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

A R T I C L E   I N F O

Article history:
Received 6 September 2016
Accepted 19 April 2017

A B S T R A C T

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 (Treg) 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...
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

Dickson 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 jejunal regions of dogs [27].

Hayashi et al. who performed 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 coccoide 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. Lactobacilli 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 CO₂, H₂, CH₄ and H₂S. 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 Bacteroidetes 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 H₂S [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, synthetize 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. Similarly, 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 thiobarbituric acid reactive substance (TBARS) assay is one of the most common methods for the measurement of the levels of
malondialdehyde [50]. Kanner et al. have investigated that lipid hydroperoxides are generated during digestion especially in the gaster, which contains absorbed oxygen and has a low pH [51]. Due to these facts, we hypothesized that the highest concentration of TBARS would be in the gaster.

Ferric reducing ability of tissue (FRAT) is a colorimetric method for measuring the total antioxidant capacity based on the ability to reduce Fe$^{3+}$ [52]. Due to the facts that Lactobacilli are able to produce catalase, an endogenous antioxidant, and are more abundant in the small intestine compared to the colon [30,41], we assumed that higher FRAT would be in the small intestine.

To test our hypothesis, individual segments of the GIT were separated. For the evaluation of the hypothesis, homogenates of gaster, jejunum, ileum, caecum and colon were prepared and the parameters of oxidative stress were analyzed. For all measurements, spectrophotometric and fluorescent methods were used.

**Empirical data**

The evaluation of our hypothesis has shown that in AGEs, there was a significant difference between the various parts of the gastrointestinal tract. Compared to the stomach, a significantly higher concentration was found in the ileum and cecum. There was a less accentuated, but significant difference between the stomach and the colon, concentrations being higher in the latter, and between the jejunum and the cecum, and in this case also, concentrations were higher in the latter. TBARS and FRAT assessment has not shown significant differences between the various tested parts of the GIT. However, the highest levels of TBARS and FRAT were found in the jejunum and the lowest in the colon and in the stomach. Evaluation of AOPP has shown that the highest concentration was in the caecum (Fig 1).

**Consequences of the hypothesis and discussion**

ROS and RNS are produced as a result of normal cellular processes and in low amounts they act as important regulatory molecules of various signaling processes. Furthermore, they mediate responses that protect the cell against oxidative stress, rather ironically [53]. Gastrointestinal tract has a key role in ROS and RNS production due to bacterial residing [54]. However, the disruption of normal cellular homeostasis by free radicals has been described in a vast number of gastrointestinal disorders, such as reflux, gastritis, enteritis, IBD, and cancers that are associated with them, pancreatitis, liver cirrhosis and the gastrointestinal complications of diabetes. Oxidative stress can be correlated with inflammation in IBD [53,55,56].

One of the ways to battle oxidative stress is targeted gene therapy in the gastrointestinal tract (GIT). One of the possible ways of gene therapy used in these types of diseases is bactofection, where therapeutic genes are delivered by bacteria that penetrate the cell membrane and release the gene into the cell. Among others, antioxidative gene therapy by bactofection has been described in colitis [25]. This study focused on the distribution of different markers of oxidative stress and antioxidant status in the GIT. It would be of great benefit to have a more detailed understanding of the specific types of oxidative stress in various segments of the GIT. This would possibly allow for more targeted therapy of different gastrointestinal disorders.

Concerning AGEs, the evaluation of our hypothesis confirms our presumption. The highest levels of AGEs were found in the caecum of the experimental animals, i.e. the GIT segment, where the residing bacteria are able to ferment carbohydrates. On the other hand, large part of the fermentation is carried out in the proximal part of the colon, where the evaluation of our hypothesis has shown low concentrations of AGEs. The gut microflora might represent the

---

**Fig. 1.** Graphs showing association between the concentration of oxidative stress markers and the individual parts of the GIT. Advanced glycation end products (AGEs), advanced oxidation protein products (AOPP), thioarbituric acid reactive substance (TBARS), ferric reducing ability of tissue (FRAT).
The key factor that leads to the overproduction of AGEs in the caecum, since some species have been shown to produce AGEs themselves and/or are able to metabolize nutritional AGEs [57]. AGEs are the products of the Maillard reaction, they are toxic and pro-inflammatory in the environment of the gut and they might up-regulate inflammatory pathways in bowel diseases. AGEs have also been theorized to be capable of changing the profile of gut microbiota and to give rise to a more detrimental microenvironment [58]. Therefore, it might be reasonable to concentrate on a specified supportive therapy against AGEs in the caecum in inflammatory bowel diseases.

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 and that they cause intestinal epithelial cell death and intestinal tissue injury [59]. It would be sensible to target AOPP and AOPP-induced cellular mechanisms as a part of a supportive antioxidant therapy of IBD.

The highest level of TBARS was found in the jejunum of the rats. The assessment of the hypothesis has not confirmed our predictions that low pH and absorbed oxygen lead to high amounts of TBARS. On the other hand, it has been shown that lipoperoxidation contributes to the damage that is caused to the small intestine in celiac disease [60]. As a supportive treatment of this disease, it could be effective to target this specific form of oxidative stress in the small intestine.

The ferric reducing ability of a tissue or a body fluid is determined by its antioxidant capacity. Its levels were found to be 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 [61]. Even though this difference was not significant in this case, it would be wise to consider boosting the antioxidant capacity of the colon in diseases of the large intestine, as are ulcerative colitis, diverticulitis or irritable bowel syndrome. Unfortunately, thus far only limited success has been shown in the use of antioxidants for the therapy of IBD.

This is likely due to factors, such as harsh environment in the GIT that affects the performance of the therapeutics, low drug concentrations at the diseased sites and/or the fact that most of the antioxidant therapeutics only target only certain fractions of ROS. In summary, this hypothesis offers some insight into the differences between the oxidative status along the rat GIT and some advice on supportive antioxidant therapy of its diseases.

Source of support

The proposed work was supported by Grant VEGA 1/0204/17 of The Ministry of Education, Science, Research and Sport of the Slovak Republic.

Conflict of interest statement

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References


