 patients deceased. These patients had higher gene expression levels of TLR4 compared to the survived patients (p = 0.036). Conclusion: TLR4 mRNA levels were associated with poor cardiac function classified according to NYHA criteria and with increased mortality in hemodialysis patients. Our findings are following nowadays knowledge of TLR4 involvement in inflammatory pathways and oxidative stress which are possible mechanisms of developing and progression of heart failure.

Keywords: TLR4; heart failure; chronic kidney disease.

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Interactive role of oxidative stress and inflammation in the development of diabetic foot

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Introduction: Diabetic foot is a chronic complication of poorly regulated diabetes mellitus (DM), manifested by ulceration. Complex interaction of hyperglycemia, inflammation and oxidative stress (OS) underlies the development of foot ulceration. The aim of our study was to investigate the OS parameters and their association with inflammatory biomarkers in patients with and without diabetic foot. Additionally, we assessed the diagnostic accuracy of prooxidant-antioxidant balance (PAB) and neutrophils to lymphocytes ratio (NLR) in determining the diabetic foot presence.

Methods: General biochemistry, PAB, superoxide dismutase (SOD), total antioxidant status (TAS) and complete blood count (CBC) were determined in 42 type 2 DM patients by standard laboratory methods. NLR was calculated as a ratio of the absolute neutrophil count to the absolute lymphocyte count. Based on the presence of foot ulceration patients were divided in groups with diabetic foot (DF, N = 23) and without this complication (DM, N = 19).

Results: DF patients had significantly higher (P < 0.01) PAB (median: 167 HKU; 25th-75th percentile: 155-195 HKU) compared to DM group (median: 134 HKU; 25th-75th percentile: 124-166 HKU) while SOD activities were significantly lower (P < 0.01). TAS did not significantly differ among the groups. NLR values in DF group (median: 2.8; 25th-75th percentile: 2.0-4.3), were significantly higher (P < 0.01) than in DM group (median: 1.7; 25th-75th percentile: 1.4-2.1). In the DF group, significant correlation was observed between PAB and NLR (r = 0.453, P < 0.05), while no such correlation was observed in the DM group. The diagnostic accuracy of both PAB (AUC = 0.776; 95% CI: 0.606-0.913; P < 0.01) and NLR (AUC = 0.776; 95% CI: 0.632-0.919; P < 0.01) was estimated as acceptable.

Conclusion: Oxidative stress and inflammation are more pronounced in patients with diabetic foot. PAB and NLR could be useful non-invasive biomarkers in the early detection of the development of the diabetic foot.

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PP69

Phenotypic changes in human coronary artery smooth muscles cells cultured on peroxynitrous acid-modified extracellular matrix

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In vascular disease, including atherosclerosis, inflammatory cells are recruited to the artery wall where they produce oxidants. Targets for these oxidants within the artery wall include extracellular matrix (ECM) proteins due to their high abundance and rates of reactions with oxidants. The vascular ECM is important for the integrity and function of the artery wall, including the control of vascular cell phenotype. During the development of atherosclerosis, vascular smooth muscle cells undergo a pronounced phenotypic switch from quiescent and contractile, to a proliferative and synthetic form. We hypothesize that ECM modifications induced by the inflammatory oxidant, peroxynitrous acid (ONOOH), might drive this phenotypic switch, and thereby contribute to the progression of atherosclerosis. ECM from cultures of primary human coronary artery smooth muscle cells (HCASMCs) was treated with increasing concentrations of ONOOH, with ELISAs used to determine the extent of modification caused by the oxidant. Cell adhesion to native and modified ECM, and subsequent proliferation, were examined by calcein-AM staining and MTS assay. Expression of mitosis, ECM and inflammatory genes was examined by qPCR, and secretion of inflammatory proteins quantified using ELISA. Extensive modification and nitration was detected on HCASMC-ECM components treated with ONOOH, as detected using specific antibodies. Modified ECM reduced adhesion of naive HCASMCs, but increased cell proliferation. mRNA expression of ECM proteins (laminin chains, fibronectin, and versican), inflammatory cytokines (IL-1B and IL-6), matrix metalloproteinase proteinase 1 (MMP-1) and vascular cell adhesion molecule 1 (VCAM-1) were up-regulated. Finally, increased secretion of IL-1B was detected from HCASMCs cultured on modified matrix. The changes to HCASMC behavior reported here are consistent with ECM remodeling and the pro-inflammatory state seen in atherosclerosis, suggesting a link between oxidant-modified ECM and the progression of atherosclerosis. These data highlight the potential of targeting oxidant generation as a preventative, or therapeutic, strategy for atherosclerosis.

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Blockade of IL-6 abrogates pancreatic and lung damage caused by acute pancreatitis in PGC-1α knock-out mice

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PPAR coactivator 1α (PGC-1α) is a transcriptional coactivator that controls mitochondrial antioxidant defence and is downregulated in pancreas of obese mice. Obesity increases the risk of local and systemic complications during acute pancreatitis. Our aim was to determine the role of PGC-1α deficiency in the inflammatory response during acute pancreatitis. We induced acute pancreatitis by seven hourly intraperitoneal injections of cerulein in wild-type and PGC-1α knockout (KO) mice with pancreatitis. The IL-6 antagonist LMT-28 (1 mg/kg) was administered orally 1 h before the first and fourth cerulein injections to blockade IL-6 receptor gp130. Edema and inflammatory infiltrate were more intense in pancreas from PGC-1α KO after cerulein-induced acute pancreatitis in comparison with wild-type mice. In wild-type mice with pancreatitis PGC-1α protein levels increased but PGC-1α was acetylated, and the mRNA expression of its target genes superoxide dismutase-2, peroxiredoxin 3 and catalase were downregulated. PGC-1α deficiency markedly enhanced nuclear translocation of phospho-p65 and recruitment of p65 to interleukin-6 (Il-6) promoter leading to increased Il-6 mRNA levels. In addition, PGC-1α KO mice exhibited increased Il-6 plasma levels than wild-type mice with pancreatitis. PGC-1α KO mice with pancreatitis exhibited increased myeloperoxidase activity in the lungs, together with alveolar wall thickening and collapse. LMT-28 abrogated the increase in pulmonary myeloperoxidase activity in PGC-1α KO mice and greatly ameliorated the pulmonary and pancreatic injury. In conclusion, PGC-1α deficiency causes a severe inflammatory response during acute pancreatitis through NF-B-dependent up-regulation of IL-6, which was prevented by blockade of IL-6 receptor gp130 with LMT-28.

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Intermittent hypobaric hypoxia and cold treatment after gastrocnemius muscle injury enhance redox balance and avoids UPS activation

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Background: Common skeletal muscle injuries in sports and their recovery process are associated with muscle regeneration. Protein breakdown and repair pathways could be key questions to understand recovery from muscle injury. The formation of new myofibers involves a metabolic activation affecting redox balance. Therefore, our objective is to evaluate the redox parameters and the pathways for protein repair and degradation in healthy and injured contralateral gastrocnemius muscles after intermittent exposure to hypobaric hypoxia or cold.

Methodology: After inducing muscle injury in the right rat gastrocnemius, 9-day treatments of 4 h/day in intermittent hypobaric hypoxia (4,500 m) (HYPO) or cold (4 °C) (COLD) were applied. A control group (CTRL) was maintained in normoxia at 27 °C. Gastrocnemius muscles of injured and healthy legs were collected and homogenized in an urea lysis buffer (6 M urea, 1% SDS). Redox status was evaluated by the analysis of oxidized proteins (AOPP), glutathione levels and the expression of 4-hydroxynonenal protein-adducts (4-HNE), Nrf2 and eNOS. Protein repair pathways were evaluated by determining the expression of chaperones (HSP90, HSP70) and the ubiquitin proteasome system by Western Blot. Results: Our results show that after 9 days of recovery, the injured legs maintain high levels of oxidised proteins, lower expression in NRF2, accumulate more ubiquitinated low molecular weight proteins and greater expression is observed in the 20S catalytic subunit. Intermittent exposure to hypobaric hypoxia (HYPO) or cold (COLD) reverses the accumulation of AOPP. Furthermore, exposure to COLD preserves GSH and increases the expression of HSP90 and eNOS, which is consistent with an improvement in vascularization. In both experimental treatments, COLD and HYPO, we observed a decrease in ubiquitination in low molecular weight proteins. Conclusions: Exposure to hypobaric hypoxia or cold for 9 days after skeletal muscle (gastrocnemius) injury attenuates the effects on protein damage, improving redox balance and downregulating repair systems.

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Mechanisms of detoxification of high manganese concentrations by the microalga *Chlorella sorokiniana*

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Many neutrophilic and acidophilic microalgal species tolerate high metal concentrations and can survive or colonize metal-polluted waters. They show significant biotechnological potential for the remediation and wastewaters processing. On the other hand, negative effects of metal pollution on microalgae may affect the function of aquatic ecosystems because these photosynthetic microorganisms represent the primary producers of O2 and biomass. However, adaptive mechanisms that microalgae employ to detoxify metal excess are largely unknown. Herein we analyzed the response of the freshwater microalga *Chlorella sorokiniana* to high but non-toxic levels of Mn2+. Manganese is a key metal pollutant, with five possible oxidation forms that can bind to a variety of different ligands. At pH below 7, it is predominantly present in Mn2+ form. Scanning electron microscopy showed that in response to 1 mM Mn2+, *C. sorokiniana* released mucilage polymers within 1 h. Electron paramagnetic resonance spectroscopy (EPR) showed that the early response involved loose Mn2+ binding to mucilage and/or the cell wall. The amount of loosely bound Mn2+ was significantly decreased after 24 h, whereas biomass showed significant accumulation of Mn, O and P, as determined by energy dispersive X-ray spectrometry, indicating the production of polyphosphates, which may sequester Mn. Further, it was found that the exposure to Mn2+ resulted in rapid and transient decrease of total free glutathione concentration; the drop was observed after 1 h, and the concentration returned to initial values after 24 h. EPR measurements showed a similar trend in the level of reduced thiols. The observed changes can be explained either by the synthesis of phytochelatins – sulfur-rich short-chain peptides that sequester metals, or by glutathionylation of proteins. Reduced thiols could not be detected in the extracellular space, indicating that *C. sorokiniana* did not release thiols in response to high Mn. These results demonstrate that the adaptive response of *C. sorokiniana* to high Mn levels involves multiple components and time phases. The early phase involves mucilage release, phytochelatins and/or protection of protein thiols, whereas the successive phase involves Mn coordination by polyphosphates and other mechanisms that remain to be resolved.

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The effect of long-term Cd exposure on the content of reduced GSH and SH groups in Ostrinia nubilalis (Hbn) larvae

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Environmental pollution caused by heavy metals, as well as their accumulation in ecosystems, is a growing global problem that negatively affects many living organisms. Cadmium (Cd) is a heavy metal whose increased concentration in the ecosystem has negative impact on the growth and development of living organisms, including insects. High Cd concentration leads to the synthesis of reactive oxygen species (ROS), which cause oxidative stress. During evolution insects have acquired mechanisms of antioxidant protection to resist these negative effects. Glutathione (GSH) is an important antioxidant molecule that, together with sulfhydryl groups (SH) of proteins, enables the removal of excess ROS and toxins from the body. *Ostrinia nubilalis* (Hbn) is an economically important species, which causes great damage to corn, and it is well known for its incredible adaptability to extreme environmental conditions. Therefore, the aim of this study was to examine how different Cd concentrations affect *O. nubilalis* larvae by monitoring the level of GSH and SH. First instar (L1) larvae were exposed to Cd contaminated diet until the larvae reached the fifth instar (L5). In total, six experimental groups were set up, five treatments (Cd I: 0.73, Cd II: 3.70, Cd III: 6.85, and Cd IV: 41.71 mg kg\(^{-1}\)) and control (C). The results showed that Cd induced a significant decrease in reduced GSH level in larvae fed on contaminated diet compared with control larvae, while the content of protein SH groups remained unchanged. The results are consistent with previous research where it has been confirmed that increased GSH levels are a result of oxidative stress. However, additional research should be conducted on this topic to better understand the mechanisms that allow this species good adaptability to extreme environmental conditions.

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PP74

The involvement of peroxisome biogenesis and crosstalk with lipid bodies in white adipose tissue response to hypothyroidism

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Peroxisomes are highly dynamic and interactive organelles playing important roles in -oxidation of fatty acids and lipid biosynthesis. Hypothyroidism is one of the most common endocrine disorders that perturbs overall lipid homeostasis, in large part by acting on lipid turnover in adipocytes. The involvement of peroxisomes in the white adipose tissue (WAT) in hypothyroidism remains unknown. Here, we examined the protein expression of peroxisome biogenesis markers (Pex11 and Pex19), peroxisome ultrastructure, localization patterns of acyl-coenzyme A oxidase 1 (ACOX1) and catalase (CAT), along with CAT activity and protein levels in rat retroperitoneal WAT depot in euthyroid and hypothyroid states. For that purpose, adult male Wistar rats were treated with antithyroid drug (0.04% methimazole) for 7, 15, and 21 days. Untreated rats served as euthyroid controls. Hypothyroidism instigated biogenesis of peroxisomes in WAT, as protein expression levels of Pex11 and Pex19 increased concomitantly with the relative abundance of peroxisomes during the course of 21-day hypothyroidism. Besides, hypothyroidism induced specific associations of peroxisomes and lipid bodies (PLB), which invade the lipid body interior (pexopodia-like). However, these PLBs were mostly deprived of ACOX1 and CAT, markers of peroxisomal maturity, and oxidative function. Moreover, CAT activity was decreased in contrast to CAT protein level that remained the same as control over the course of hypothyroidism. Our results indicate that inconsistency in metabolic recruitment with the biogenesis of peroxisomes could be an important factor in defining the metabolic response of WAT in hypothyroidism. Our study lays the foundation for future research focusing on deciphering the exact redox and metabolic milieu of proliferating peroxisomes and their crosstalk with lipid bodies in hypothyroid white adipocytes.

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PP75

Nitric Oxide a key regulator for cardiac regeneration in cryo-injury model of myocardial infarction in adult zebrafish

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Objective: The objective of the study is to understand the role of nitric oxide (NO) on cardiac regeneration and its association to structural recovery in cryo-injury model of myocardial infarction using adult zebrafish. Experimental Protocol: Wild type adult zebrafish of both the sexes were used in this study and they were grouped into control, cryo-injury, cryo-injury treated with N -Nitro L-arginine methyl ester hydrochloride (LNAME) of 5 mg/kg body weight and cryo-injury treated with sodium nitroprusside (SNP) of 1mg/kg body weight. Animals were subjected to various structural and molecular analyses at various time point (0, 3, 7, 15, 30, 45 and 60 days post-operative period).Results: The data indicates that the NO inhibition markedly enhances the fibrous accumulation with concomitant reduction of degradation in cryo injured heart when compared to cryo and cryo treated with NO donor. It also produce inhibition of cell population and migration and integration when compared to cryo and cryo treated with SNP. Molecular study also revealed that the inhibition of NO potentially suppress nitric oxide synthase system. Conclusion: Taken together the present data demonstrated that the NO plays vital role on cardiac regeneration via regulating the fibrin and collagen synthesis and degradation in time dependent manner.

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Oxidative damage in reperfusion after stroke: ferroptosis and the role of the mitochondrial sodium/calcium exchanger NCLX

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Ferroptosis is a form of regulated cell death driven by iron-dependent accumulation of lipid hydroperoxides that cause membrane damage. It is now known to play a critical role in important pathological mechanisms such as ischemic cerebral stroke, where brain iron concentration and lipid peroxides levels are increased. Lipid peroxidation can be initiated by reactive oxygen species, which are known to be produced by the mitochondria during hypoxia-reoxygenation. The mitochondrial sodium/calcium exchanger NCLX is involved in this process, since its activation during acute hypoxia drives superoxide production at complex III. The inhibition of Na+ import through NCLX is enough to block this pathway and inhibit reactive oxygen species production during hypoxia. Taking this into account, our hypothesis is that NCLX could be involved in the production of lipid peroxides during hypoxia-reoxygenation, which would lead to membrane integrity loss and subsequent cell death by ferroptosis. In order to test this idea, we developed a method for detecting lipid peroxidation in cell culture models of hypoxia-reoxygenation and ischemia-reperfusion by labelling the cells with Bodipy 581/591 C11, a lipophilic fluorescent ratio-probe used for indexing levels of lipid peroxides. By using this model and other ferroptosis readouts, we are assessing the role of NCLX in triggering ferroptosis in hypoxia-reoxygenation and ischemia-reperfusion.

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Relationship between klotho protein concentration, telomeres attrition and oxidative stress in ESRD patient before and after haemodialysis session

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OBJECTIVE: It is well known that end stage renal disease (ESRD) is followed by a number of systemic complications halmarked by chronic low-grade inflammation, CVD, anemia, hypertension, hyperphosphatemia. The aim of this study was to estimate level of the Klotho (KL) protein, leukocytes’ telomere length (LTL) and redox status of ESRD patients before and after haemodialysis session. PATIENTS AND METHODS: 130 ESRD patients on regular haemodialysis program (3 times/week, 4-5 h/session), among which 38 (29.2%) have diabetes mellitus, were enrolled in the study. Redox status was determined by measuring two prooxidants and products of its activity: total oxidative status (TOS), prooxidant–antioxidant balance (PAB) and two antioxidants: paraoxonase-1 (PON1 and total antioxidative status (TAS). KL protein was assayed using a commercially available sandwich ELISA test, while LTL was determined with modified qPCR test. Routine biochemical and hematological parameters was obtained by conventional methods on automated analyzers. To assess the efficacy of hemodialysis Kt/V ratio was calculated. RESULTS: KL protein in HD patients was significanly lower compared to reference values before, so as after HD session. Wilcoxon’s paired test revealed significant increase in KL protein after hemodialysis (p = 0.033). Patients with KL values < 23.3 pg/mL (25th percentile value) were significantly older (p = 0.008), with decreased number of leukocytes (p = 0.022) and decreased TAS concentration (p = 0.031). Significant positive correlation was found between KL before and after hemodialysis session (r = 0.460, p < 0.001), between LTL before and after hemodialysis (r = 0.302, p < 0.002) session and Kt/V and KL after hemodialysis (r = 0.272, p = 0.009). Logistic regression using Kt/V < 1.3 (cut-off value for inadequate hemodialysis), showed that low KL after hemodialysis session is significant predictor of inadequate Kt/V status (OR: 0.994 (0.988-0.999), p = 0.039). CONCLUSION: Klotho protein diminishing and short LTL superposition mediated through advanced prooxidative conditions represent additional risk factors, which could explain, at least in part, earlier cardiovascular morbidity and mortality in HD patients.

Keywords: klotho protein; telomeres attrition; oxidative stress; ESRD, hemodialysis.

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Mitochondria and inflammation: tissue specific effects during endotoxemia

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Sepsis and endotoxemia are complex inflammatory syndromes that may involve bioenergetic dysfunction and lead to multi-organ failure. To unravel the importance of the bioenergetic dysfunction related to the inflammatory insult (endotoxemia) in different relevant organs (heart, brain and pancreas), Sprague-Dawley rats were i.p. injected with LPS 8 mg/kg or vehicle, and after 6 h were euthanized. An increase in plasma TNF-α levels was observed along with an 18-fold increase in systemic NO levels (p < 0.0001). In pancreas mitochondria a 30% decrease in O₂ consumption, ATP production and complexes activities were observed (p < 0.01). Brain cortex mitochondria showed mitochondrial inner membrane hyperpolarization, increased mitochondrial O₂⁻ levels by 1.5-fold, and a 40% decrease in ATP production (p < 0.05). Cardiac mitochondria exhibited a 36% and a 20% decrease in ATP production and mitochondrial inner membrane potential, with an increase in H₂O₂ production (p < 0.05). Both brain and heart showed mitochondrial protein nitration, correlated to systemic and tissue NO levels. Moreover, a recovery in cardiac mitochondrial function was observed when systemic NO levels were modulated with L-NAME or c-PTIO administration. Our results showed mitochondrial dysfunction (pancreas > heart > brain); along with increased ROS/RNS levels. Bioenergetic failure might lead to organ dysfunction with NO functioning as a key modulator in the pathogenesis this syndrome.

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Redox-dependent metabolic remodeling of white adipose tissue during cold acclimation: The role of Nrf2

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Understanding regulatory mechanisms which lie at the basis of depot-specific white adipose tissue (WAT) remodeling is required in order to open the door for potentially new pharmacological approaches. Although the functional differences between visceral and subcutaneous WAT depots are imposing, there is little evidence concerning the impact of redox regulation on their distinctive metabolic responses to various physiological challenges. Nuclear factor-erythroid factor 2-related factor 2 (Nrf2) has been well established as the master regulator of white adipocyte adaptive antioxidant response, with its recently emerged role in regulating glucose and lipid metabolism. In order to further investigate depot-specific Nrf2-mediated redox-regulated metabolic responses, we exposed Mill Hill rats to low temperature (4 ± 1 °C) for 45 days. Therefore, we examined protein expression of Nrf2, glutathione (GSH) levels and protein expression of the first line antioxidant defense (AD) enzymes, as well as protein expression of enzymes involved in lipid metabolism in two representative visceral and subcutaneous WAT depots, mesenteric and inguinal WAT, of room temperature- and cold-acclimated rats. Our results showed higher protein levels of Nrf2 in mesenteric WAT, unlike inguinal WAT where protein expression of Nrf2 remained at the control level. In accordance with Nrf2, increased protein expression of manganese and copper, zinc-superoxide dismutase and elevated levels of GSH were observed in mesenteric WAT, alongside with steady protein levels of AD components in inguinal WAT. Such depot-specific redox profiles correlate with elevated protein expression of key enzymes involved in lipid biosynthesis, mitochondrial and peroxisomal oxidation and oxidative phosphorylation in mesenteric WAT, but also with consistent protein expression of the same enzymes in inguinal WAT. Our results suggest that depot-specific WAT metabolic plasticity is redox-dependent and neatly regulated on transcriptional level with Nrf2.

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A chronic exposure to urban polluted air hampers cardiac mitochondrial dynamics in mice

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The World Health Organization estimates that 91% of the world’s population breathe low quality air. As a consequence, 7 million premature deaths occur every year due to air pollution exposure, mostly by cardiovascular diseases including stroke and ischemic heart disease. We have previously shown that breathing air pollution fine particulate matter (PM2.5) impairs cardiac mitochondrial function – both in acute and chronic mice models of exposure – leading to deficient contractile function, aggravated ischemia/reperfusion injury, and progression to heart failure. Now, we aim to evaluate whether a long term PM2.5 exposure could interfere with mitochondrial remodeling mechanisms, such as biogenesis or changes in fusion and fission rates, which could lead to the restoration of myocardial mitochondrial function and bioenergetics. Male 8-week-old BALB/c mice were exposed to urban air (UA, 27 ± 8 µg PM2.5/m³) or filtered air (FA, 2 ± 1 µg PM2.5/m³) in whole-body exposure chambers located in Buenos Aires City downtown for 12 weeks. In UA-exposed mice, cardiac tissue oxygen uptake and mitochondrial active state respiration significantly decreased by 32% and 48%, respectively (p < 0.01), and the GSH/GSSG ratio decreased by 27%. Moreover, mitochondrial H2O2 production increased by 39% while ATP production decreased by 17% (p < 0.05). Protein expression of PGC-1α was significantly decreased by 45% in heart homogenates from UA-exposed mice. No changes were found in mTFA levels in cytosol or isolated mitochondria. Likewise, no translocation to mitochondria of the fission protein DRP-1 was observed. Lastly, the expression of the fusion protein OPA-1 was significantly decreased by 62% in mitochondria from UA-exposed mice. Taken together, these results suggest that UA exposure disturbs cardiac mitochondrial repair mechanisms and dynamics, which might contribute to impaired heart mitochondrial function in this group. Ultimately, our data highlights the importance of considering the impact of environmental factors on cardiac mitochondrial physiopathology during the onset and development of cardiovascular diseases.

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PP81

Strawberry wine antioxidant properties in the protection against free radicals

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Fruit and derived products like wine are rich source of natural active compounds which exhibit beneficial health effect on human organism. Those compounds can affect on activity of enzymes of antioxidant protection which are important in the protection against free radicals. The aim of this study was to investigate in vitro activity of strawberry wine by monitoring activities of antioxidant protection enzymes and lipid peroxidation (malondialdehyde level) in isolated rat synaptosomes. Fruit wines were produced from strawberry fruit in the controlled conditions of microvinification. Synaptosomes were isolated from the brain of Wistar albino rats. All analyzed wine samples influenced on the activity of antioxidant protection enzymes and decreased malondialdehyde level. Activity for superoxide dismutase in synaptosomes was in range (4.82-5.31 U/mg) while catalase activity was (0.027-0.032 U/mg). Glutathione peroxidase activity was in range (0.0115-0.0127 U/mg), as well as malondialdehyde level (1.77-2.21 nmol/mg). Obtained results indicate that fruit wines possess promising antioxidant properties which can protect against free radicals generated during oxidative stress.

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Deregulated inflammasome response in Rett syndrome

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Rett syndrome (RTT), a devastating neurodevelopmental disorder, is mainly caused by mutations in the X-linked MECP2 gene. To date, RTT is considered a spectrum disease, since patients show multisystem disturbances associated with mitochondrial dysfunctions and OxInflammatory status. Several studies demonstrated that dysregulations of inflammatory signalling and immune system are key players in RTT pathophysiological mechanisms. Inflammasomes are known to be multi-protein complexes crucially involved in innate immune responses against pathogens and ROS-related cellular stress. The assembly of NLRP3/ASC inflammasome lead to pro-caspase-1 activation and the following maturation of IL-1β and IL-18. Our previous work showed a deregulation of the inflammasome system in primary dermal fibroblasts from RTT patients. To further investigate how this pathway may be altered in RTT immunocompetent cells, we used RTT and control peripheral blood mononuclear cells (PBMC) that were subjected to lipopolysaccharide (LPS)+ATP pro-inflammatory stimulation. RTT cells showed increased basal levels of nuclear NF-B, together with enhanced levels of NLRP3 and ASC protein expression, as well as higher co-localization of the two proteins. LPS+ATP treatment induced a higher release of IL-1β in RTT PBMC, as compared to stimulated control cells, although the levels of cytosolic inflammasome components decreased. Taken together, our findings confirm our previous data from RTT fibroblasts, which seem to be a reliable model to study the disease. On the other hand, the challenged inflammasome machinery observed in PBMC from RTT patients further support the idea that a deregulation of this system, both in immunocompetent and non-immunocompetent cells, may perpetuate the subclinical inflammatory state observed in the pathology.

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Thioredoxin reductase inhibition by new trans-platinum(II) complexes elicit cytotoxicity in cisplatin resistant ovarian cancer cells

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The DNA-alkylating drug cisplatin is widely used for the treatment of solid tumors. However, tumor chemoresistance often occurs and limits the effectiveness of the therapy. In this process, the overexpression of enzymes belonging to the thiol redox systems such as Thioredoxin reductase (TrxR) is involved. TrxR is a selenoenzyme and a key antioxidant protein involved in the redox homeostasis of the cell. Here, we characterize the activity and the mechanism of action of two new trans-triphenylphosphino dialkylamino platinum(II) complexes. In particular, we investigated the effect of the complexes on the viability and redox homeostasis of ovarian cancer cells both sensitive (A2780) and resistant (A2780cis) to cisplatin. First, the complexes were found able to accumulate in the cell thus exerting their cytotoxic effect by overcoming the mechanisms of cisplatin resistance. Despite a decreased DNA platination activity when compared to cisplatin, the new complexes showed a potent inhibitory activity on TrxR in the sub-micromolar range of concentrations. The inhibition of TrxR likely involves the interaction with the distinctive selenocysteine present in the active site of the redox enzyme. By studying the activity of the complexes on the ovarian cancer cells, we found that they decrease TrxR specific activity triggering ROS production and inducing an imbalance of the redox homeostasis with impact on free thiol levels and glutathione ratio (GSH/GSSG). In conclusion, the new trans-platinum(II) complexes, having a strong inhibitory activity on TrxR, exert a cytotoxic effect and overcome cisplatin resistance in ovarian carcinoma cells.

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Cocoa/methylxanthines supplementation impact on brain glutathione level in aged mice

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Aging is an unrestrainable process which is known to initiate oxidative stress and cause oxidative tissue damage, as has been reported in numerous studies concerning neurodegenerative diseases in elderly. Our objective was to investigate prevention of glutathione depletion in aged healthy C57BL/6 male mice long-term supplemented with cocoa powder or methylxanthines at quantity equivalent to human daily cocoa powder dose of 7.3 g (two tablespoons). The activities of brain antioxidant enzymes, glutathione peroxidase (GSH-Px) and glutathione reductase (GR), as well as the glutathione content were measured. It was found that cocoa powder and cocoa relevant combination of theobromine and caffeine induce increasing of glutathione level. GSH-Px activity was affected only in methylxanthines supplemented group in terms of increase compared to control, while GR activity remained unmodified after both dietary interventions. Because of the unchanged brain antioxidant enzymes activities in cocoa treated group of mice, different mechanism may contribute to glutathione buffer system and thus play a role in maintaining redox balance.

Keywords: brain; glutathione; cocoa; antioxidant enzymes; aging.

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Systemic response to acute aerobic exercise in the circulatory system: a possible cross-talk between plasma extracellular vesicles and blood monocytes

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Extracellular vesicles (EVs) mediate cell-cell communication in different physiological conditions, as well as during physical exercise (PE). Since peripheral blood mononuclear cells (PBMCs) can release and internalize plasma-EVs, the aim of this work was to investigate the relationship between the modulation exerted by fitness levels and acute aerobic exercise (EX) - 30’ on treadmill, 70% HRmax – on EV-cargo and PBMC protein expression. Analysis focused on molecules involved in the stress response, such as antioxidant enzymes and heat shock proteins, as well as markers of oxidative stress (lipid peroxidation (LP) and protein carbonylation (PCO)). To this aim, two groups of healthy young males, categorized by different VO$_{2}$max (Untrained, UTS, $n=7$, 41.8 yrs ± 3.8 and Trained, TS, $n=7$: 48.5 yrs ± 3.2), were included in the study. Plasma-EVs and PBMCs were isolated from blood samples collected at baseline, 3 hrs and 24 hrs after EX. Our data demonstrated that UTS-EVs had higher PCO and LP levels than TS-EVs while in PBMC this difference is only seen for PCO ($p<0.05$), with no modulation by EX. Plasma-EVs shuttle antioxidant enzymes (SOD1, SOD2, Catalase and TrxR1) and HSP70, present in PBMCs. EVs and PBMCs basal levels of SOD1 and TrxR1 were not different between groups, with an EX-related increase of SOD1 exclusively in PBMCs of UTS 24 hrs post-EX ($p<0.05$). SOD2 basal levels were significantly higher in PBMCs and EVs from UTS compared to TS ($p<0.05$), while Catalase was significantly higher in UTS-EVs ($p<0.05$), but not in UTS-PBMCs. No modulation of SOD2 or Catalase was exerted by EX. HSP70 appears modulated by fitness levels and EX only in PBMCs, with a higher basal expression in TS than in UTS and a significant increase in UTS at 24 hrs post-EX. These data suggest a link between PBMCs and plasma-EVs, with a possible communication following the exercise-induced modifications concerning redox homeostasis.

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Lon protease, protein oxidation and mitochondrial DNA damage: an influence beyond the mitochondrial area

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As the main energy producer of the eukaryotic cell, the mitochondria is also a major source of reactive oxygen species and a prime target for oxidative damage to proteins. These characteristics place it at the heart of the accumulation of damaged proteins and the critical role of protein maintenance systems. Particularly involved in the elimination of oxidized proteins within the mitochondrial matrix and the maintenance of mitochondrial DNA, Lon protease is a key player in the quality control of mitochondrial proteins and thus in the mitochondrial functions. Therefore, Lon constitutes an important line of defense of the mitochondrial matrix against protein oxidative damage. The results obtained with HeLa cells under-expressing Lon confirmed this activity by showing that Lon deficiency was enough to induce an oxidative stress. Indeed, in addition to increased protein carbonylation, we have also observed an increase in lipid peroxidation induced protein modification in Lon depleted cells. The influence of Lon on the cellular protein quality control status even extends beyond the mitochondrial area since Lon knockdown is accompanied by a decrease in two proteasome peptidase activities. Given the context of oxidative stress associated with Lon deficiency, we wanted to open up perspectives by looking for proteins with modified levels of oxidation when Lon is under-expressed. A 2D Oxi-DIGE experiment followed by a mass spectrometry analysis were used to identify proteins with a variation in carbonylation level when comparing the mitochondrial-enriched fractions of HeLa cells expressing Lon with those deprived of this protease. Mitochondrial or not, these proteins were found to belong to energy metabolism and the protein quality control. Finally, in agreement with the role of Lon in the maintenance of mitochondrial DNA, we obtained preliminary results suggesting damage to the mitochondrial genome related to a Lon deficiency.

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UV induced changes in profile and structure of protein in rats plasma are retained by topically applied cannabidiol

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UV radiation as a main environmental factor that reaches human skin, is known to induce a multiple changes in the metabolism of skin-building cells. Moreover, its harmful effects can be transmitted into deeper layer of human body. Therefore, the aim of this study was to analyse the proteomic changes occurred in plasma of chronic UVA/UVB irradiated rats and define the effect on these changes of skin topically applied protective compound – cannabidiol (CBD). Obtained data allowed to describe changes in the expression of significant proteins including: keratin and 14-3-3 protein and numerous of signalling and anti-inflammatory proteins (NFκB inhibitor, phosphoglycerate mutase 2, protein kinase C, protein S100) in plasma of UV-irradiated rats. CBD partially prevented these changes especially in the case of UVB irradiated animals. Moreover, the effects of UVA/UVB on rats skin led in plasma to the oxidative stress manifested by increased level of lipid peroxidation products–protein adducts formation. However, CBD treatment prevented these changes, what was the most effective in the case of decreasing 4-HNE–protein adducts level following UVB irradiation. Additionally, CBD skin topical treatment leading to the penetration of CBD into the blood also caused direct modifications to the plasma protein structure by creating CBD adducts with molecules, such as proline-rich proteins, transcription factor 19, and N-acetylglucosamine-6-sulfatase, what significantly changed the activity of these proteins. In conclusion, it can be suggested that CBD may be an effective compound against systemic oxidative stress induced by UV radiation, but its potency requires careful analysis as to ensure it poses no risk to the metabolism of other cells/tissues/organs of the body.

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Antioxidant properties of a new pyridoxine derivative with NO-donating ability

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Cancer is one of the leading deaths causes in the world. Due to the use of anticancer drugs high doses, various side effects are often observed, including cardiotoxicity. The search for new drugs for adjuvant chemotherapy of tumors with low toxicity, antioxidant and NO-donor activity, and cardioprotective action is urgent. The aim of work was to study the cytotoxic, antioxidant and NO-donor properties of the hybrid compound B6NO (bis-(4,5-hydroxymethyl-2-methyl-3-hydroxy) pyridinium salt of 2-nitroxy-butane-1,4-diacid) for normal (Vero and FetMSC) and tumor cells (HeLa and HepG2). It was shown that B6NO exhibits antioxidant properties in the concentration range from 5 to 80 μM, while reducing the ROS content to the control level during the oxidative stress induction by tert-butyl hydroperoxide. At the same time, the B6NO antioxidant activity on tumor cells was significantly lower. It was determined that B6NO chelates iron ions by 94%, while vitamin B6 in equimolar concentration bound ferrous ions by no more than 10% which indicates B6NO ability to block the Fenton reaction. In addition, it was revealed that the hybrid compound B6NO inhibits process of initiated lipid peroxidation more effectively than pyridoxine. When using the DAF-FM DA dye, it was determined that B6NO significantly increases the intracellular nitrogen monoxide accumulation in the normal cells, which is provided both by optimizing the nitrogen monoxide synthesis pathways via eNOS and by biotransformation of nitrate groups. The B6NO compound showed low toxicity, both in the cells and laboratory animal’s models. The B6NO increased or did not decrease doxorubicin and cisplatin cytotoxicity in experiments on tumor cells in vitro. Thus, the results indicate a high potential of the B6NO as a compound for tumors adjuvant chemotherapy.

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Keywords: antioxidant activity; vitamin B6.

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Improper enzymatic defensive responses in Rett syndrome disorder

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Rett Syndrome (RTT) is a neurodevelopmental disorder linked in most case to a mutation in the X-linked gene encoding for MeCP2, an epigenetic regulator. The molecular mechanism by which the deficiency of MeCP2 leads to the pathology is not yet completely clear. Increasing evidences suggest a critical role of redox imbalance in RTT pathogenesis. Therefore, in this study, we decided to evaluate the levels and activities of key enzymes involved in cellular defensive response against increased reactive oxygen species (ROS) production in primary fibroblasts obtained from controls and RTT patients. First, we evaluated transcripts levels of catalytic and modulatory subunits of $\gamma$-glutamylcysteine synthetase (GCLC & GCLM), before and after treatment with 4-hydroxynonenal (4HNE 1, 5, and 10 $\mu$M at 2 and 24 h). RTT responded to the oxidant insult with a significant and dose-dependent decrease in GCLC mRNA, while for GCLM we observed a significant downregulation only with 10 $\mu$M 4HNE. Moreover, both gene expression and enzymatic activity of GSH peroxidase (GPX) and GSH reductase (GR) showed altered levels in RTT after 4HNE stimulus. The increased susceptibility to 4HNE prompted us to evaluate also RTT response to ferroptosis, an iron-dependent cell death associated with lipid peroxidation and inability to eliminate lipid peroxides. In basal conditions, protein level of GPX4, the negative regulator of ferroptosis, was higher in RTT. Nevertheless, after treatment with several doses of erastin (specific GPX4 inhibitor) or RSL3 (inhibitor of the cystine/glutamate antiporter), cell death rate increased in RTT compared to controls. In addition, 10 $\mu$M erastin or 5 nM RSL3 for 3 h induced changes in GPX and GR enzymatic activity, more evident in RTT cells. In conclusion, our results highlighted in RTT altered expression and activity of enzymes involved in cellular antioxidant response. These defects could suggest an increased vulnerability to ferroptosis, which however requires further investigation.

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Blood levels of RCAN1 and MDA as possible biomarkers in Alzheimer’s Disease

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Alzheimer’s disease (AD) is the most common form of dementia and is estimated to affect more than 46 million people around the world. The most studied biomarkers for AD diagnosis are β-amyloid deposits and glucose consumption in the brain determined by imaging techniques, as well as β-amyloid, tau, and phosphorylated tau determination in cerebrospinal fluid. However, these biomarkers present severe problems due to their invasiveness, high cost and low availability. Consequently, the study of biomarkers in peripheral blood is still of great interest. In previous studies, we proposed an initial set of biomarkers composed of 3 proteins that can be measured in serum samples: regulator of calcineurin 1 (RCAN1), clusterin, receptor for advance glycation end products (RAGE). This work aimed to analyse blood levels of these proteins in a new retrospective cohort study of 143 individuals over 65 years old. Moreover, we included blood levels of malondialdehyde (MDA), a biomarker for oxidative stress to this study. For that purpose, we measured RCAN1, clusterin, RAGE and MDA levels in blood samples from cognitively healthy individuals, subjects with mild cognitive impairment (MCI) and Alzheimer’s disease (AD) patients. Preliminary results from this transversal study show that age is significantly higher in MCI and even more in AD patients compared to the control group. This result supports the fact that the risk of AD is correlated to age. No statistically differences have been found in clusterin or RAGE blood levels between the three clinical groups. However, blood levels of MDA and RCAN1 were significantly lower in AD patients compared to MCI and control subjects. These results differ from other studies performed in brain samples of AD patients where these markers are increased. These findings may suggest a poor clearing of MDA and RCAN1 from brain to peripheral blood in AD patients.

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PP91

Inorganic nitrate prevents the loss of intestinal claudin-5 induced by broad-spectrum antibiotics but has no impact on gut microbiome diversity: is nitrate fuelling bacteria metabolism?

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Dietary nitrate is a redox signalling molecule with critical physiological functions both in the gut and systemically. In the distal bowel, nitrate may interact with the local microbiota, modulating not only the structure and function of local bacterial communities but also the epithelial barrier function. Although some data has been emerging on the effect of nitrate on oral microbiota, its impact on intestinal bacteria remains elusive. This study investigates the impact of nitrate on intestinal microbiota and the expression of local tight junction proteins. Rats were divided in 4 groups and exposed to the following regimens for 7 days: 1) antibiotics, 2) antibiotics + nitrate, 3) nitrate and 4) tap water. Occludin and claudin-5 were analysed by immunoblotting in the colon. Nitrate and nitrite were measured in intestinal tissue by HPLC and fecal bacterial DNA was studied by DGGE before and after treatment. Nitrate increases claudin-5 expression in rats exposed to a therapeutic dose of broad spectrum antibiotics in comparison to animals exposed to antibiotics alone (p = 0.016) but decreases the expression of occludin (p = 0.003), suggesting that different proteins may be modified by different mechanisms by nitrate. As expected dietary nitrate increases intestinal nitrate concentration (p = 0.038). Curiously, in the presence of antibiotics, dietary nitrate increases tissue nitrate concentration by c.a. sixfold in comparison to both controls and rats exposed to antibiotics without supplementation (p < 0.0001). Antibiotics eradicated most of gut flora (p = 0.0016), reducing microbiota richness by 56% while nitrate showed a tendency to attenuate such microbial loss (48%, p = 0.068). In conclusion, although nitrate consumption may be recommended during antibiotic therapy, functional studies are mandatory to ascertain the impact of this anion on intestinal barrier function and bacterial metabolic pathways, which may recycle this anion and likely trigger different redox signalling pathways along the gut.

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Graphene quantum dots protect SH-SY5Y cells from SNP-induced neurotoxicity by ROS/RNS scavenging

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We here investigated the ability of graphene quantum dots (GQD), graphene nanoparticles with antioxidative capacity, to protect SH-SY5Y human neuroblastoma cells from oxidative/nitrosative stress induced by iron-nitrosyl complex sodium nitroprusside (SNP). Although GQD diminished the levels of nitric oxide (NO) in both cell free condition and SNP-exposed cells, NO scavengers (PTIO and uric acid), displayed only slight protection from SNP, suggesting that NO scavenging was not the main protective mechanism of GQD. Moreover, GQD significantly protected SH-SY5Y cells from neurotoxicity of light exhausted SNP, incapable of producing NO, implying the existence of protective mechanism independent of NO-scavenging. GQD lowered the increase in the concentration of hydroxyl radical (•OH) and superoxide anion (O2•−) caused by SNP both in the cell-free condition and inside cells, as well as ensuing oxidative stress and lipid peroxidation. Nonspecific antioxidants (glutathione, NAC), •OH scavenger (DMSO), and iron chelators (DTPA, BPDSA), but not superoxide dismutase, mimicked the cytoprotective activity of GQD, suggesting that GQD protect cells by neutralizing •OH generated in the presence of iron released from SNP. GQD were readily internalized by SH-SY5Y cells, while extensive washing of cells pre-incubated with GQD only partly reduced their protective activity, suggesting that GQD exerted neuroprotective effect both intra- and extracellularly. By demonstrating that GQD protect neuroblastoma cells from SNP-induced neurotoxicity by both extracellular •OH/NO scavenging and some unknown intracellular mechanism, our results suggest that GQD could be valuable candidate for treatment of neurodegenerative and neuroinflammatory disorders associated with oxidative/nitrosative stress.

Keywords: GQD; neurotoxicity; oxidative/nitrosative stress; •OH/NO scavenging.

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PP93

Oxidative modifications of lysozyme induced by AAPH-derived peroxyl radicals lead to loss of enzymatic activity and inter-protein crosslinking involving specific Trp residues

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Lysozyme (Lyso) is an anti-bacterial enzyme present in multiple biological fluids, and released from the cytoplasmic granules of macrophages and neutrophils at sites of infection and inflammation. As consequence, Lyso is frequently exposed to peroxyl radicals and other oxidants generated in biological environments. Lyso is a small protein (14.3 kDa) that contains a high number of Trp residues (six). We therefore hypothesized that exposure of lysozyme to peroxyl radicals would generate specific modifications and protein crosslinks via radical-radical reactions involving Trp residues. Furthermore, such Trp oxidation might modulate Lyso activity. Lyso was incubated with AAPH (2,2-azobis(2-methylpropionamidine) dihydrochloride) as a peroxyl radicals source. Enzymatic activity was assessed, while oxidative modifications were detected and quantified using electrophoresis and liquid chromatography (UPLC) with fluorescence or mass detection (MS). Computational models of AAPH-Lyso interactions were developed. Exposure of Lyso to AAPH (10 and 100 mM for 3 h, and 20 mM for 1 h), at 37 °C, decreased enzymatic activity. 20 mM AAPH showed the highest efficiency of Lyso inactivation (1.78 moles of Lyso inactivated per peroxyl radicals). Conversion of Met to its sulfoxide, and to a lesser extent, Tyr oxidation to 3,4-dihydroxyphenylalanine and diTyr, were detected by UPLC-MS. Extensive transformation of Trp, involving short chain reactions, to give kynurenine, oxindole, hydroxytryptophan, hydroperoxides or di-alcohols, and N-formyl-kynurenine was detected. Trp62, Trp63 and Trp108 were the most affected residues. Interactions of AAPH inside the negatively-charged catalytic pocket of Lyso, with Trp108, Asp52, and Glu35, suggest that Trp108 oxidation mediates, at least partly, Lyso inactivation. Crosslinks between Tyr20-Tyr23 (intra-molecular), and Trp62-Tyr23 (inter-molecular), were detected with both proximity (Tyr20-Tyr23), and chain flexibility (Trp62) appearing to favor the formation of covalent crosslinks. Our results show that oxidation of specific Trp residues would be critical for Lyso inactivation, and would (partially) explain protein crosslinking.

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A new approach to modulate cellular damage during chronic inflammation in atherosclerosis

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The excessive production of hypochlorous acid (HOCl) by myeloperoxidase (MPO) during chronic inflammation is a key driver of lesion formation in atherosclerosis. HOCl is a potent chemical oxidant that reacts readily with almost all biological molecules, causing extensive cellular damage. This has sparked interest in the development of therapeutic approaches that decrease HOCl formation as a means prevent the development of atherosclerosis and other chronic inflammatory pathologies. Thiocyanate (SCN\(^-\)) supplementation decreases lesion development in murine atherosclerosis. This is attributed to the preferential formation of hypothiocyanous acid (HOSCN) rather than HOCl by MPO, but the cellular pathways are poorly understood. In this study, we examined the ability of SCN\(^-\) and its selenium analogue, selenocyanate (SeCN\(^-\)) to modulate HOCl-induced damage to macrophages and vascular smooth muscle cells, which play a critical role in lesion development and stability in atherosclerosis. Use of SeCN\(^-\) may offer an advantage owing to the ability of selenium to upregulate antioxidant defenses. Addition of SCN\(^-\) prevented HOCl-induced cell death, altered the pattern and extent of intracellular thiol oxidation, and decreased perturbations to calcium homeostasis and pro-inflammatory signaling. Protection to a similar or greater extent was observed with SeCN\(^-\). In each case, amelioration of damage was detected with sub-stoichiometric ratios of the added compound to HOCl. The effects of SCN\(^-\) are consistent with conversion of HOCl to HOSCN. Whilst SeCN\(^-\) prevented HOCl-induced damage to a similar extent to SCN\(^-\), preliminary experiments revealed that the resulting product hyposelenocyanous acid (HOSeCN) induced cell death, but the extent of toxicity was dependent on the specific cell type. These results provide new insight into the protective mechanisms involved in the reduction of lesion formation seen with SCN supplementation. However, further characterization of the biological reactivity of HOSeCN is required to better assess the suitability of SeCN\(^-\) to modulate HOCl-induced damage in vivo.

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Free radical scavenging capacity of some Moldovan red wines

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Presence in wine of various compounds like saccharides, acids, tannins, phenolic compounds, vitamins, etc., argues the positive effects of this beverage on human health. In this regard, wine antioxidants are widely studied and their bio-protective effects are already recognized. In this study, eight red wines from the vineyards of the Scientific-Practical Institute of Horticulture and Food Technologies (Chisinau, Republic of Moldova), which are located in the central part of the country, were tested for the antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH•). The DPPH• scavenging reaction is widely diffused as an easy to use method of antioxidant capacity evaluation of plant extracts, food materials or of single compounds. The H-atom transfer reaction from a free radical scavenger to DPPH• causes a decrease in absorbance which can be followed by a common spectrophotometer set in the visible region. The antioxidant test was done on young red wine varieties from 2020 vintage: Cabernet Sauvignon, Codrinschii, Come Bojole, Crimposie, Feteasca Neagra, Mugurel, Negru de Ialoveni, Saperavi. The analysis was performed at room temperature of 20 ºC and the results were expressed as grams of ascorbic acid equivalents per liter (g AAE/L). The antioxidant capacity of the analyzed red wines ranged from 0.84 ± 0.01 to 1.86 ± 0.02 g AAE/L (average antioxidant activity was 1.331 g AAE/L). Come Bojole wine showed the strongest antioxidant capacity against the DPPH free radical, and the lowest value was registered for the wine Cabernet Sauvignon. Accordingly, the IC50 values for the studied wines were determined. Kinetic curves were plotted for each sample and different kinetic behaviour was observed.

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Quercetin facilitates cell chemosensitivity in cisplatin resistant cancer cells through inhibition of Trx/TrxR/Prx system and PI3K/Akt/mTOR signaling

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Cellular redox state is largely determined by Trx/TrxR/Prx system which controls the thiol-disulfide balance and consumption of H2O2 and plays the important role in the regulation of redox signaling. Trx/TrxR-regulatory redox pathways may be the significant part of key events in the stress response and/or resetting redox homeostasis resulted in the change of most vital functions of cells, including proliferation, differentiation, apoptosis. In this study, we evaluated the effect of quercetin, a natural flavonoid, on gene expression of Trx/TrxR system and PI3K/Akt/mTOR signaling pathway in human ovarian carcinoma SKOV-3 cells resistant to cisplatin. Under formation of cisplatin resistance, the significant elevation of gene expression of Trx/TrxR/Prx system (TRX1, TRX1, TRXDR1, TRXDR2 – up to 4-8 folds; PRDX1, PRDX2, PRDX3, PRDX6 – up to 2-7 folds) as well as overexpression of PI3K, AKT, MTOR (up to 10-15 folds) and NRF2 (up to 8 fold) genes was observed. The treatment of resistant cells with quercetin (100 μM) depressed these genes to the values of the expression in wild cells and caused the significant increase of sensitivity to cisplatin. G2/M arrest of cell cycle and inhibition of proliferation by quercetin was more effective in resistance SKOV3/CDDP cells in comparison with wild cells. The results can confirm the suggestion about the ability of quercetin to destroy the redox-dependent basis of cancer cell resistance and to return sensitivity to chemotherapeutic agents.

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Glutathione and redox-dependent regulation under the cancer cell drug resistance to cisplatin

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Tripeptide GSH is associated not only with the control and maintenance of redox cell homeostasis, but also with the processes of proliferation and cell differentiation. It has been resumed the importance of GSH and the GSH/GSSG ratio in the regulation of tumor cell viability, in the initiation of tumor development, progression, and drug resistance. The aim of our study was to determine the role of GSH/GSSG ratio in regulation of expression of redox-dependent genes (isoforms of glutaredoxin, thioredoxin, peroxiredoxin) which are important for antioxidant defense under development of cancer cells resistance to cisplatin (CDDP) possessed pro-oxidant action. Under development of resistance of human ovarian carcinoma SKOV-3 cells to CDDP co-ordinative enhanced expression of genes encoding glutathione synthetase (GS), heavy and light subunits of γ-glutamylcysteine synthetase (γ-GCSH, γ-GCSL, γ-GCS respectively) was found in the resistant cells in compare with the wild cells. In addition, the growth of GSH/GSSG ratio as index of cellular redox state, enhanced expression of redox-regulated genes of isoforms of glutaredoxin (GLRX1, GLRX2), thioredoxin (TRX2), peroxiredoxin (PRDX6) as well as elevated level of transcription factor Nrf2 were observed in both types of resistant cells. The mechanism of development of cancer cells resistance to CDDP has been suggested to include the activation of GSH synthesis de novo owing to the increase of expression of γ-GCSH, γ-GCSL and GS genes, redox-dependent elevation of expression of GLRX1, GLRX2, TRX2, PRDX6 which are co-ordinate regulated by redox-dependent transcription factor Nrf2 and elevated level of GSH/GSSG ratio.

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A role for NADPH oxidase in mediating lipotoxicity and inflammation in β-cells

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The role of H2O2 on β-cell function and viability during lipotoxicity and inflammation is still a matter of debate. While increased H2O2 levels were shown to be involved in β-cells dysfunction, others have proposed that H2O2 acts as a signaling molecule, essential for insulin secretion. However, little is known about the dynamic changes in H2O2 levels and the subcellular source of H2O2. We therefore used pancreatic islets from mice expressing roGFP2-Orp1 in the mitochondrial matrix or in the cytosol, exposed to palmitate and cytokines to investigate real-time dynamic changes in H2O2 levels with subcellular resolution. We observed a transient increase in cytosolic H2O2 levels upon palmitate and cytokines treatment. Increase of cytosolic H2O2 coincided with a parallel decrease in NAD(P)H levels in both conditions. Mitochondrial H2O2 levels were slightly increased with palmitate and unchanged upon cytokines treatment. Next, to investigate the source of cytosolic H2O2, we employed NOX2 KO islets. We observed no changes in H2O2 levels in NOX2 KO islets upon palmitate and cytokines treatment. Interestingly, under same conditions, NOX2 KO islets were protected against apoptosis and insulin secretion was preserved. In conclusion, we suggest that NOX2-derived H2O2 is involved in β-cells dysfunction and death upon lipotoxicity and inflammation.

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Optimization of oxidative stress-induced premature senescence model using VH10 fibroblasts for assessment of natural compounds

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Senescence is a complex process resulting in irreversible cell cycle arrest, however, with maintenance of active cellular metabolism. Stress-induced premature senescence (SIPS), a type of premature senescence induced by various stressors including oncogenic or oxidative stress, may serve as a useful tool for assessment of natural substances with promising modulatory effects on senescence. The human foreskin fibroblasts VH10 were reported to be applied for establishment of SIPS model using chronic low dose rate exposure of gamma rays, in which the oxidative stress has been proposed to play a critical mechanistic role [Loseva et al., Proteomes. 2 (2014) 341-362]. Therefore, we used these cells for development of SIPS model using oxidative stress conditions elicited by \( \text{H}_2\text{O}_2 \). In our model, VH10 fibroblasts were exposed to different concentrations of \( \text{H}_2\text{O}_2 \) (10, 25, 50 and 100 umol/l) for 30 or 60 min. Reduced viability of treated cells has been detected already at the low concentrations of \( \text{H}_2\text{O}_2 \) (10 umol/l) at both time points. However, the cells with enlarged and flattened morphology were observed mainly following exposure to 100 umol/l \( \text{H}_2\text{O}_2 \) for 60 min. The same cells were also positively labelled for standard senescence marker, SA-b-galactosidase, reaching the maximum labelling 72 h post-exposure (59 and 73% for 30 and 60 min, respectively). Furthermore, we focused on evaluation of cytotoxicity profile of astaxanthin (ASX), a carotenoid with promising anti-aging properties. In VH10 fibroblasts, 24 h treatment with ASX at concentrations up to 33.5 umol/l did not show any cytotoxic effect. These findings have shown that \( \text{H}_2\text{O}_2 \) can serve as an effective inductor of premature senescence in VH10 fibroblasts. Further experiments are needed to elucidate potential protective and antisenesence effects of natural molecules, such as ASX, in our model.

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Coenzyme Q10 as anti-ageing agent in human dermal fibroblast. Comparison of bioavailability and efficacy of the reduced and oxidised form

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Coenzyme Q10 (CoQ10) is an endogenous lipophilic quinone found in equilibrium between its oxidised (ubiquinone) and reduced (ubiquinol) form, ubiquitous in biological membranes and endowed with antioxidant and bioenergetic properties, both crucial to the ageing process. CoQ10 biosynthesis decreases with age in different tissues including skin and its biosynthesis can be modulated by HMG-CoA reductase inhibitors (statins). Statin-induced CoQ10 deprivation has previously been shown to be associated with the development of a senescence phenotype in human dermal fibroblasts (HDF). Here pro-ageing effect of statin-induced CoQ10 deprivation in HDF were confirmed by senescence-associated secretory phenotype (SASP) markers (p21, IL−8, CXCL1, and MMP-1) upregulation and concomitant decreased expression of extracellular matrix components (elastin, collagen type 1). Moreover, by compared the bioavailability of exogenously added CoQ10, in form of ubiquinone or ubiquinol, to CoQ10-deprived HDF, we observed a striking higher bioavailability of the reduced form associated with an enhanced efficacy in rescuing the senescent phenotype quantified in terms of -galactosidase positivity, p21, collagen type 1, and elastin at the gene and protein expression levels. In particular 15 µg/mL ubiquinol significantly rescued cellular and mitochondria CoQ content highlighting an effective subcellular delivery. In conclusion, our results highlight the pivotal role of CoQ10 for vital skin and strongly support the use of both forms as a beneficial and effective anti-ageing skin care treatment.

Keywords: Coenzyme Q10; ageing; skin; mitochondria; oxidative stress; senescence; SASP.

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Skin carcinogenesis mouse models

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Skin cancer is the most common form of cancer, with rising incidence over the years. It is estimated that 2-3 million cases of non-melanoma skin carcinomas, namely basal cell and squamous cell carcinomas, are diagnosed annually. Ultraviolet radiation (UVR) is the major etiologic factor in the development of skin malignancy causing DNA damage, gene mutations, immunosuppression, and oxidative stress in the skin. Skin carcinogenesis has been studied on various mouse models. Nevertheless, there are no studies comparing different hairless and nude mice for their carcinogenic ability. This study compared the development of squamous cell carcinoma, on 4 different hairless mouse models; SKH-hr1, SKH-hr2, SKH-hr2+apoE hypercholesterolaemic mice and Nude partially immunodeficient mice. 8-10 male mice of each group were exposed to UV radiation 3 times per week for 36 weeks. The condition of the skin and skin cancer development was evaluated by clinical evaluation – photodocumentation, histopathological assessment, measuring various biophysical parameters (stratum corneum hydration, transepidermal water loss, sebum, melanin, erythema, thickness, blood circulation, skin roughness), and blood antioxidant status. Taking into consideration all the parameters above, the most suitable mouse model for future studies of skin carcinogenesis is considered to be the SKH-hr2+apoE model. A significantly adequate carcinogenesis was also obtained by SKH-hr2 and Nude mice.

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PP102

Moringa Oleifera leaf extract influences the enzymatic antioxidant system capacity in C2C12 skeletal muscle cells

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Moringa oleifera is a multi-purpose herbal plant with numerous health benefits including nutritional and medicinal advantages and antioxidant capacity. Its benefits are essentially due to the presence of large amounts of essential amino acids, carotenoids and a very wide range of vital antioxidants, antibiotics and nutrients including vitamins and minerals. Recently, Moringa oleifera leaf extract (MOLE) exposure was demonstrated increasing oxidative metabolism of C2C12 skeletal muscle cells. A rise in oxidative metabolism could induce a larger reactive oxygen species (ROS) release as a byproduct thus leading to a redox imbalance. The aim of this study was to evaluate the influence of MOLE on redox status in skeletal muscle cells in order to match with the increased oxidative metabolism. To this purpose, differentiated C2C12 skeletal muscle cells were treated with MOLE and analyzed for total antioxidant capacity (TAC) and glutathione levels as marker of redox status; enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) engaged in antioxidant defense; and lipid peroxidation (TBARS) and protein carbonyls (PrCar) as markers of oxidative damage. MOLE improved glutathione redox homeostasis, CAT, SOD, and GPx enzymatic activities while no changes were found in TAC. TBARS and PrCar levels resulted unchanged probably due to the enhancement in the antioxidant enzymatic network. Taken together, these data indicate that MOLE exposure, besides improving oxidative metabolism, may be beneficial to skeletal muscle cells by enhancing the enzymatic antioxidant system capacity.

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Fractal analysis of chromatin condensation in the human sperm nuclei

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The disequilibrium of reactive oxygen/nitrogen species (ROS/RNS) is one of the causes of male infertility. To examine the role of ROS/RNS in reproduction, we modulated the cell concentration of $\text{O}_2^{-}\cdot$ and NO using the superoxide dismutase (SOD) mimic, M40403, which selectively removes $\text{O}_2^{-}\cdot$ and consequently increases the NO bioavailability. The fine chromatin changes are difficult to observe via routine light/electron microscopy, therefore we used fractal analysis to analyze DNA compaction. We used normospermic semen samples from ten human subjects. After purification in Cook density gradient, the sperm-rich fraction was rinsed in modified Tyrod medium (TM). Purified samples were divided into three groups. One group was evaluated immediately after resuspension in TM and served as the control. For the other two groups, untreated and SOD mimic-treated group (50 µM), the sperm was resuspended in TM and evaluated after incubation at 37 °C / 6% CO₂ for 3 hours. Air-dried and methanol fixed sperm smears were stained with toluidine blue and analyzed on Leica DMLB microscope. Images from ten randomly selected fields were analyzed in ImageJ plugin FracLac to get fractal dimension defined as a measure of complexity. Average values of fractal dimensions (mean ± SEM) are: control group – 1.016 ± 0.553; untreated group – 0.955 ± 0.291; treated group – 1.146 ± 0.096. Also, average values of lacunarity are: control group – 0.734 ± 0.129; untreated group – 0.727 ± 0.129; treated group – 0.901 ± 0.297. Results show that SOD mimic remodels chromatin condensation by increasing the euchromatin area, potentially leading to a resume in transcriptional activity. This hypothesis would be consistent with our published findings of up-regulated mRNA expression of eNOS, MnSOD, and catalase in SOD mimic-treated spermatozoa ex vivo (Otasevic et al., 2013). Therefore, SOD mimic modulates the redox environment via direct chromatin remodeling in spermatozoa. Fractal analysis has proven to be a good method for the analysis of chromatin condensation in human sperm nuclei.


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PP104

Potential health benefits of the nutrient, ergothioneine

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The low-molecular weight thione, ergothioneine, can accumulate at high levels in cells and tissues in humans and other animals from dietary sources, especially mushrooms. The existence of a selective transporter for ergothioneine (OCTN1) responsible for its uptake, and the avid accumulation and retention by the body suggest that ergothioneine may be important for the human body. Indeed, numerous studies have shown that low blood ergothioneine levels (compared to healthy age-matched individuals) are associated with a wide range of disorders including mild cognitive impairment, dementia, Parkinson’s disease, chronic kidney disease, frailty, and cardiometabolic disorders. This suggests that a deficiency in this compound may predispose an individual to increased risk of these age-related disorders and that supplementation may be beneficial as a prophylactic or therapeutic agent against disease. Numerous studies have demonstrated the antioxidant and other cytoprotective properties of ergothioneine. Here we summarise some of the latest findings and potential therapeutic applications of this compound.

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Different immunoexpression of NOSs in testes of hypothyroid rats

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Nitric oxide released from three isoforms of nitric oxide synthase (NOS), endothelial (eNOS), neuronal (nNOS) and inducible (iNOS), found in the mammalian testis is the major regulator of the spermatogenetic process. It is established that germ cells express eNOS, Leydig cells iNOS, while Sertoli cells express minor amounts of nNOS in physiological conditions. There is no data on their presence and localization in hypothyroidism. This work aimed to determine the immunoexpression of NOS isoforms in rat testes in hypothyroidism. Two-month-old male Wistar rats were fed standard pelleted food ad libitum. The rats were divided into four groups. Hypothyroidism was induced by 0.04% methimazole in drinking water for 7, 15 and 21 days, respectively. Control group received tap drinking water. Isolated testes were examined by immunohistochemistry on 2 µm semi-fine sections using antibodies against iNOS, nNOS and eNOS, respectively. The immunopositive reaction for all examined NOS isoforms was visible in seminiferous tubule of both euthyroid control and hypothyroid groups, but with different patterns and intensity. eNOS was found in the cytoplasm and the nuclei of spermatogenic cells, whereas iNOS and nNOS were found exclusively in the nuclei. Hypothyroidism induces increased immunoexpression of eNOS and nNOS on day 7, while iNOS was increased on days 7 and 15. Prominent content of eNOS was found in nuclei of late spermatocytes and early spermatids; iNOS in early spermatocytes and spermatids and nNOS in early/late spermatocytes and early spermatids. All three isoforms were also found to be highly expressed in spermatozoa. On day 21, NOSs expression and localization were mainly restored to euthyroid, control level. Hypothyroidism was associated with a significant increase of each of the three NOSs expression in early/late spermatocytes, early spermatids and spermatozoa on days 7 and 15 compared to the euthyroid control. The physiological meanings and mechanisms of their induction in hypothyroidism remain elusive.

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Role of Sirt3 in sex-related response to high fat diet-induced metabolic and oxidative stress in mice

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Metabolic dysregulation is affected by various physiological processes, with males and females having different regulation of metabolic homeostasis. Little is known about the mitochondrial Sirt3 protein in the context of sex-related differences in development of metabolic dysregulation. To test our hypothesis that the role of Sirt3 in response to high fat diet (HFD) is sex-related, we measured metabolic, antioxidative and mitochondrial parameters in liver of Sirt3 WT and KO mice of both sexes fed with standard or HFD for ten weeks. We found that combined effect of Sirt3 and HFD was evident in more parameters in males (lipid content, glucose uptake, ppar, cyp2e1, cyp4a14, Nrf2, MnSOD activity) than in females (protein damage and mitochondrial respiration), pointing towards a higher reliance of males on the effect of Sirt3 against HFD-induced metabolic dysregulation. Male-specific effect of HFD also includes reduced Sirt3 expression in WT, and alleviated lipid accumulation and reduced glucose uptake in KO mice. In females, with generally higher expression of genes involved in lipid homeostasis, either HFD or Sirt3 depletion compromised mitochondrial respiration and increased protein oxidative damage. This work presents new insights into sex-related differences in the various physiological parameters with respect to nutritive excess and Sirt3.

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Delphinidin sensitizes ovarian cancer cells to 3-bromopyruvic acid

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3-Bromopyruvic acid (3-BP) is a promising anticancer compound. We found that two human ovary cancer (OC) cell lines, PEO1 and SKOV3, showed relatively high sensitivity to 3-BP (IC50 of 18.7 and 40.5 µM, respectively). However, further sensitization of OC cells to 3-BP would be desirable. The anthocyanidin delphinidin (D) has also been reported to be cytotoxic for cancer cell lines. Our screening showed that from among several flavonoids tested, D was the most toxic to PEO1 and SKOV3 cells. Combined action of 3-BP and D was mostly synergistic in PEO1 cells and mostly weakly antagonistic in SKOV3 cells. Viability of MRC-5 fibroblasts, used as control cells, was not affected by both 3-BP and D at concentrations of up to 100 µM. Combined action of 3-BP and D decreased the level of ATP and the level of reactive oxygen species (ROS) detectable with dihydroethidine, cellular mobility and cell staining with phalloidin and Mitotracker Red in both cancer ovary cell lines, but increased DCFDA-detectable ROS level and decreased mitochondrial membrane potential and mitochondrial mass only in PEO1 cells. Glutathione level was increased by 3-BP+D only in SKOV3 cells. These differences may contribute to the lower sensitivity of SKOV3 cells to 3-BP+D. Our results point to the possibility of sensitization of at least some OC cells to 3-BP by D.

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PP108
CKD, ROS and heart failure in mice – is there really a link?

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CKD and heart failure are frequently recorded comorbidities in the clinical situation. Previously, experimental studies were performed to induce CKD and analyze secondary cardiac effects, thereby giving evidence of ROS involvement. To achieve better understanding, we performed a comparative study inducing CKD via different Adenine feeding protocols, in C57BL6N and Sv129 mice. High-dose Adenine (≤ 0.3%) impaired health condition, accompanied by food rejection, starvation and ultimately autophagy in cardiomyocytes. Low-dose Adenine (0.15%) displayed slowly progressing CKD, without evidence of starvation-induced autophagy. Plasma creatinine levels were increased in both strains at different time points, while cardiac BNP mRNA expression was only partially increased. Extensive myocardial calcification and mild fibrosis were observed in the hearts of Adenine-fed Sv129 mice, although cardiac function was not altered after 13 weeks. Interestingly, protein levels of antioxidant enzymes were significantly regulated in opposing directions in BL6N (upregulation) and Sv129 mice (downregulation), however no difference in markers for global oxidative stress were found. These results suggest that an early response to increased ROS seems to be involved in development of secondary cardiac effects. In addition, our results point out to a strong genetic background influence, which might be responsible for the differences in antioxidant response and cardiac effects.

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PP109

Fish based food modifies oxidative stress in excessive physical activity exposed dogs

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Intense and prolonged exercise can result in excessive generation of free radicals resulting in oxidative damage to both proteins and lipids in dogs cells. Even though exercise shows many health benefits, on the contrary, intense and vigorous exercise can result in excessive generation of free radicals resulting in oxidative damage to both proteins and lipids. It is likely that intensified periods of physical training with minimal recovery may influence redox homeostasis, inducing a state of chronic oxidative stress and inflammation. The aim of the present study was to evaluate the effects of changing regular dogs diet almost excluded in n-3 PUFA with fish enriched food on the metabolic parameters such as lipid status and oxidative stress in excessive physical activity exposed dogs. After treatment with fish based food, levels of blood glucose and total as well as LDL cholesterol significantly decreased which is important because recently was shown that dogs are prone to hyperlipidemia. The values of triglycerides did not change. We found significantly higher activities of glutathione peroxidase (GPx) and catalase (CAT) after the treatment. The level of lipid peroxidation (MDA) significantly decreased after supplementation, as a possible consequence of higher activities of aforementioned enzymes. Hypolipidemic as well as hypoglycemic effects of fish enriched food represent the innovation. Namely, this is the confirmation of positive results of fish based food on recently proven frequent hyperlipidemia in dogs.

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Antioxidant status in hypertensive disorders of pregnancy

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Background and aim: Pregnancy is a physiological condition that can be associated with maternal hypertension leading to possible complications in childbirth outcome. Enzymes activities of paraoxonase 1 (PON1) and superoxide-dismutase (SOD) may be prone to changes during pregnancy with hypertension. PON1 has different activity distribution among HDL subclasses that can also be changed. The aim of our study was to estimate total PON1 and SOD activities as well as PON1 activity distribution on HDL subclasses through high-risk pregnancies.

Methods: 79 pregnant women with high-risk for preeclampsia development were included and 46 of them developed some hypertensive disorder in pregnancy. Plasma samples were obtained. SOD activity, total PON1 activity and relative proportion of PON1 activiity on different HDL subclasses were determined in 1st, 2nd and 3rd trimester and prior to delivery.

Results: SOD activity was significantly lower in 2nd and 3rd trimesters of pregnancy when compared to 1st trimester (P < 0.001) whereas total PON1 activity was significantly higher in 3rd than in 1st trimester (P < 0.05) in group of women with hypertension. Women with hypertension in 1st and 3rd trimester and prior to delivery were characterized by significantly increased total PON1 activity comparing to group of women with no hypertension (P < 0.05). SOD activity was significantly higher in pregnant women having hypertension than in women without it in 1st trimester (P < 0.05). Relative proportion of PON1 activity on HDL3c subclasses was significantly higher in group of women with this complication when compared to women without it in 1st trimester and prior to delivery (P < 0.05).

Conclusions: SOD activity decreased whereas total PON1 activity increased during pregnancy with hypertension. Pregnant women with hypertension had higher activities of total PON1 and SOD as well as relative proportion of PON1 activity on HDL3c subclasses than women without this complication in 1st and 3rd trimester and prior to delivery.

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Redox status of human subcutaneous adipose tissue and blood: relationship with obesity

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Background and aim: Abdominal obesity is an important risk factor for many metabolic disturbances which could lead to cardiovascular disease development. The aim of this study was to examine possible difference in redox status of blood and subcutaneous adipose tissue regarding subjects’ obesity. Methods: Ten obese subjects (7 female, 3 male, BMI = 35.0 ± 7.0, 36.9 ± 10.8 years) and 10 lean subjects (5 female, 5 male, BMI = 23.4 ± 3.5, 38.8 ± 10.4 years) were subjected to abdominal subcutaneous adipose tissue biopsies for redox status determination, during their intragastric balloon placement intervention or different aesthetic interventions, respectively. Blood samples were drawn immediately before intervention. In patients’ blood and adipose tissue homogenates we have performed following analyses: total oxidative status – TOS, prooxidant-antioxidant balance – PAB, advanced oxidation protein products – AOPP, ischemia-modified albumin-IMA, malondialdehyde – MDA and total sulphydrl groups – SHG and superoxide-dismutase – SOD activity by spectrophotometric methods. Results: AOPP, IMA and PAB were significantly higher in blood (p < 0.05 for all three parameters), but there were no difference in adipose tissue homogenates of obese subjects compared to lean controls. TOS and MDA showed comparable levels in their blood, but its values were significantly higher in adipose tissue of obese subjects (p < 0.05 for both parameters). Antioxidative enzyme SOD activity was significantly higher in lean subjects – blood, but statistically significant was only in adipose tissue samples compared to obese subjects. Conclusion: Redox status parameters showed its distinct nature, i.e. protein related products revealed difference in blood, while lipid related products showed significant difference in obese subjects’ adipose tissue samples compared to lean subjects’ adipose tissue samples. SOD as cellular enzyme also indicated more subtle changes in tissue samples. Results of this preliminary study emphasized oxidative stress burden in adipose tissue and blood of obese people, which contributes to plethora of metabolic disturbances connected with obesity.

Keywords: redox status; obesity; adipose tissue; abdominal obesity; metabolic disturbances.

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2,3-dinor metabolites of oxylipins are major excreted biomarkers of oxidative stress and inflammation in obesity

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Obesity, insulin resistance, high blood pressure and dyslipidemia are independent risk factors of cardiometabolic disease and are all associated with increased levels of endogenous oxylipins, biomarkers of oxidative stress, a key mechanism in physiological ageing. Obesity can induce systemic oxidative stress through various mechanisms, and several oxidized molecules, like oxLDL, have been associated with increased adiposity. Recently, we showed that the gut microbiota of Colombians was described by five consortia of co-abundant microorganisms, differentially associated with diet, lifestyle, obesity and cardiometabolic disease risk. This finding led us to hypothesize that urinary oxylipins would reflect the intensity of oxidative metabolism linked to gut microbiota dysbiosis. We examined a convenience sample of 105 community-dwelling individuals whose fecal microbiota was previously characterized and enriched in one of the five consortia mentioned above. We also collected health information in these individuals, including anthropometry and blood chemistry. Participants (age: 40.2 ± 11.9 years, 47.6% women) were grouped according to their body mass index (BMI), microbiota composition and cardiometabolic health status. We quantified the urinary excretion of 22 oxylipins, adjusted by urinary creatinine levels, using HPLC-QqQ-MS/MS, including isoprostanes, prostaglandins, and metabolites. Eight out of the 22 oxylipins measured were under the LOQ of the method (tetranor-PGDM-lactone, tetranor-PGDM, 20-OH-PGE2, 15-keto-PGF2α, 15-keto-15-E2t-IsoP, 15-E1t-IsoP, PGE1 and PGF1α). The levels of unmetabolized oxylipins, total prostaglandins, and total isoprostanes did not show statistically significant differences among individuals grouped by cardiometabolic status or gut microbiota composition. In contrast, the levels of unmetabolized oxylipins were not associated to BMI, the total urinary content of their metabolites showed a significant elevation with increased BMI. The drivers of these differences were mainly the metabolites from 15-F2t-IsoPs (2,3-dinor-15-F2t-IsoP and 2,3-dinor-15-epi-15-F2t-IsoP) and Prostaglandin-D (2,3-dinor-11-PGF2α). This suggests that isoprostanes, especially the 2,3-dinor metabolites, vary in their ability to detect associations with BMI. Our results suggest that 2,3-dinor metabolites could be more sensitive biomarkers of oxidative stress than their parent oxylipins and other metabolites in the context of obesity.

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Extracellular histones induce endothelial pyroptosis through oxidative stress mechanisms leading to severe phenotypes in sepsis

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Circulating extracellular histones has gained relevance as cytotoxic mediators in the pathophysiology of sepsis, promoting cell death and tissue damage. Extracellular histones act as damage-associated molecular patterns, which induce oxidative stress that lead to activate NLRP3 inflammasome and pyroptosis, a programmed cell death mechanism that produces inflammation. Despite the inflammasome activation during sepsis in immune cells has been proposed, there is no information about mechanisms of the inflammasome and pyroptosis activation in endothelial cells. Our study highlights the role of extracellular histones inducing NLRP3 inflammasome and pyroptosis activation in endothelial cells through the induction of oxidative stress. Likewise, we demonstrated how histone hyperacetylation attenuates this process. Furthermore, we demonstrated that pyroptosis occurred in septic shock patients and circulating histone levels correlated with the expression of pro-inflammatory cytokines, the release of endothelial adhesion factors and septic shock severity. We propose histone-mediated pyroptosis as a new target to develop therapeutic interventions against sepsis.

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Genetically encoded biosensor G-geNOp for detection and quantification of intracellular nitric oxide in isolated skeletal muscle fibres

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Nitric oxide (NO) is constantly produced by skeletal muscle and is involved in several processes in this organ, such as blood flow, contractile activity and glucose homeostasis. NO may play a prominent role on glucose uptake in skeletal muscle associated with the effect of insulin and in the development of insulin resistance manifested in type 2 diabetes, and possibly in ageing. To study the role of NO in pathophysiology is necessary to detect and quantify the presence of this molecule in intracellular compartments. Different methodological approaches have been developed and used in the last 20-30 years to provide measurement of NO. However, there are limitations in terms of reliable intracellular NO values, since biological samples need to be processed, and this is prone to artefacts. Recently, it has been developed new NO detectors such as the genetically encoded biosensor G-geNOp. This probe is suitable for the detection and quantification of intracellular NO in live cells using a fluorescence microscopy approach [Eroglu E, et al., Nat Comm, 2016]. We have expressed biosensor G-geNOp in mouse skeletal muscle fibres in order to evaluate intracellular NO flux in real time using fluorescence microscopy imaging. Previously, the plasmid with the coding sequence of G-geNOp was microinjected and electroporated into FDB muscle, follow by fibre isolation after 4 days. We have assayed the addition of different NO donors (SNAP and SNP) to the medium of fibres that expressed G-geNOp and monitored intracellular NO flux during time courses. The fluorescence signal of G-geNOp demonstrated that after the addition of NO donors, NO crossed the sarcolemma and was internalized into fibres, where after the reaction with the biosensor, the fluorescence was quenched, which indicated the presence of NO. In conclusion, G-geNOp is a suitable biosensor for detection and monitoring intracellular flux of NO in skeletal muscle fibres.

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Effect of multiple botanical extracts on vascular tone and antioxidant activity

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The aim of the present study was to evaluate the relationship between the antioxidant capacity of batches of botanical extracts enriched or not in polyphenols and their effect on ex-vivo vascular reactivity. The total polyphenol content (TPC) of 31 different batches of botanical extracts was determined according to the Folin-Ciocalteu method. The effect of extracts (2 mg/ml) on vascular reactivity were evaluated by incubating, rat aorta segments in organ bath (20 ml) with cumulative volumes of extracts to reach a final volume of 2 ml of extracts. Moreover, the antioxidant capacity for extracts containing more than 400 mg of TP expressed in mg Gallic Acid Equivalent (GAE)/g extract, were evaluated by the PAOT-liquid® technology on 20 µL of extracts. Correlations between vascular reactivity and polyphenol content as well as antioxidant capacity were analyzed. The mean values of total polyphenols in the batches of extracts tested varied from 0.950 mg GAE/g to 917.38 mg GAE/g. Among the batches tested, those showing vasorelaxation values greater than 50% after addition of cumulative volumes of extracts between 50 µl and 300 µl were grape seed extracts. Moreover, we observed a significant correlation between TPC of all batches tested and vasorelaxation intensity of aorta segments, for 50 µl (p = 0.04), 100 µl (p = 0.003), 200 µl (p < 0.001) and 300 µl (p < 0.001) cumulative volumes in organ bath. PAOT-liquid® values for the extract tested varied from 67.5 ± 4.36 mg (GAE) L⁻¹ to 13543.5 ± 253.12 mg (GAE) L⁻¹. Interestingly, we found also a significant correlation between the TPC introduced in the organ bath and the associated antioxidant capacity for the cumulative volumes comprised between 50 µl and 1500 µl. Among botanical extracts, the polyphenols rich extracts obtained from grape seed extracts showed the best antioxidant capacity and had positive effect on vascular tone. Dietary supplementation with polyphenol rich extracts contribute to maintain a good cardiovascular function.

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Protocol design for the evaluation of chronic antioxidant supplementation on oxidative stress and cognition status in post COVID-19 patients

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Post COVID-19 sequelae include several complications including cognitive impairment, a situation associated with increased oxidative stress. In a pilot study, we intend to recruit 20 patients having a MOCA (MOntreal Cognitive Assessment questionnaire) score ≤ 25 after their discharge from hospital for COVID-19 infection requiring a long stay (> 30 days) in Intensive Care Unit. The goal of our study was to check how a blend of polyphenols (French Grape (Vitis vinifera L.) and North-American Wild Blueberry (Vaccinium angustifolium A) extracts) at 800 mg enriched with vitamins and minerals at nutritional doses (Memophenol™) and given each day during 6 months could potentially decrease oxidative stress and improve cognitive status when compared to a placebo. For that, we propose to investigate a large battery of tests including the determination of antioxidants (vitamins C and E (alpha- and gamma-tocopherol), beta-carotene, glutathione, thiol proteins, total polyphenols, paraoxonase, glutathione peroxidase), trace elements (copper, zinc, selenium), oxidative damages to lipids (lipid peroxides, oxidized LDL) and inflammatory biomarkers (myeloperoxidase), respectively before supplementation, 3 and 6 months after. In parallel, the evolution of the MOCA score will be followed. Actually 8 patients have been included in the study.

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Oxidative stress status in COVID-19 patients hospitalized in intensive care unit for severe pneumonia. A pilot study

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Background: A key role of oxidative stress has been highlighted in the pathogenesis of COVID-19. However, little has been said about oxidative stress status (OSS) of COVID-19 patients hospitalized in intensive care unit (ICU). Material and Methods: Biomarkers of the systemic OSS included antioxidants (9 assays), trace elements (3 assays), inflammation markers (4 assays) and oxidative damage to lipids (3 assays). Results: Blood samples were drawn after 9 (7–11) and 41 (39–43) days of ICU stay, respectively in 3 and 6 patients. Vitamin C, thiol proteins, reduced glutathione, -tocopherol, -carotene and PAOT® score were significantly decreased compared to laboratory reference values. Selenium concentration was at the limit of the lower reference value. By contrast, the copper/zinc ratio (as a source of oxidative stress) was higher than reference values in 55% of patients while copper was significantly correlated with lipid peroxides (r = 0.95, p < 0.001). Inflammatory biomarkers (C-reactive protein and myeloperoxidase) were significantly increased when compared to normals. Conclusions: The systemic OSS was strongly altered in critically ill COVID-19 patients as evidenced by increased lipid peroxidation but also by deficits in some antioxidants (vitamin C, glutathione, thiol proteins) and trace elements (selenium).

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Oxidative stress status and its correlation with redox status as measured by an electrochemical (PAOT®) methodology: a pilot study in critical COVID-19 pneumonia survivors

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Background: In most serious COVID-19 forms which required prolonged stay in intensive care unit, pulmonary, cardiovascular, renal, neurological and psychological sequelae have been reported after the infection. All these complications can be sustained by chronic inflammatory problems and/or increased oxidative stress. Material and Methods: Biomarkers of the systemic oxidative stress status (OSS) including enzymatic and non-enzymatic antioxidants, total antioxidant capacity of plasma (PAOT®-Sore), trace elements, oxidative damage to lipids and inflammation markers, were investigated in 12 patients admitted to a revalidation center for post COVID-19 pneumonia. Results: From blood samples collected two months after hospital discharge and one month after admission to the revalidation center, vitamin C, thiol proteins, reduced glutathione, gamma-tocopherol and beta carotene were significantly decreased compared to reference values. By contrast, lipid peroxides and markers of inflammation (neutrophils, myeloperoxidase) were significantly higher than the norms. Lipid peroxides was strongly correlated with Cu ($r = 0.95$, $P < 0.005$) and Cu/Zn ratio ($0.66$, $P = 0.020$). Using an electrochemical method (PAOT®), total antioxidant capacity (TAC) evaluated in saliva and urine negatively correlated with copper and lipid peroxides. Similar findings were obtained for PAOT®-skin score. Conclusions: Systemic OSS was strongly altered in patients admitted in revalidation after COVID-19 infection. This suggests the need for supplementing these patients with antioxidants.

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Evaluation of anti-tumoral and/or anti-angiogenic compounds on the MDA-MB-231 and HMEC redox balance

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Angiogenesis, the formation of new blood vessels from other pre-existent ones, is highly regulated by a balance between stimulators and inhibitors under physiological conditions. However, a persistent angiogenesis is related to many pathological conditions, including cutaneous, ophthalmic and inflammatory diseases, as well as cancer. Interestingly, reactive oxygen species (ROS) have been found to stimulate or block the angiogenic response, acting as a double-edged sword in endothelial cells, because high or sustained ROS concentration is detrimental, while transient or low levels of ROS can activate signaling pathways that promote angiogenesis. Moreover, elevated rates of ROS have been found in almost all cancers, where they promote many aspects of tumor development and progression. However, tumor cells also express higher levels of antioxidant proteins to detoxify ROS, suggesting that a delicate balance of intracellular ROS levels is required for cancer cell function, and an alteration in this balance could regress the tumor.2 Our group has studied the redox potential of several anti-tumoral and anti-angiogenic compounds (HT, DMF and GR-24) in human breast cancer cells (MDA-MB-231) and in human endothelial cells (HMEC). Our data show that in presence of these compounds, tumor and endothelial cells decrease the reduced sulfhydryl group levels, suggesting that these compounds can diminish the levels of intracellular reduced glutathione. On the other hand, we have seen that DMF and GR-24 reduced the cell viability of HMEC in the H2O2 cytotoxicity assay, indicating that these compounds could sensitize cells to oxidative stress. Additionally, DMF, GR-24 and HT modulate the catalase and superoxide dismutase activities in tumor and endothelial cells, and HT was capable of reducing intracellular ROS production in both cell types. Taking altogether, our data reveal that these compounds alter the redox balance of tumor and endothelial cells, and their antitumor and antiangiogenic activity could be, at least in part, due to the imbalance in the cellular redox state.

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Characterisation of protein iodination and chlorination generated by myeloperoxidase

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During inflammation, innate immune system cells are activated and generate oxidants including hydrogen peroxide (H$_2$O$_2$). The heme enzyme myeloperoxidase (MPO), which is also released from these cells, uses H$_2$O$_2$ and halide ions including chloride (Cl$^-$) and iodide (I$^-$) to form hypohalous acids (HOX; X = Cl, I) that are primary defences against pathogens. Hypochlorous acid (HOCl) is the major species formed under most conditions. Excessive or misplaced formation of HOCl damages host tissues, and especially proteins, with this linked to multiple inflammatory diseases. As MPO can utilize multiple substrates, this study aimed to examine whether other halides, and particularly I$^-$, might reduce HOCl generation and subsequent protein damage, by increasing HOI formation. For this strategy to be successful, knowledge of the chemistry of hypoiodous acid (HOI) is required, but this is poorly explored. In this study we therefore examined whether and how HOI, generated by a peroxidase/H$_2$O$_2$/I$^-$ system + Cl$^-$, modifies proteins. Experiments were performed using both MPO, and lactoperoxidase (LPO) which generates HOI but not HOCl. Serum albumin and the extracellular matrix (ECM) proteins fibronectin and anastellin (a domain of fibronectin) were used as model target proteins. SDS-PAGE was used to examine structural changes, with specific chemical changes determined using LC-MSn. Using top-down proteomics, increasing levels of iodination were detected on anastellin as I$^-$ concentrations were raised. Peptide analysis allowed the identification of specific iodinated tyrosine residues, with some initial sites of chlorination replaced by iodinated species when the concentration of I$^-$ was increased at a constant Cl$^-$ concentration. This is consistent with the formation of HOI in place of HOCl and confirms that I$^-$ can act as a competitive substrate for MPO. Further studies are needed to clarify the biological implications of protein iodination when compared to chlorination in the context of inflammatory diseases.

Keywords: inflammation; myeloperoxidase; iodide; hypoiodous acid.

Note: Kathrine V. Jokumsen and Valerie H. Huhle contributed equally.

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Mitochondrial-targeted hydrogen sulfide donor, AP39 reduce hydrogen peroxide-induced mitochondrial dysfunction in human neuronal cells

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Oxidative stress hallmarks the degeneration of dopaminergic neurons in Parkinson's disease. Although the mechanisms responsible for the neuronal degeneration are not fully elucidated, evidence suggests that mitochondrial dysfunction is a contributing factor. Recently, a mitochondrial-targeted hydrogen sulfide (H₂S) donor, AP39, consisting of a triphenylphosphonium targeting motif linked to a H₂S-donating moiety (dithiolethione) showed to promote cellular bioenergetics. We explored the ability of AP39 to protect neuronal mitochondrial function in an environment of oxidative stress induced by hydrogen peroxide (H₂O₂). The neuroblastoma SH-SY5Y cell line was differentiated in low FBS and retinoic acid media for 7 days. Differentiation was detected by immunocytochemistry using antibodies against microtubule-associated protein 2. Non-differentiated (SH-SY5Y) and differentiated (d-SH-SY5Y) cells were exposed to AP39 (0.1, 0.3 and 1 μM) for 24 h. Cell viability was ascertained with an MTT assay. H₂S bioavailability and its co-localization within the mitochondria was detected by fluorescence microscopy using 7-azido-4-methylcoumarin (AzMC) and Mitotracker red probes, respectively. Following AP39 treatments, H₂O₂ (100 μM) was administered for 2 h. To assess the potential of AP39 to protect against H₂O₂ insults, parameters of mitochondrial oxygen consumption were evaluated using a Seahorse XF24 analyser and mitochondrial-specific superoxide (mt-ROS) were detected using MitoSOX red probe. AP39 did not affect cell viability in both SH-SY5Y and d-SH-SY5Y. AzMC fluorescence demonstrated a significant increase in H₂S bioavailability, co-localized within the mitochondria. In SH-SY5Y, 0.3 μM of AP39 stimulated mitochondrial bioenergetics, while in d-SH-SY5Y, 1 μM of AP39 was necessary to promote the mitochondrial oxygen consumption. In both, SH-SY5Y and d-SH-SY5Y, AP39 reduced H₂O₂-induced mitochondrial damage by improving the basal and maximal respiratory capacity and abrogating mt-ROS. These suggest that targeted delivery of H₂S to the mitochondria may provide a promising approach in the management of neuronal disorders associated with oxidative stress-induced mitochondrial dysfunction.

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PP122

Non-invasive analysis of metabolism biomarkers aiming to discriminate thyroid hyperplasias vs carcinomas prior to thyroidectomy

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The metabolic syndrome contributes to tumor development. However, the use of metabolic biomarkers has not yet been implemented. Taking into account that the metabolic syndrome is associated with mitochondrial dysfunction and the accumulation of mutations in mtDNA we aimed to evaluate its value as biomarkers in thyroid cancer. Methods. The study was conducted in two phases, with cohorts of 26 and 45 patients respectively, undergoing a thyroidectomy due to suspicion of thyroid cancer. Tumor development and metabolic alterations were analyzed in tumor and surrounding healthy tissue as well as in peripheral blood mononuclear cells (PBMCs). Results. We noted a significant correlation between metabolic disorders at the systemic level, measured in PBMCs and tumor tissue. Two mitochondrial DNA fragments were identified in plasma samples. The origin of these fragments and their correlation with metabolic markers in both the tumor tissue and PBMCs was evaluated. One of them, a fragment of the mitochondrial ND1 gene was found to be associated with markers of good general or systemic metabolic status, with balanced mitochondrial activity and antioxidant levels. The second fragment, derived of the mitochondrial ND4 gene, was associated with bad metabolic status markers and originated from the tumor. Conclusion. Therefore, these results suggest that the assessment of metabolic parameters in PBMCs can inform both metabolic state systemic and its impact on the tumor, and that circulating mitochondrial DNA fragments can be used as biomarkers for the stratification of patients according to their level of metabolic risk of thyroid cancer.

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Immunocytochemical localization of glutathione peroxidase (GSH-Px) in Duchenne-Becker patients’ erythrocytes

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Oxidative stress plays an important role in progression of Duchenne-Becker muscular dystrophy (DBMD), therefore, increased activity of antioxidative enzymes confirmed in previous studies in many tissues and cells, including erythrocytes it not surprising. Erythrocytes of DBMD patients are also characterized by structural and functional abnormalities, but data regarding internal AD enzymes localization are scarce. Hence, we used immunogold labeling to study GSH-Px presence and localization in erythrocytes from DBMD patients and age-matched healthy subjects. DBMD patients’ erythrocytes have a significantly increased presence of GSH-Px compared to healthy subjects. GSH-Px is found predominantly on the surface of erythrocytes in the healthy subjects, while in the DBMD patients it is distributed throughout the erythrocytes. Striking differences in GSH-Px presence and localization in erythrocytes from DMBD patients, suggesting that GSH-Px is involved and represents an important indicator in the assessment of oxidative status in DMBD.

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Phenolic acids induce changes in thiol group reactivity of defatted human serum albumin

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Phenolic acids are widely distributed in plant-based foods and, therefore, they are consumed on daily basis by humans. The binding of phenolic acids to human serum albumin (HSA) could lead to changes in its thiol group reactivity. The influence of chlorogenic, caffeic, ferulic, p-coumaric and sinapic acid on the pseudo first rate constant (k') for HSA thiol group’s reaction with 5, 5'-dithiobis-(2-nitrobenzyoic acid) (DTNB) were investigated. The commercial HSA was defatted and reduced with dithiothreitol. Phenolic acids were dissolved in DMSO to prepare 25 mmol/L stock solutions. The 0.25 mmol/L deffated HSA solution in sodium phosphate buffer, 100 mmol/L, pH = 7.4 was used for determination of k’ with DTNB. The final reaction mixture with molar ratio of HSA/phenolic acid 1:1 was incubated at 37 °C for one hour before spectrophotometric measurements in six repeats for each sample. The pseudo first order constant for defatted HSA reaction with DTNB was 30.78 ± 1.75 x 10^-3 s^-1. There was no significant change in k’ after incubation of HSA with sinapic and caffeic acids. The interactions between HSA with ferulic, p-coumaric and chlorogenic acid lead to significant increase of HSA thiol group reactivity with increase of mean k’ from 7.4 – 16.5%. The highest k’ constant was obtained for chlorogenic acid (35.86 ± 1.93 x 10^-3 s^-1, p < 0.001 compared to k’ for deffated HSA), followed by p-coumaric acid’s constant (34.58 ± 1.37 x 10^-3 s^-1, p < 0.01 compared to k’ for deffated HSA) and ferulic’s constant (33.07 ± 1.59 x 10^-3 s^-1, p < 0.05 compared k’ for deffated HSA). The obtained results indicate that HSA thiol group reactivity change could play an important role in ferulic, chlorogenic and p-coumaric acids’ antioxidant effects in vivo after consumption of food that contains high amounts of these bioactive molecules.

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A novel bioinspired proteasome activator against aging

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Proteasomes are constituents of the proteostasis network that regulate the proteolysis of normal and abnormal (in any way) proteins. Since proteasome function and content are diminished with progressive aging, interventions aiming to maintain functional proteasomes have been performed to promote longevity and to improve healthspan. Proteasome activation has been achieved either through genetic means or through natural compounds in human primary fibroblasts and in Caenorhabditis elegans. This activation resulted in cellular and organismal lifespan extension. The elevated proteasome function also conferred lower paralysis rates in various Alzheimer’s disease (AD) nematode models accompanied by decreased Aβ deposits, thus ultimately decelerating the AD phenotype progression. Similar positive results were also produced in human neuroblastoma cells and in murine cortical neurons challenged with increased concentrations of αβ peptide in the presence of proteasome-activating compounds.

In the present study, a novel hybrid compound combining the structural features of the natural antioxidant vitamin E and hydroxytyrosol (the main polyphenolic constituent of olive oil) in one scaffold was designed and synthesized. The new analogue, namely MK151, was evaluated for its ability to activate the proteasome in the test tube using highly purified human 20S proteasome. MK151 was administered to primary human fibroblasts throughout their replicative lifespan and conferred lifespan extension and healthspan amelioration. Likewise, improvement of organismal healthspan was also observed in wild-type C. elegans populations. In total, our results suggest that proteasome activation exerts downstream positive outcomes on cellular and organismal aging models and they unveil the need for the identification of anti-aging compounds with proteasome-activating properties.

Keywords: ubiquitin-proteasome system; proteasome activation; anti-aging; health span.

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Mapping and quantification of damage and cross-links formed on fibronectin by peroxynitrous acid

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Fibronectin is an abundant glycoprotein present in plasma and tissue extracellular matrices. Particularly high concentrations are present at sites of tissue damage, due to its key role on wound repair. At such sites it is exposed to oxidants generated by activated leukocytes, and particularly peroxynitrous acid (ONOOH) formed from nitric oxide and superoxide radicals (ONOOH reacts rapidly with the abundant tyrosine (Tyr) and tryptophan (Trp) residues present in proteins, resulting in the formation of 3-nitrotyrosine (3-nitroTyr) and di-tyrosine from Tyr, and 6-nitrotryptophan (6-nitroTrp) from Trp. Previous studies have shown that human plasma fibronectin is modified by ONOOH, but the location and extent of modification, and the role of protein structure are poorly understood. In this study we provide a detailed analysis of the sites of modification, the relative abundance of these, as well as the sites of intra- and inter-molecular cross-links (Tyr-Tyr, Trp-Trp, Tyr-Trp) as detected by LC-MS/MS. Extensive formation of 3-nitroTyr, and lesser levels of 6-nitroTrp, are detected at some, but not all, Tyr and Trp residues. Considerable variations in the extent of modification are detected between residues. Significant methionine oxidation was also detected. The cross-link sites provide novel data on the interactions between different modules of the protein in the conformation present in human plasma; this has allowed a new model to be proposed for its unknown quaternary structure. Comparison of the pattern of modifications detected with ONOOH with those generated by the alternative inflammatory oxidant, hypochlorous acid (HOCl), and a myeloperoxidase/H₂O₂/Cl⁻ enzymatic system, shows that these induce damage to significantly different extents, and at different sites, though there are some common features. The characterization and quantification of these modifications offers the potential to use these products as specific biomarkers of extracellular matrix modification and turnover in pathologies associated with inflammation-associated fibrosis and extracellular matrix changes.

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Protein cross-link formation by oxidation of disulfide bonds

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Cross-links formed within and between proteins are believed to be a major cause of alterations in protein structure and function, and may drive the accumulation of protein aggregates in human diseases. Protein cross-links can be either reversible (usually disulfide bonds) or irreversible (via formation of carbon-carbon or carbon-heteroatom bonds). Oxidation of cysteine (Cys) residues present on the same, or different, proteins to give intra- or inter-molecular disulfides, is a widespread process. These can be formed deliberately, via enzymatic reactions, or as a result of unintended oxidation. We show here that new intra- or inter-molecular disulfide cross-links can also be formed starting from an existing disulfide bond. This provides a mechanism for cross-linking of proteins that contain no free Cys residues. Oxidation of the disulfide bond by singlet oxygen (\(\text{O}_2\)) gives a reactive species (a zwitterion peroxide or thiosulfinate) that reacts with a Cys residue on a second protein, to give novel inter-protein disulfide cross-links. This is shown to occur on oxidation of multiple proteins (\(\alpha\)-lactalbumin, lysozyme, beta-2-microglobulin), and subsequent incubation with the Cys-containing protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH). These reactions generate inter-protein cross-links as detected by SDS-PAGE, immunoblotting and mass spectrometry (MS). The cross-link yield is dependent on the \(\text{O}_2\) concentration, requires a disulfide bond present on the original protein, and the presence of a free Cys residue on GAPDH. Peptide mass mapping using MS with 18O-labeling has allowed identification of the residues involved for some proteins (e.g. Cys25 from the Cys25-Cys80 disulfide in beta-2-microglobulin, with Cys149 or Cys244 of GAPDH). The formation of these cross-links results in a loss of GAPDH enzymatic activity, suggesting that these reactions have functional consequences. These data provide ‘proof-of-concept’ for a novel mechanism of protein cross-link formation which may account, in part, for the accumulation of cross-linked proteins in multiple human pathologies.

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Crosslinking of C-reactive protein to serum albumin in human plasma via disulfide bond oxidation

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Crosslinking between proteins is a significant cause of protein dysfunction, and is associated with the accumulation of aggregates in aged and diseased tissues. Crosslinks can be formed between multiple amino acid side chains, and these can be both reversible or irreversible. Disulfides formed by enzymatic reactions, or as a result of oxidant-mediated reactions, are a major class of reversible crosslinks. Whilst these are commonly generated via oxidation of Cys thiol groups, we have shown that these can also be generated by ‘oxidant-mediated thiol-disulfide exchange reactions’ via oxidation of an initial disulfide to a thiosulfinate or zwitterionic peroxide intermediate, and subsequent reaction with another thiol. These types of reactions can result in adduction of either low-molecular-mass (e.g. GSH) or another protein. The latter gives rise to new intermolecular protein-protein crosslinks. In this study, we show that reaction of the single disulfide present in C-reactive protein (CRP, a major human plasma inflammatory protein) with the biological oxidants HOCl, ONOOH or \(\text{O}_2\)\textsuperscript{1}, can give rise to reversible disulfide bond formation with human serum albumin (HSA). This occurs in an oxidant dose- (or illumination-time-) dependent manner, and can be detected both with the isolated proteins, and in human plasma containing high, but not low, levels of CRP. This inter-protein crosslink has been detected by both immunoblotting with antibodies against both proteins, and by mass spectrometry (MS). The latter method has provided direct evidence for a crosslink involving Cys36 of CRP and Cys34 of HSA. The extent of protein-protein crosslinking depends on the nature of the initial oxidant and the extent of oxidant exposure. The crosslinks can be reversed by dithiothreitol and tris(2-carboxyethyl)phosphine hydrochloride. These data indicate that disulfide bond oxidation in proteins can be a source of novel inter-protein crosslinks, which may help rationalize the accumulation of crosslinked proteins in aged and diseased tissues.

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Production of BioSeNPs in fungus Phycomyces blakesleeanus is accompanied by decrease in intracellular thiols as detected by in vivo EPR

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Selenium represents an essential microelement for many organisms, and exists in several oxidation states. Selenite (Se⁴⁺) is often toxic due to its water solubility and bioavailability, but can be reduced to insoluble Se⁰ by many microorganisms including fungi. Fungus Phycomyces blakesleeanus reduces Se⁴⁺ to Se⁰ and forms selenium nanoparticles (BioSeNPs) as witnessed by red coloration of the mycelium after 24h of treatment with 2, 5 and 10 mM Se⁴⁺. SEM micrographics and EDS spectra confirmed presence of BioSeNPs with characteristic peaks at 1.4 (SeLα), 11.2 (SeKα) and 12.5 (SeKβ) keV. One of the mechanisms of selenite toxicity is considered to be production of reactive oxygen species that then triggers response of cellular antioxidative systems, among which important part is played by thiols such as glutathione. Glutathione is also believed to play a more direct role in reduction of Se⁴⁺ to Se⁰ as proposed by the Painter reaction. Thiol specific biradical EPR probe (RSSR) was used for EPR in vivo detection of intracellular thiol-group modifications in mycelium of fungus P. blakesleeanus treated by 0.1, 0.5, 1, 2, 5 and 10 mM selenite (Na₂SeO₃) for 24 h. Decrease of available –SH groups is detected as the decrease in ratio of mono- and biradical peak in the spectrum of the given sample. This ratio was 43.05 for control, and didn’t change with 0.1 mM treatment (42.23), but halved to 23.55 with 0.5 mM Se⁴⁺. It further decreased 10 × to minimal values of 4.31, 4.21, 4.1 and 3.5 with 1, 2, 5 and 10 mM Se⁴⁺, respectively. This cannot be solely attributed to biomass decrease as it never decreased more than 45%. P. blakesleeanus forms BioSeNPs from Se⁴⁺ and intracellular thiols are involved in this process either through neutralization of ROS or directly in the reduction of Se⁴⁺, but most probably both.

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Synergistic anticancer effect of glycolysis inhibition and oxidative phosphorylation suppression

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There is no effective therapy for melanoma, a malignant tumor of melanocytes with an increasing incidence. High energy demands of melanoma cells are predominantly satisfied by aerobic glycolysis. When glycolysis is suppressed, these metabolically plastic cells switch to oxidative phosphorylation. The aim of this study was to investigate the antimelanoma effects of simultaneous inhibition of glycolysis by 2-deoxy-D-glucose (2DG) and oxidative phosphorylation by rotenone (ROT). 2DG synergized with ROT in inducing death of B16 melanoma, but not primary mesenchymal cells. Combined treatment stimulated caspase activation, but not PARP cleavage and DNA fragmentation. Disintegration of plasma membrane and inability of caspase inhibitors and necrostatin to suppress toxicity of 2DG/ROT implied that combined treatment induced necrosis, rather than apoptosis and necroptosis. 2DG/ROT stimulated ATP depletion, mitochondrial superoxide production, and mitochondrial swelling, but not depolarization of mitochondria. 2DG/ROT-induced toxicity was suppressed by antioxidant α-tocopherol, but not mitochondrial depolarization inhibitor cyclosporine. Combined treatment induced the translocation of hexokinase II, a suppressor of voltage-dependent anion channel (VDAC) opening, and cytochrome c from mitochondria in the cytoplasm, while VDAC opening inhibitor DIDS suppressed 2DG/ROT toxicity. Our results suggest that 2DG/ROT treatment stimulates mitochondrial swelling, release of hexokinase II and subsequent opening of VDAC in the outer mitochondrial membrane. These events allow cytochrome c to exit and activate caspases, which are unable to stimulate PARP and consequent DNA fragmentation in the energy-depleted state. On the other hand, superoxide synthesized in mitochondria upon 2DG/ROT treatment also exits through VDAC and triggers energy-independent necrosis. Simultaneous inhibition of glycolysis and oxidative phosphorylation appears to be promising strategy for further development of novel anticancer therapeutics.

Keywords: glycolysis; oxidative phosphorylation; mitochondria; necrosis; 2-deoxy-D-glucose; rotenone; antitumor therapy; melanoma.

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Antioxidant capacity of Karnozin Extra® formulation

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Aim: L-carnosine is a naturally occurring dipeptide (beta-alanyl-L-histidine) highly present in excitable tissues. Karnozin Extra® is a commercially available L-carnosine-based formulation enriched with coenzyme Q10, L-carnitine, vitamin E, Northern bilberry and grape seed extracts. The current study aimed to evaluate in vitro antioxidative effect of Karnozin Extra® and pure L-carnosine. Methods: The neutralizing effect on hydroxyl (‘OH) radicals was determined by spectrophotometrical measurement at 532 nm of 2-thiobarbituric (TBA) reactive substances, which are formed by degradation of 2-deoxy-D-ribose by ‘OH radicals. The neutralization of nitroso (‘NO) was determined spectrophotometrically at 546 nm after the formation of the pink-coloured complex with Griess reagent. The degree of lipid peroxidation was quantified by the measurement of absorbance of the adduct formed in a reaction between thiobarbituric acid and malondialdehyde. Results: Karnozin Extra® exhibited a significantly higher antioxidative effect with regards to scavenging ‘OH and ‘NO free radicals compared to pure L-carnosine. The inhibition of lipid peroxidation was higher with Karnozin Extra® when compared to L-carnosine. Conclusion: Karnozin Extra® represents a potent antioxidative formulation that has a potential for application in diseases characterized by elevated oxidative stress.

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The connection between PON1192 activity phenotypes and redox status in end stage renal disease patients on maintenance haemodialysis

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Background: PON-1 antioxidative HDL-associated enzyme in RR PON1192 activity phenotype subjects has lower antioxidative capacity, which is considered as risk factor regarding cardiovascular and cardiovascular-related diseases. The aim of this study was to determine the PON1192 activity phenotype distribution in end stage renal disease patients (ESRD) on haemodialysis program, and to determine redox status of these patients according to their phenotype’s subgroups. Methods: In 125 ESRD patients and 22 healthy participants (control group, CG) we have measured paraoxonase (POase) and arylesterase (ARE) PON1 activity, as well as redox status parameters (total oxidative status – TOS, prooxidant-antioxidant balance – PAB, advanced oxidation protein products – AOPP and total antioxidant status – TAS, total sulphhydryl groups – SHG) in serum samples. According to POase/ARE ratio PON1192 activity phenotype (QQ, QR, RR) were determined. Results: PON1192 activity phenotypes distribution was as follows: ESRD vs. CG 39 (31.2%) vs. 4 (18.2) QQ, 32 (25.6%) vs. 15 (68.2%) QR and 54 (43.2%) vs. 3 (13.6%) RR phenotype ($\chi^2 = 15.9, p < 0.001$). ESRD patients had significantly lower POase and ARE PON1 activities vs. CG (POase: 278 (170-469 vs. 744 (671-922, P < 0.001 and ARE: 38 (26-52) vs. 173 (138-227), P < 0.001) and also in distinct subgroups regarding their PON1 192 phenotypes (P < 0.001 for all three phenotypes). TOS so as AOPP concentration were the highest in RR patients and the lowest in QQ ones, while values in QR patients were in between (p < 0.001 for both parameters). Also, a significant negative correlation was found between AOPP and ARE activity ( = -0.378, p < 0.001), and TOS and ARE activity ( = -0.265, p < 0.005), while a significant positive correlation was found between AOPP and POase activity ( = 0.311, p < 0.005), and TOS and POase activity ( = 0.356, p < 0.001). Conclusion: ESRD patients on haemodialysis program have RR as the most common PON1192 phenotype and the highest level of oxidative stress, which increases the risk of cardiovascular disease development.

Keywords: PON1 phenotypes; redox status; hemodialysis.

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Fatty acid composition and antioxidant activity of grape seed oils

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Grape (Vitis Vinifera L.) is a perennial grown fruit, and it is of worldwide interest for nutritional and medicinal purposes. Seeds represent the underrated but valuable part of a berry. The composition of grape seeds depends on the grape variety, environmental factors, and maturation degree. In large, seeds contain between 6 and 20% of oil. This oil is a rich source of unsaturated fatty acids, tocopherols and phytosterols, and it is well known for its anti-inflammatory, antioxidant and antimicrobial properties. The present study aimed to determine fatty acid composition and to investigate antioxidant potential of seed oils obtained from six different grape varieties, emphasizing insufficiently explored autochthonous Serbian varieties. Fatty acids were identified and quantified using gas chromatography. Linoleic acid was the most abundant among all samples and ranged from 61.42 to 67.59%. Varieties Merlot and Drenak were found as the richest sources of this fatty acid. Grape seed oils contained significant amounts of oleic acid; Serbian autochthonous variety Jagoda pointed out with the highest content. Alpha-linolenic acid was also quantified in all samples. Total phenolic content was determined by the Folin-Ciocalteu method, while the antioxidant activity of methanolic extracts was evaluated using FRAP assay. The oil obtained from Jagoda seeds stands out as the most potent. Antioxidant activity significantly correlated with total phenolic content (r = 0.977; p < 0.01), suggesting the substantial contribution of polyphenol compounds to this bioactivity. Future investigations are needed to reveal the compounds responsible for antioxidant activity. In addition, the importance of determining the chemical composition and bioactivity of Serbian traditional grape varieties must be emphasized.

Keywords: grape seed oil; fatty acids; polyphenols; antioxidant activity; FRAP.

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Cannabidiol as a modulator of apoptosis of psoriatic and healthy keratinocytes

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In the development of psoriasis, the formation of characteristic skin lesions is accompanied by metabolic changes in keratinocytes. However, UVB phototherapy used to remove metabolically altered cells also affects healthy keratinocytes. Therefore, cannabidiol, non-psychoactive phytocannabinoid, is proposed as a therapeutic agent for psoriatic keratinocytes and as a protective agent for healthy cells. In psoriatic keratinocytes, decreased expression of the anti-apoptotic AKT kinase is observed and increased expression of pro-apoptotic PAGM5 and PAGM5-protein adducts leads to activation of the internal pathway as increased caspase 3 and 9 expression and increased cytochrome c release are observed. On the other hand, apoptosis increased by UVB radiation is rather UVB effects on the extrinsic pathway (with an increase in caspase 8 expression) in both healthy and psoriatic cells. CBD alleviates the effects of UVB radiation by increasing AKT levels in healthy cells and Bcl2 levels in psoriasis cells, with changes in Bcl2 levels probably not due to CBD having an effect on the Nrf2 pathway as its activity is reduced. This may be due to the increased expression of Nrf2 inhibitors (Bach1, Keap1) or the observed increased level of adducts of Keap1 with other proteins. Increased apoptosis and necrosis in psoriatic keratinocytes and after UVB irradiation of both psoriatic and healthy keratinocytes, and its reduction by the use of cannabidiol, were confirmed by microscopic examination using annexin and propidium iodide staining. In conclusion, UVB causes a dramatic increase in the death of both psoriatic and healthy keratinocytes, while cannabidiol partially mitigates this effect on healthy keratinocytes, so it can be used as a protective factor to reduce the side effects of phototherapy.

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Keywords: psoriasis; keratinocytes; cannabidiol; UVB radiation; apoptosis.

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Citrullinated histones protect from cell death and vascular damage because the attenuation of oxidative stress

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NETosis is a key process in the defense of a pathogenic infection during innate immunity activation, which consists on the neutrophil “explosion” and the NET formation, containing DNA, histones and other nuclear proteins. During sepsis, an exacerbated response of the body to an infection, the NETosis occurs. Histone H3 citrullination is a requirement to activate NETosis. So, it is expected that histones released during NETosis are citrullinated. Our group investigated the molecular mechanisms activated by extracellular native histones, and those mechanisms activated by extracellular citrullinated histones. We demonstrated that citrullinated histones have a protective role in endothelial cells, and they did not affect cell viability, possibly due to they are not able to activate oxidative stress. Nevertheless, citrullinated proteins induce inflammatory patterns as well as regulatory endothelial mechanisms. Furthermore, septic patients shown elevated levels of circulating histone H3 citrullinated, concluding that the histone citrullination is produced during the first stages of sepsis, probably due to the NETosis processes.

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Mindfulness based stress reduction (MBSR) program leads to a reduction in physiological evaluated stress

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Background: Oxidative stress has complex interactions with our lifestyle habits that negatively affect our health. Increasing evidence suggests that chronic psychosocial stress enhances oxidative stress, which in turn may contribute to aging and etiology of many lifestyle-related degenerative diseases. Mindfulness practice is defined as “paying attention in an intentional and non-judgmental way to the present moment”. Past studies investigating the link between mindfulness and stress response demonstrated that Mindfulness-Based Stress Reduction (MBSR) program is an effective stress management technique which have beneficial effects on emotional and psychological responses to stressors. In contrast, there have been less studies of its effect on physiological parameters, such as oxidative stress.

Methods: In this study, we evaluated the effectiveness of MBSR program on a sample of 42 people (age 30-66 years). In particular, we analyzed blood pressure, plasma concentration of carotenoids and salivary cortisol levels, before (baseline) and after an MBSR training (8 weeks). Cortisol was measured by an Enzyme Immunoassay kit. Carotenoid concentration was evaluated by Raman spectroscopic technique. Levels of perceived stress, anxiety and awareness were assessed by Perceived Stress Scale, State Anxiety Inventory, and Mindful Attention Awareness Scale questionnaires, respectively. Student’s t was used for statistical analysis (P < 0.05).

Results: Mindfulness practice significantly reduced salivary levels of cortisol (P < 0.01), blood pressure in hypertensive people (P < 0.01) and increases blood concentration of carotenoids (P < 0.05). An increase in awareness and a decrease in perceived stress and anxiety were also observed. All the parameters analysed showed a statistically significant improvement (P < 0.01).

Conclusions: These preliminary data are a first indication that the MBSR program is an effective tool to ameliorate antioxidant defence (as indicated by carotenoids data) confirming positive effects on blood pressure and psychological outcomes. Further studies on pro-inflammatory cytokine levels and overall redox related mechanisms are needed to better evaluate MBSR systemic effects.

Keywords: blood pressure; carotenoids; cortisol; mindfulness-based stress reduction; stress.

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S-Adenosyl methionine administration enhances nitrosative stress in acute pancreatitis leading to blockade of the trans-sulfuration pathway and accumulation of homocysteine

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Acute pancreatitis (AP) is an inflammatory process of the pancreas that frequently leads to local and systemic complications. Currently, AP is the third leading cause of hospitalization due to gastrointestinal disease in Western countries, and its incidence has increased progressively during recent years. Depletion of reduced glutathione (GSH) and S-adenosyl methionine together with dysregulation of the trans-sulfuration pathway are features of AP that may contribute to its severity. The aim of this work was to determine whether administration of S-adenosyl methionine (SAM) could prevent GSH depletion and restrain the dysregulation of the trans-sulphuration pathway in cerulein-induced AP in mice. Metabolomic and proteomic analysis confirmed that AP causes depletion of methionine, S-adenosylmethionine, 5'-methylthioadenosine, cystathionine, cysteine and GSH in pancreas, without changes in homocysteine levels. Regarding enzymes involved in the trans-sulfuration pathway, an increase in adenosyl homocysteinase and cystathionine gamma-lyase levels and a decrease in S-methyl-5'-thioadenosine phosphorylase levels were observed in AP. Cystathionine β-synthase (CBS) protein levels did not change during AP, but tyrosine-nitration of CBS was observed due to Nos2 mRNA induction that may decrease its activity. SAM administration enhanced both Nos2 up-regulation and CBS nitration leading to accumulation of homocysteine in AP. Furthermore, SAM treatment triggered enrichment of the euchromatin marker H3K4me3 in the promoters of Tnf-α, Il-6, and Nos2 and enhanced the mRNA up-regulation of Tnf-α, Il-6, and Nos2. Accordingly, SAM administration increased inflammatory infiltrate and edema in pancreas with acute pancreatitis. In conclusion, S-adenosyl methionine treatment enhanced the inflammatory response as well as tyrosine-nitration of cystathionine β-synthase in acute pancreatitis aggravating the blockade of the trans-sulphuration pathway and leading to pancreatic homocysteine accumulation without preventing GSH depletion.

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Protection from HFD-induced metabolic stress in adult female mice is attributed to the combined effect of Sirt3 and ovary hormones

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Aim: High fat diet (HFD) is an important factor in the development of metabolic diseases, with liver as metabolic center being highly exposed to its influence. However, the effect of HFD-induced metabolic stress with respect to ovary hormone depletion and sirtuin 3 (Sirt3) is not clear. Here we investigated the effect of Sirt3 in liver of ovariectomized female mice on metabolic stress upon HFD. Methods: 129S Sirt3 WT and KO female mice were fed standard-fat diet (SFD) or HFD for ten weeks following ovariectomy or sham surgery. Metabolic, oxidative and mitochondrial parameters were examined in the liver by Folch, gas chromatography and lipid hydroperoxide analysis, histology and oil red staining, RT-PCR, Western blot, antioxidative enzyme activities, and oxygen consumption analysis. Results: In SFD-fed WT mice, ovariectomy increased Sirt3, accompanied by maintained mitochondrial function, increased fatty acids synthesis, lower levels of lipid hydroperoxides, and the absence of weight gain. Combination of ovariectomy and Sirt3 depletion reduced pparα, Scd-1 ratio, MUFA proportions, CII-driven respiration, and increased lipid damage. HFD compromised mitochondrial function and activated peroxisomal ROS scavenging enzyme catalase in sham mice, whereas in combination with ovariectomy and Sirt3 depletion, increased body weight gain, expression of NAFLD- and oxidative stress-inducing genes, and impaired response of antioxidative system. Conclusion: This study provides evidence that protection against harmful effects of HFD in female mice is attributed to the combined effect of female sex hormones and Sirt3, thus contributing to preclinical research on possible sex-related therapeutic agents for metabolic syndrome and associated diseases.

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Detection of extracellular superoxide in isolated human immune cells and in an animal model of arterial hypertension using hydropropidine probe and HPLC analysis

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Superoxide formation is a hallmark of cardiovascular disease with the involvement of different tissues and cell types. Identification of the cellular sources and subcellular localization of superoxide formation is important to understand the underlying disease pathomechanisms. In the present study, we used HPLC quantification of the superoxide-specific oxidation products of hydroethidine (HE or DHE) and its derivative hydropropidine (HPr) for measurement of intra- and extracellular superoxide formation in isolated leukocytes and tissues of hypertensive rats. Superoxide generation by isolated leukocytes from human subjects as well as tissue samples of hypertensive rats (infusion of angiotensin-II for 7 days) was investigated using HPr+ and HE fluorescent probes with HPLC or plate reader detection. Both fluorescent dyes were used to test for intra- and extracellular superoxide formation using the supernatant or cell/tissue pellet for analysis. We demonstrate the correlation of impaired functional parameters (blood pressure, vascular function, and oxidative burst) and increased superoxide formation in different organ systems of hypertensive rats using the HPr+/HPLC method. In the cell model, the differences between HE and HPr+ and especially the advantage of the extracellular specificity of HPr+, due to its cell impermeability, became evident. Plate reader-based assays showed much higher background signal and were inferior to HPLC based methods. In conclusion, the HPr+/HPLC assay for superoxide determination is highly reliable in isolated immune cells and an animal model of arterial hypertension. In particular, the cell impermeability of HPr+ made it possible to differentiate between intra- and extracellular superoxide formation.

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Cannabidiol treatment of keratinocytes, before and after hydrogen peroxide exposure, reduces protein adducts formation with lipid peroxidation products in membrane proteome

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The topical use of low concentrations of hydrogen peroxide (H$_2$O$_2$) in clinical applications (especially in dermatology) is due to its antibacterial/antifungal properties and participation in wound healing. However, apart from its beneficial effects, H$_2$O$_2$ when long-term used, may also participate in another reactive oxygen species formation and reveal pro-inflammatory effects. H$_2$O$_2$ may promote the oxidation of lipids, proteins and DNA. Cannabidiol (CBD), major non-psychoactive phytocannabinoid from Cannabis sativa L., may help to normalize negative effects of H$_2$O$_2$ applications due to its antioxidative and anti-inflammatory properties. CBD can modify the redox, inflammatory and survival status of cells through its action in molecular signalling by interactions with components of the endocannabinoid system and membrane receptors.

Our previous studies, as well as literature data clearly indicate protective effect of CBD against oxidative modifications of proteins and lipids in skin cells. Thus, the aim of this study was to analyse CBD effects on membrane proteome of keratinocytes. 419 proteins were identified and quantified using SDS-PAGE/nanoHPLC/QExactiveOrbiTrap analysis. Obtained data showed that H$_2$O$_2$ application (200 μM) as well as CBD treatment – applied before and after H$_2$O$_2$ – changed significantly proteomic profile of keratinocyte membranes. Moreover, it has been observed that CBD treatment dramatically reduced H$_2$O$_2$-induced level of protein adducts generated by highly reactive lipid peroxidation products (4-HNE, MDA, 4-ONE). Together with this, it has been observed that CBD significantly reduced the level of 4-HNE adducts with antioxidant proteins (glutaredoxin, glutathione S-transferase and peroxiredoxins) which may reduce cellular antioxidant response. Therefore, our study indicates that CBD counteracts the effects of H$_2$O$_2$-induced oxidative stress on skin keratinocytes, which confirms the possibility of its protective action in clinical applications.

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Hydroxyl radical scavenging potential of the late embryogenesis abundant proteins (LEA) proteins from *Ramonda serbica* – in silico approach

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*Ramonda serbica* Panc. is a resurrection plant that can survive long desiccation periods (extreme loss of cellular water). The accumulation of late embryogenesis abundant proteins (LEAPs) is a crucial step in desiccation tolerance mechanism. Based on *in vitro* studies, LEAPs can be involved in antioxidative defense, ion sequestration, structural stabilization of both membranes and enzymes during freezing or drying, while by forming intracellular proteinaceous condensates they increase structural integrity and intracellular viscosity of cells during desiccation. Here we investigated the antioxidative potential of LEAPs identified by de novo transcriptomics of *R. serbica*, based on their primary and secondary confirmation. In our previous work [1], we displayed the antioxidative capacity of 20 free proteogenic amino acids (FAA) through determining their hydroxyl radical (·OH, generated in Fenton reaction) scavenging rate by using electron paramagnetic resonance. These results served as a basis for generating a model for prediction of ·OH scavenging activity for selected proteins. In addition, the model was built based on protein primary sequences, hydrophobicity, 3D structure and predicted solvent accessible area. Manually curated data for peptides and proteins with experimentally determined ·OH scavenging rate were used for training and testing. The model was fed into machine learning algorithm and ·OH scavenging potential scale was created using IC50 values. By applying our model, we classified 164 LEAPs according to their potential for ·OH scavenging. Further work will focus on the experimental evaluation of the obtained model by measuring of the rate of ·OH scavenging in the presence of recombinantly produced LEAPs.

Keywords: desiccation tolerance; electron paramagnetic resonance (EPR); intrinsically disordered proteins; machine learning; resurrection plants; secondary structure.

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PP142

The negative impact of the hominin's DPP4 gene inherited from neanderthals to pandemic of COVID-19

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Background: According to preliminary sequences from 2010, 99.7% of the nucleotide sequences of the modern human and Neanderthal genomes are identical, compared to humans sharing around 98.8% of sequences with the chimpanzee. In contrast, the difference between chimpanzees and modern humans is approximately 1,462 mtDNA base pairs. Materials and Methods: Neanderthal-inherited genetic material is found in all non-African populations and was initially reported to comprise 1 to 4% of the genome. This fraction was later refined to 1.5 to 2.1%. We had gone through many researches of Neanderthals affected gene flow in humans. Results: It is estimated that 20% of Neanderthal DNA currently survives in modern humans. Modern human genes involved in making keratin, a protein constituent of skin, hair, and nails, have especially high levels of introgression. For example, approximately 66% of East Asians contain a POUF23L variant introgressed from Neanderthals, while 70% of Europeans possess an introgressed allele of BNC2. Our finding shines a light on an enzyme called dipeptidyl peptidase4 (DPP4). Scientists already know the protein allows another coronavirus, which causes Middle Eastern respiratory syndrome (MERS), to bind to and enter human cells. The new analysis, of DPP4 gene variants among COVID-19 patients, suggests the enzyme also provides SARS-CoV-2 with a second door into our cells, along with its usual infection route via the angiotensin-converting enzyme 2 (ACE2) receptor on cell surfaces. Conclusion: Most Europeans, Asians, and Native Americans harbor a handful of genes from Neanderthals, up 1.8% to 2.6% of their DNA. Studies of ancient DNA in Neanderthal fossils have shown the hominin's DPP4 gene subtly differs from the typical human one. Conclusion: The hominin’s DPP4 gene inherited from Neanderthals plays a major role in Immune System Disorders and Lower Immune response in many diseases. This gene plays a major role in affecting humans with COVID-19 and spreading it through the world. All humans contain this gene from 1 to 4%. East Asians, Europeans, Middle and south Americans conveys more, hence, native Africans contain less amounts of hominin’s DPP4 gene. Therefore, East Asians, Europeans, Middle and south Americans are prone to severe COVID-19.

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Age-related changes in antioxidant defence system of Wistar rats brain

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Oxidative stress generated from increased oxygen species play an important role in the aging. The hypothesis of oxidative stress is in line with possible mechanism for aging-related neurodegenerative diseases. Usually used biomarkers for investigations are major product of lipid peroxidation, activities of enzymatic defense system and activity of acetylcholine esterase (AchE) in brain. Our experiment performed on Wistar rats divided in two groups young (3-6 months), n = 8 and aged (18 month), n = 8. Animals were caged in groups of 4, with ad libitum access to water and food under 12:12 h light/dark cycle at control room temperature (22-24 °C). After one week of acclimation, rats were decapitated and their brains and blood were collected. Brains were removed from the skull within 30s after decapitation and collected: cortex, hippocampus, thalamus, Nc caudatus. Tissue were snap-frozen until experimental work. Lipid peroxidation (MDA), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and acetylcholine-esterase were examined after tissue preparation. Our results showed statistically significant decreasing (p < 0.01) in MDA concentration in aged Wistar rats in cortex and (p < 0.001) in hippocampus and increasing in thalamus, p < 0.05). SOD were statistically decreased in aged rats p < 0.001 in cortex, thalamus and Nc caudatus as well as GR decreased in aged rats in cortex and hippocampus, (p < 0.05) and GSH-Px which activity was statistically decreased in hippocampus, p < 0.05). In all parts of brain activity of AchE were decreased in aged rats, p < 0.05). Present study data suggest age-related changes in antioxidative defence in Wistar rats brain. Brain as a aerobic organ with highest oxygen consumption is a tissue more susceptible to oxidative damage. Increased oxidative stress in brain may lead to neurodegenerative disorders, our future investigation will address it.

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Drug induced cytotoxicity in various in vitro models

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In vitro hepatic toxicology models provide a valuable role in evaluating drug cytotoxicity and elucidating the underlying mechanism. Different cellular-based (e.g., immortal hepatocarcinoma, isolated primary hepatocytes, or in vitro differentiated cell lines) models are available, each having its cost-benefit. The method of cell culture (e.g., monolayer or 3D) can also influence cellular behavior, hence the suitability of a given model. Acetaminophen (APAP) – a widely used OTC drug in treating pain and fever – can induce cell death due to the formation of the reactive metabolite NAPQI. The biotransformation of APAP to NAPQI is mediated by CYP450 enzymes found predominantly in hepatocytes. The exact mechanism of APAP-induced in vivo hepatocyte death is still under investigation. On the other hand, in vitro models propose an even wider range of programmed mechanisms involved. In our experiments, we used APAP to evaluate the performance of different in vitro hepatic toxicology models. This study also compares the usability of different techniques (fluorescent microscopy, flow cytometry, spectrophotometric) to assess cell viability, oxidative stress, glutathione depletion, and caspase activation. This study was based on two cellular models (HepG2 hepatocarcinoma and in vitro differentiated HepaRG cell line) using three culture methods (2D monolayer, 3D spheroid, and 3D nano scaffold). Our results show that APAP sensitivity correlates with CYP450 expression, a function of both the cellular and culture methods. We show that while the hepatocytes in differentiated HepaRG culture are sensitive to APAP, resistant non-parenchymal cells behave as a strong background for particular methods and limit their use. We also observed that while the predominantly initiated cell death pathways in HepG2 and HepaRG were fundamentally different, careful methodological considerations enabled the detection of overlapping phenomena.

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Ascorbate and cell death

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Our understanding of ascorbate’s physiological functions is well established due to scientific efforts of the past decades. However, the potential use of ascorbate in cancer therapy is still under investigation. Ascorbate, when administered in high (“pharmacologic”) doses, can act as a pro-oxidant. Metal ions catalyze this auto-oxidative process in the presence of oxygen through the Fenton reaction. The concomitantly formed reactive oxygen species can be used to target cancer cells sensitive to oxidative stress. Recent advances in the mechanism of pharmacologic ascorbate-induced cell death reinforce the promise of its’ potential application in cancer therapy. Ascorbate administered at different concentrations may activate different cellular mechanisms accordingly. Our results propose two caspase independent mechanisms at moderate ascorbate dose induced cytotoxicity: necroptosis and autophagy. Specific inhibitors of the latter processes were able to alleviate cell death while activation of key protein markers (RIPK1 and LC3-II) was observed parallelly. Furthermore, ascorbate was found to inhibit the iron-linked, oxidative stress-mediated programmed cell death process: ferroptosis. Ascorbate inhibited the decrease of cell viability, ROS and lipid peroxide formation induced by specific ferroptosis inducers erastin and RSL-3. Our results show that this interrelationship is significant at physiological ascorbate concentrations and may interfere with ferroptosis’s clinical application.

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Antinociceptive and anti-inflammatory effect of *Erythrina americana* flowers: an alternative for pain management

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Although pain is a physiological alert mechanism activated by detecting a harmful or potentially harmful stimulus, pain is frequently related to inflammatory processes and can persist even after the injury has healed. For this reason, its treatment has always been the object of study since pain that is not relieved (chronic pain) is recognized worldwide as a health problem that reduces the quality of life of those who suffer from it. The genus *Erythrina* is made up of a great diversity of species, at least 120. The different parts of the tree are used in traditional medicine to treat various health disorders, among which the relief of pain and inflammation stands out. However, few studies demonstrate antinociceptive and anti-inflammatory activity. To evaluate analgesia in nociceptive and inflammatory pain, first, Wistar rats were divided into six groups. Then the animals were treated with different doses of ethanol extract from the flowers of *E. americana* (100, 178, 316, and 562 mg/kg), diclofenac (17 mg/kg), or vehicle (tween 1%) by the intragastric route. Pain stimulation was performed by subcutaneous administration of 2% formalin (50 µl/animal) to the hind paw and, the nociceptive response was measured in periods of 1 minute every 5 minutes for one hour. The results showed that the oral administration of the extract significantly reduced the nociceptive behavior produced by formalin administration. It is suggested that antioxidant compounds such as rutin or chlorogenic acid in flowers of *E. americana* are responsible for the analgesic activity, which could provide beneficial effects in pain management.

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Identification of products and effects of coexistent compounds on reaction of kynurenic acid with HOBr and HOCl

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Kynurenic acid (KYNA), a tryptophan metabolite, acts as antagonist or agonist of several receptors. Hypobromous acid (HOBr) and hypochlorous acid (HOCl) are generated by eosinophils and neutrophils. At inflammation sites, KYNA may encounter HOBr and HOCl to generate products. When KYNA was incubated with HOBr under neutral conditions, KYNA generated a product almost exclusively. The product was identified as 3-bromokynurenic acid (3-Br-KYNA). KYNA reacted with HOCl, generating two products. The major product was identified as 3-chlorokynurenic acid (3-Cl-KYNA) with its oxidative decarboxylation product, 3-chloro-4-hydroxy-2(1H)-quinolinone (3-Cl-HQN) as a by-product. When KYNA was incubated with HOCl in the presence of NaBr, the concentration of 3-Br-KYNA increased with decreasing concentration of the chlorinated derivatives in a dose-dependent manner with NaBr. Free amino acids suppressed the reactions of KYNA with HOBr and HOCl. Taurine suppressed the HOCl reaction but not the HOBr reaction.

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3-methyladenine protects melanoma cells against energy stress-induced necrosis by autophagy-independent decrease in oxidative stress and partial involvement of JNK

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We investigated the effect of 3-methyladenine (3MA), a class III phosphatidylinositol 3-kinase (PI3K)-blocking autophagy inhibitor, on the melanoma cell death induced by simultaneous inhibition of glycolysis by 2-deoxyglucose (2DG) and mitochondrial respiration by rotenone. We have elsewhere shown that 2DG/rotenone caused oxidative stress, ATP depletion, swelling of mitochondria, ultimately leading to necrosis. Energy stress is known to induce autophagy, a tightly regulated self-degradation process, which by recycling damaged organelles and macromolecules provides building blocks and energy. However, 2DG/rotenone did not induce proautophagic beclin-1 expression and autophagic flux in melanoma cells despite activation of AMP-activated protein kinase (AMPK) and subsequent inhibition of mammalian target of rapamycin complex 1 (mTORC1). 3MA, but not autophagy inhibition with other PI3K and lysosomal inhibitors, attenuated 2DG/rotenone-induced mitochondrial damage, oxidative stress, ATP depletion, and cell death. 3MA increased both AMPK and mTORC1 activation in energy stressed cells, but neither AMPK nor mTORC1 inhibition reduced its cytoprotective effect. 3MA reduced superoxide generation and c-Jun N-terminal kinase (JNK) activation, and both antioxidant and JNK blockade mimicked its protective activity. Therefore, 3MA prevents energy stress-triggered melanoma cell death through autophagy-independent decrease of oxidative stress and JNK activation. Our results warrant caution in use of 3MA as an autophagy inhibitor.

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Profile of oxidative stress biomarkers in COVID-19: correlation with clinical inflammatory and biochemical parameters

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Introduction: Disturbed redox homeostasis plays multiple roles in COVID-19 pathogenesis. Still, data on the level of oxidative stress by-products in COVID-19 patients are scarce. Aim: To assess the profile of oxidative stress by-products during the course of acute COVID-19 and get more insight into the origin of the systemic oxidative stress in these patients.

Material and methods: Malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG) and advanced oxidation protein products (AOPP), as well as clinical inflammatory and biochemical parameters were determined in plasma of 58 COVID-19 patients, on admission, as well as 7 and 14 days upon admission. Results: The highest levels of MDA and AOPP were observed at the time of diagnosis. Based on correlations between 8-OHdG and ALT activity (p = 0.028) or creatinine concentration (p = 0.003) subclinical liver and kidney damage contribute to systemic oxidative stress in the early phase of disease. Seven days upon admission, a significant drop in MDA and AOPP levels was observed, while plasma concentration of 8-OHdG increased (p < 0.005). At this point, a significant correlation of AOPP with inflammatory biomarkers such as CRP (p = 0.016) and absolute number of neutrophils (p = 0.041) was found. 14 days upon admission, a noticeable increase was observed in AOPP (p = 0.004) and MDA levels (p = 0.038), compared to the second point, but without reaching initial values. Moreover, significant correlation was observed between AOPP (p = 0.046) or MDA (p = 0.029) levels with IL-6, as one of the key inflammatory players. Regarding the biochemical parameters, significant correlation of AOPP levels was found with the activity of plasma non-functional enzymes AST (p = 0.001) and LDH (p = 0.030).

Conclusions: Significant changes in MDA, 8-OHdG and AOPP levels exist during the course of COVID-19. Correlation between by-products of oxidative damage and clinical immunological or biochemical parameters confirms the suggested involvement of neutrophils networks, IL-6 production, as well as liver and kidney damage involvement in systemic oxidative stress.

Keywords: COVID-19; oxidative stress; MDA; AOPP; 8-OHdG.

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PP150

NADPH oxidase may be the key-player in skin response to the dietary factors: fibroblasts-keratinocytes co-culture studies

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A huge data point to an extreme sensitivity of the human skin to environmental conditions (such as the sun light, air contaminants, cigarette smoke) as soon as to the habitual diet. The chronic exposure to some Ambiental and dietary factors can accelerate or slow down the appearance of the aging sings as well as ameliorate or worsen the dermatological pathologies. Although we know that environment and the diet affect the health of our skin, the molecular mechanisms underlying these phenomena are still obscure. Our recent studies conducted on fibroblasts-keratinocytes co-culture model have demonstrated how fibroblasts-derived Reactive Oxygen Species (ROS) can affect the redox state, proliferation rate and the signaling molecules balance in keratinocytes. Moreover, we showed that the NADPH oxidase activity in fibroblasts was significantly sensitive to external treatments with AAPH (water soluble ROS-generator). Here we present a new data indicating that the dietary-derived bio-active molecules, such as resveratrol, ergothioneine and apocynin affect instantly the NADPH oxidase activity in living fibroblasts altering the redox fibroblasts-to-keratinocytes signaling. Our preliminary data indicate that food-derived bio-active molecules may act on our skin modulating the NADPH oxidase activity in fibroblasts and, thus, changing the fibroblasts-keratinocytes redox signaling axis.

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