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**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 antibiotherapy, 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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