Medical Hypotheses 103 (2017) 81–85

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Association between oxidative status and the composition of intestinal microbiota along the gastrointestinal tract

Marianna Gyuraszova, Alexandra Kovalcikova, Roman Gardlik *

Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, 811 08 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 6 September 2016
Accepted 19 April 2017

ABSTRACT

Studies have shown that the microbiota along the gastrointestinal tract (GIT) plays an important role when it comes to the maintenance of its proper functions. Many studies exist that have analyzed the composition of the bacterial community in the different regions of the GIT of humans and model animals. Microbial imbalance leads to several systemic disorders, including cardiovascular and renal disease. The imbalance between the production of reactive oxygen species (ROS) and their elimination by antioxidants leads to oxidative stress. Oxidative stress plays an important role in a variety of physiological processes, as well as disease. The continuous formation of ROS in the GIT is the result of the interaction between intestinal mucosa, symbiotic bacteria and dietary factors. It has also been proven that ROS play a role in the pathogenesis of several GI disorders, including IBD. We hypothesized that the levels of advanced glycation end products (AGEs) would be the highest in the ileum, caecum or colon, where the microbiota mostly consist of butyrate producing bacteria, *Bacterioides, Clostridium, Ruminococcus* or *Bifidobacterium*, which derive energy through carbohydrate fermentation. We also assumed that advanced oxidation protein products (AOPP) mostly act in the segments, where bacteria reside and which are responsible for the amino acid fermentation, such as caecum or colon. Lipid hydroxyperoxides are generated during digestion in the stomach, which contains absorbed oxygen and has a low pH. According to this we hypothesized that the highest concentration of thiobarbituric acid reacting substances (TBARS) could be in the stomach, which, however, has not been confirmed. Because *Lactobacilli* are able to produce catalase, an endogenous antioxidant, and are abundant in the small intestine, we hypothesized that antioxidant capacity (measured by ferric reducing ability) would be the highest here. The highest levels of AGEs were found in the caecum. The highest level of TBARS was found in the jejunum of the rats. The assessment of our hypothesis also revealed high levels of AOPP in the caecum. It has been shown that AOPP contributes to the progression of IBD. The ferric reducing ability of tissue was the lowest in the colon of the experimental animals, which is in accordance with previous studies that show that rat colon has a lower total antioxidant capacity than the small bowel. In summary, we offer some insight into the differences between the oxidative status along the GIT of rats and some advice concerning supportive antioxidant therapy of gastrointestinal diseases.

Background

Many studies have shown that intestinal microbiota plays an important role in maintaining proper function of the gastrointestinal tract (GIT). Bacterial microbiota in the GIT plays a role in the motility of the GIT, in the development of the epithelial layer in the intestine, nutrient absorption and in the modulation of immune responses [1,2]. Several studies have analyzed the composition of the bacterial community in the different regions of GIT in humans and various animal species, such as cats or chicken [3–10].

In nutrient rich environment, the composition of gut microbiota is relatively balanced, although interindividual differences are typical [11]. Dysbiosis of the microbial population in the GIT may lead to inflammation and to the development of inflammatory bowel disease (IBD), such as ulcerative colitis, Crohn's disease and irritable bowel syndrome. Intestinal microbiota plays an important role in the development of local and systemic immunity. The microbial content has been shown to have an effect on the expansion of B and T cells in Peyer's patches and mesenteric lymph nodes, especially CD4+ T cells, including FOXp3-expressing T regulatory (T_{reg}) cells [6]. In addition, quantitative and/or qualitative microbial imbalance may also lead to several systemic disorders including obesity, type 1 diabetes and type 2 diabetes, cardiovascular and

* Corresponding author.
E-mail address: romangardlik@gmail.com (R. Gardlik).

http://dx.doi.org/10.1016/j.mehy.2017.04.011
0306-9877/

renal disease [12–14]. It has been shown that intestinal dysbiosis is associated with the worsening of renal failure [15]. Furthermore, the accumulation of uremic toxins is related to microbial status because many of these toxins are derived from microbial metabolism [16].

The hypothesis

Oxidative stress and GIT

Reactive oxygen species (ROS), reactive nitrogen species (RNS) and their byproducts play an important role in the destruction of damaged cells and in maintaining the redox homeostasis. However, imbalance between ROS production and their elimination by antioxidants leads to oxidative stress. Oxidative stress plays a role in a variety of physiological processes as well as disease [17–20]. The continuous formation of ROS in the GIT is the result of the interaction between intestinal mucosa, symbiotic bacteria and dietary factors [21]. The organism is able to recognize pathogens and manage commensal bacteria. ROS are considered to have an antimicrobial effect via a phagocytic pathway. During phagocytosis, professional phagocytes consume oxygen and release superoxide into the extracellular space. ROS are thus able to damage macromolecules such as lipids and proteins [22,23]. ROS play a role in the pathogenesis of several GI disorders, including IBD. We have previously shown that antioxidative gene therapy may be effective for the alleviation of experimentally induced colitis in mice and rats [24,25].

Composition of the intestinal microbiota along the GIT

Dicksved et al. have observed that the most abundant bacterial phyla in the human stomach are Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria [26]. It was found, that Firmicutes, Fusobacteria, Bacteroidetes and the Proteobacteria phyla dominate the duodenum and jejunum of dogs [27].

Hayashi et al. who perfomed a molecular analysis of the human intestinal microbiota, have shown that in the ileal microbiota streptococci, lactobacilli, 'Gammaproteobacteria', the Enterococcus group and the *Bacteroides* group are the most abundant. Caecal microbiota mostly consists of the *Clostridium coccoides* group, the *Clostridium leptum* subgroup, and the *Bacteroides* group [3]. Similarly, in chicken, it has been shown that the major groups of the chicken ileum and caecum microbiota are lactobacilli, *Enterococcus cecorum* and butyrate producing bacteria [28].

Another study, which dealt with human intestinal microbiota, has shown that microbiota in the jejunum differ from the ileum, the ascending colon and the rectum. The genus of *Streptococcus* dominates in the jejunum, whereas *Bacteroides* and *Clostridium* are mostly abundant in clusters in the distal ileum, ascending colon and rectum [29]. Similarly, the study, which mapped the mouse GIT, has demonstrated that the microbiota in the stomach and small intestine are different from those in the large intestine and feces. Lactobacillaceae are dominant in the stomach and small intestine, while anaerobes such as Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae, and Ruminococcaceae are dominant in the large intestine [30].

Oxidative stress in different parts of the GIT

Anaerobic bacterial fermentation of non-digestable carbohydrates leads to the production of metabolites, such as electron sink products lactate, pyruvate, ethanol, succinate, as well as gases, such as CO_2, H_2, CH_4 and H_2S. Moreover, short chain fatty acids (SCFA), such as butyrate, acetate and propionate arise from this microbial fermentation of carbohydrates. Most of the saccharolytic fermentation takes place in the proximal colon [31]. Dietary carbohydrate intake affects transit and pH in the colon. In the proximal colon, an environment with slightly acidic conditions, butyrate producing bacteria dominate, such as Firmicutes, which results in a fourfold higher concentration of butyrate. In contrast, in the distal part of the colon, where the pH is maintained at 6.5, Bacteroidetes dominate, which mainly produce acetate and propionate. The Bacteriodetes are part of a mutual cross-feeding bacterial community. The gasses, which are produced during the fermentation, are consumed by other members of the community [32,33]. In the conditions of carbohydrate depletion, mainly in the distal part of the colon, saccharolytic fermentation is replaced by proteolytic fermentation. Proteins are fermented to SCFA, branched chain fatty acids and nitrogenous compound. The oxidative or reductive deamination of amino acids, a result of amino acid fermentation, cause ammonia formation [34]. Bacterial degradation of cysteine and methionine leads to the formation of toxic H_2S [35,36]. The decarboxylation of amino acids leads to the production of amines. Intestinal bacteria catalyze the reaction of amine with nitrite to produce nitrosamine, which rapidly induces oxidative stress [37,38]. Species, which are able to ferment proteins include *Bacteroides*, *Eubacterium*, *Peptococcus*, *Fusobacterium* and *Clostridium* [34].

The products of fermentation stimulate epithelial signaling via ROS production [39]. On the other hand, it has been shown that short chain fatty acids stimulate glutathion-S-transferase, which reduces oxidative stress [40]. It has been shown that the species of *Lactobacillus* are able to produce catalase, an antioxidant that converts hydrogen peroxide to water, which alleviates colitis and reduces tumors in the colon of mice [41,42]. On the other hand, the Enterobacteriaceae family, such as *Salmonella* or *Escherichia coli*, synthesize catalase to deactivate hydrogen peroxide, which in physiological levels protects against pathogens, to survive in the host [43]. Huycke and Moore have shown that *Enterococcus faecalis*, a commensal bacterium, produces hydroxyl radicals in the intestine [44]. *Bifidobacterium* strains stimulate hydrogen peroxide and nitric oxide production and contribute to the maintenance of the physiological immune status and homeostasis [45]. *Faecalibacterium prausnitzii* is one of the most abundant bacteria in the human intestine and the changes of its abundance have been linked to dysbiosis and to several disorders [46]. Sokol et al. have shown the anti-inflammatory effect of this bacterium in a mice model of colitis [47].

Evaluation of the hypothesis

Advanced glycation end products (AGEs), a marker of carbonyl stress, are substances, which are produced by non-enzymatic reactions of reducing sugars with amino acid, peptides or free amino groups of protein [48]. Due to this circumstances, we hypothesized that the highest concentration of these substances would be in the segment of GIT such as ileum, caecum or colon, where the microbiota mostly consist of bacteria including butyrate producing bacteria, *Bacterioides, Clostridium, Ruminococcus* or *Bifidobacterium*, which derive energy through carbohydrate fermentation. Similary, advanced oxidation protein products (AOPP), a marker of oxidative damage of proteins, are formed during oxidative stress by an interaction between proteins and chlorinated compounds [49]. We hypothesized that AOPP mostly act in the segments, where bacteria reside and which are responsible for the amino acid fermentation, such as caecum or colon.

Malondialdehyde is the end product of lipid peroxidation. The tiobarbituric acid reactive substance (TBARS) assay is one of the most common methods for the measurement of the levels of