INFLUENCE OF THE MICROFLORA ON GASTROINTESTINAL NITRIC OXIDE GENERATION

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Interactions between intestinal bacteria and the host play an important role in physiological regulation of gut function and in development of various diseases. Nitric oxide (NO) exhibits a variety of biological actions in the gut including regulation of regional blood flow, gut motility, water and electrolyte transport and immunity. In patients with inflammatory bowel disease (IBD) the mucosal production of NO from NO synthases is greatly increased but its role in the pathophysiology of IBD is still unclear. Denitrifying bacteria in soil generate NO from nitrate (NO_3^-) and nitrite (NO_2^-) as a part of the nitrogen cycle. In this project we wanted to investigate whether the micro-organisms residing in the gastrointestinal (GI) tract could contribute to NO generation and under which conditions this would occur *in vivo*.

We developed several new methods to directly measure gaseous NO *in vivo* in the colon of newborn infants and in the entire GI tract of conventional and germ-free animals. In addition, in *in vitro* experiments we investigated NO generation and consumption of NO by different gut bacteria.

We started this project by monitoring the initial bacterial colonization in newborn infants with repeated measurements of intracolonic hydrogen gas (H_2), fecal short-chain fatty acids (SCFA) and NO. These markers were virtually undetectable at birth but increased in a particular pattern - bacterial products (H_2 and SCFA) appeared first followed by NO some days later. Interestingly, in some apparently healthy infants colonic NO levels increased to levels similar to those seen in adults with inflammatory bowel disease, indicating a vivid activation of the immune system in response to the emerging bacterial flora.

Next we investigated if bacteria could be an alternative source of gastrointestinal NO in addition to the mucosa. We found that in conventional rats, NO levels were distinctly compartmentalized with very high levels in the stomach, intermediate levels in the cecum and lower levels in the small intestine and colon. In contrast, in germ-free rats, NO was low throughout the gastrointestinal tract. When we fed rats nitrate, gastric NO increased greatly in conventional but not in germ-free animals, thereby confirming nitrate to be a substrate for bacterial NO generation. We went on to demonstrate that lactic acid producing bacteria (Lactobacilli and Bifidobacteria) can generate considerable amounts of NO from nitrite *in vitro*. A combined mixed faecal flora was capable of NO generation not only from nitrite but also from nitrate.

In the final study we demonstrate that intestinal NO generation can be stimulated *in vivo* by dietary supplementation with substrate (nitrate) and lactobacilli. Furthermore, *in vitro* studies show that the generation of NO by some probiotic bacteria can be counteracted by rapid NO consumption by other strains (*E. coli* and *S. aureus*).

We conclude that commensal bacteria can be a significant source of NO in the gut in addition to the NO produced in the mucosa. NO generation by gut bacteria differ profoundly from classical mammalian synthesis via NO synthases as bacteria use nitrate and nitrite as substrates instead of L-arginine. Future studies will clarify the biological role of the bacteria-derived intestinal NO in health and disease and if an imbalance in generation *vs* consumption has any significance in the patho-physiology of intestinal disorders. Direct minimally-invasive measurements of intestinal gases including NO and H_2 may also be useful to study the dynamics of the microbial colonization process and host-microbial interactions early in life.

Keywords: Nitrite, nitrate, IBD, lactobacilli, probiotics, germ-free, SCFA, H_2, *newborn infants*