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INTESTINAL GASES AND THE MUCOSAL BARRIER FUNCTION

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INTRODUCTION: THE GASTRO-INTESTINAL BARRIER

The gastro-intestinal (GI) barrier is a complex functional unit that separates the environment, i.e. the gastro-intestinal lumen, from the host. The barrier is by far the largest of the body comprising about 200 m^2, compared to the respiratory mucosa (100 m^2) and the skin (2 m^2). This barrier is also the most challenged one, because the respiratory mucosa is either equipped with squamous epithelium that protects much better compared to GI mucosa, or is sterile under normal conditions, e.g. in the lung. The skin mucosa consists of keratinized squamous epithelium that forms a hardly penetrable barrier. In contrast, the intestinal epithelium is the most permeable barrier because one of the major tasks of the GI tract is uptake of nutrients and fluids. Thus, the dilemma of the gut is that it has to fulfil two opposite tasks:

1. Uptake of nutrients and fluids that requires a high permeability, and
2. Protection against microbes, toxins and other harmful agents that requires a tight barrier.

Both task are indispensable for life and can be achieved simultaneously only if the GI barrier function is carefully balanced. Otherwise, either malabsorption (in case of loss of permeability and uptake functions) or inflammatory diseases (in case of loss of the barrier integrity) would be the consequences. Recent studies clearly showed that any impairment of the GI barrier is a crucial step for the development of acute diseases such as systemic inflammatory response syndrome (SIRS) and sepsis in the critically ill, and chronic diseases such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), allergic diseases, joint diseases etc. The mechanisms of selection of the particular type of disease that develops following GI barrier impairment are unclear, but likely dependent on the rapidiness and the extent of GI barrier disturbance.

After birth, there is a phase in which the GI barrier is not developed yet; therefore, newborns are characterized by a kind of physiological immaturity of the GI barrier. This might explain why the first months of life are obviously a particular sensitive time window for protection against diseases or the development of allergy and other immune diseases. It fits also with the known fact that pre-term babies are at particular risk for the development of *Candida* infections and Gram-negative sepsis. It s clear now that the GI tract, which is primary sterile, needs to be adequately colonized in order to develop a normal GI immune system and, finally, a normal GI barrier function. Any disturbance of this colonization will at last delay the development of the mucosal defence system, or even impair it for longer times. This observation made clear that the bacterial flora of the GI tract has a central role

for the GI barrier. Only the controlled interaction between bacterial flora and GI immune cells allows the development and maintenance of an intact GI barrier.

In recent years, great attempts have therefore been made on studying the GI barrier and the GI bacterial flora, and on modulating this functional unit by diet, life-style changes, by pre- and probiotics and by other means. However, the role of gases has not been addressed so far in this respect. This is surprising, considering the fact that many individuals suffering from symptoms related to an impaired GI function such as IBD, IBS etc. report bloating and flatulence. Therefore, the possible interactions between intestinal gases and the GI barrier will be discussed in the present review.

DO INTESTINAL GASES IMPAIR THE GI BARRIER?

Gases could affect the GI barrier by mechanical distension once high amounts of gases are generated that cause the feeling of distension, discomfort or even pain. Gases could also affect the GI barrier by their possible pharmacological effects, since cellular receptors have been identified for particular gases that mediate pharmacological effects.

Mechanical distension could lead to an impairment of the mechanical barrier (epithelial injury) resulting in bacterial translocation, enhanced secretion, leaky gut and malabsorption. In more advanced stages, impairment of the mucosal immune system and the enteric nervous system (ENS) may occur. The latter can lead to abdominal pain, enhanced secretion and motility, diarrhoea, and finally to depression and other psychological effects.

A number of potential targets of action of intestinal gases on the GI barrier are imaginable. They can be classified into three areas:

1. The secretory components of the GI barrier (impaired mucin, IgA, or chloride secretion, enhanced bacterial attachment to the epithelium, reduced production of antimicrobial agents such as bacteriocins or defensins),
2. The mechanical components of the GI barrier (effects on cell-cell contact, tight junctions, APC regions, effects on epithelial growth and apoptosis), and
3. The immunologic components of the GI barrier (modulation of the T cell response, activation of lymphocytes, mast cells or macrophages, support and priming of dendritic cells).

However, data confirming such effects of intestinal gases are lacking until now; therefore, any consequences remain speculative. The question is what do we know at all about intestinal gases? It is surprising to note that many patients that consult a gastroenterologist consider "gases" as their main problem.

KNOWLEDGE ABOUT INTESTINAL GASES IN THE PAST AND AT PRESENT

A particular interesting source of information on gases and bloating is a monograph dated 1831 and published in Germany by an unknown practitioner (*Anonymous*, 1831). He reports the intestinal gases known at this time which, apart from NO, have not changed until now:

- Wasserstoffgas: Hydrogen (H_2)
- Sauerstoffgas: Oxygen (O_2)
- Stickgas: Nitrogen (N_2)
- Kohlenwasserstoffgas: Methane (CH_4)
- Schwefelwasserstoffgas: Sulphur Hydrogen (H_2S)
- Kohlensaures Gas: Carbomonoxyde (CO) and Carbodioxide (CO_2)

Moreover, this practitioner reports on the thought on causes of bloating 175 years ago:

- Gases are mostly a result of bacterial digestion
- Gas production is dependent of what you eat (in particular, raw food is of risk)
- Gas production increases in individuals that lack exercise (increase of age and wealth)
- Gas production increases if the power of the digestive organ decreases (maldigestion, malabsorption)
- Normal versus abnormal is a question of gas composition rather than amount (e.g. N_2 is increased in patients)

Finally, he stated some additional important facts such as "Bloating remains substantially ignored, without proper clinical classification, known pathophysiology, and effective treatment. It is not even clear to what extent the complaints of individual patients correlate with objective evidence of abdominal distension. This uncertainly regarding the subjective or objective origin of the complaints further adds to confusion." How true this is until today!

What can be added since then? Until today, bloating as a medical problem remains substantially ignored, without proper clinical classification, known pathophysiology, and effective treatment. It is still not even clear to what extent the complaints of individual patients correlate with objective evidence of abdominal distension. This uncertainly regarding the subjective or objective origin of the complaints still exists and therefore, confusion could not be reduced.

EXPERIMENTAL DISTENSION OF THE GI TRACT BY GASES

The simple question whether increasing amounts of intestinal gases cause increasing intensities of GI symptoms cannot be answered easily. It is generally believed that patients with functional gut disorders manifest poor tolerance to intestinal gas loads but the mechanism of this dysfunction is unknown. *Harder* et al. (2003) therefore explored the relationship between amount of intestinal gas load versus its distribution on symptom production and gut motility. To do this, the group examined 14 healthy subjects with no GI symptoms, and infused them a gas mixture either into the jejunum or rectum for one hour during blocked rectal gas outflow. They visualized gas infusion by scintigraphic images of 133xenon labelled gas and measured abdominal perception, distension, and gut tone by duodenal and rectal barostats. By doing this, they found that a similar magnitude of gas retention (720 ml) produced significantly more abdominal symptoms with jejunal compared with rectal infusion whereas abdominal distension was similar. Jejunal gas loads were associated with proximal contraction and colonic loads with distal relaxation. According to these data, the volume of gas within the gut determines abdominal distension whereas symptom perception depends on intraluminal gas distribution and possibly also on the gut motor response to gas loads. This might, at least in part, explain the enormous variability

in symptoms the patients with bloating are suffering from.

In large animal studies, morphologic effects of experimental distension of small intestine have been studied (*Allen* et al., 1988). Intraluminal hydrostatic pressures of 0, 9, and 18 cm H_2O were induced in jejunal segments by installing Tyrode's solution for different time intervals (0, 1, 4 hours). Analysis of morphologic changes in the bowel wall was performed by light and electron microscopy (LM, EM). On decompression of the intestinal segments, progressive peristaltic contractions resumed in all segments. Experimental distension of equine small intestine resulted in oedema of the villi and submucosa, separation of the epithelial cells adjacent to the basement membrane in all distended segments, and thus impairment of the intestinal barrier, which may lead to bacterial translocation and inflammation.

Secondly, the intestinal microcirculation and the intramural vascular patterns of the small intestine were evaluated after intraluminal distension (25 cm of H_2O, 120 min) and decompression (60 min) in anesthetized horses (*Dabareiner* et al., 1993, 2001). The readouts were micro-angiography (by injection of a blue-coloured radiopaque medium), micro-corrosion (by injection of methyl-methacrylate for scanning EM), and vascular filling (LM). After intraluminal distension and decompression, the distended segments had short villi, which were separated by expanded crypts, and had mesothelial cell loss, neutrophil infiltration, and oedema in the sero-muscular layer. The number of perfused vessels was significantly decreased in the sero-muscular layer and, to a lesser extent, in the mucosal layer of the distended segments, compared with controls. After decompression, the morphologic lesions progressed in mucosal and serosal layers and the number of observed vessels increased in all intramural layers; however, vascular density did not return to the predistension state. Evaluation of the intestinal blood flow of the equine small intestine after intraluminal distension and decompression revealed a significant decrease in mesenteric blood flow to the distended intestine (from 21.4 to 13.4 ml/min per kg). Blood flow increased significantly during the decompression period (340% of baseline blood flow). An increase in microvascular permeability was documented by the determination of the osmotic reflection coefficient. Oxygen delivery and oxygen content decreased significantly during the distension period and increased during decompression. This process was accompanied by a significant increase in oedema and neutrophil infiltration after distension and decompression.

Third, the role of nitric oxide (NO) was studied in ischaemia-reperfusion experiments in feline small intestine to address the question of whether NO synthesis inhibition affects intestinal barrier function after ischaemia-reperfusion. *Kubes* (1993) showed that ischaemia-reperfusion-induced mucosal and microvascular permeability increases were dramatically augmented by NG-nitro-L-arginine methyl ester (L-NAME) infusion, and this effect was reversed by infusion of L-arginine (125 $nmol.ml^{-1}.min^{-1}$) suggesting that indeed NO plays a significant protective role for microvascular barrier function. Another approach to study effects of intraluminal distension of the small intestine is to prepare extracorporeal circuits from the jejunum of healthy horses. This allows to subject one segment to distension (intraluminal pressure, 25 cm H_2O) followed by decompression, and another segment without distension control segment) *ex vivo*. Using these means, *Nieto* et al.

(2002) showed that intestinal vascular resistance increases during intraluminal distension and returns to baseline values after decompression. Albumin clearance rate increased after distension, compared with baseline and control values, whereas the contractile response induced by cisapride, erythromycin, and metoclopramide decreased following distension.

Several conclusions can be drawn from these equine experiments. Intestinal pressure leads to oedema of the villi and submucosa, expansion of the crypts, disruption of the epithelial barrier and neutrophil infiltration into the seromuscular layer. Intestinal pressure changes vessel functions by a decrease of perfused vessels that persists after decompression, by oedema of the villi and submucosa, and by expansion of the crypts resulting in a decrease in mesenteric blood flow (followed by a compensatory increase during decompression), in oxygen delivery and tissue oxygen content. Intestinal pressure causes an increase in vascular resistance, in microvascular permeability, in albumin clearance rate, while the contractility response to prokinetics is decreased. The clinical impact of such findings cannot be judged definitively, because analogous human experiments are lacking and the experimental approaches in horse studies may be somewhat artificial. However, the likeliness of similar mechanisms in humans must be considered.

MECHANISM OF DISTENSION – HUMAN STUDIES

The number of human studies is limited. Therefore, only preliminary conclusion can be drawn. An intra-abdominal volume load, produced by colonic gas infusion, induces in healthy subjects an increment in tonic activity of the abdominal muscles that can be measured by electromyography (*Tremolaterra* et al., 2006) and this response is probably mediated via viscerosomatic reflexes (*Martinez* et al., 1999). This adaptation is impaired in patients complaining of bloating who fail to contract their abdominal muscles. Hence patients with bloating do have objective abdominal distension but it may not necessarily be due to a true increment in intra-abdominal volume, but to abdominal wall dystony with abdominal redistribution and protrusion of the anterior wall. Under normal conditions, the abdominal wall likely adapts to its content.

Does "pathological gas production" exist? Everybody produces intestinal gases. Gas production is a "physiological" consequence of digestion and bacterial fermentation, and by far not a pathological condition per se. However, if huge amounts of gases are produced, they might cause problems. The amount of intestinal gases depends on diet, composition of the colonic flora, small bowel bacterial overgrowth, small bowel malabsorption (e.g. lactose intolerance), and functional outlet obstruction (impaired anal evacuation of gases, faecal retention prolonging the fermentation process) (*Azpiroz*, 2005). Clinical evidence suggests that healthy subjects propulse and evacuate also large intraluminal gas loads without symptoms whereas IBS patients who do not necessarily produce more gas than healthy subjects might have an impaired evacuation or an impaired perception of distension. Hence, intestinal distension is not a cause of bowel dysfunction but rather a consequence of impaired adaptation.

GASES AND IRRITABLE BOWEL SYNDROME – IS THERE A UNIFYING FRAMEWORK?

There is clinical and experimental evidence that small intestinal bacterial overgrowth (SIBO) may explain bloating occurring in 92% of patients suffering from IBS. For example, it could be shown that IBS patients have an enhanced total hydrogen excretion after lactulose ingestion. The prevalence of abnormal lactulose breath test is 84% in IBS patients. There is a correlation between the pattern of bowel movement and the type of excreted gas. Most importantly, eradication of SIBO results in a 75% improvement of IBS symptoms (*Lin* et al., 2004).

Results from scintigraphic studies using gas labelled with radioactive xenon confirmed that indeed the small bowel is responsible for impaired gas transit (*Salvioli* et al. 2005; *Azpiroz*, 2005). The ileo-caecal region is an area with sphincteric function likely implicated in this dysfunction. Gas retention is due to impaired propulsion in more proximal parts of the small bowel. Interestingly, impaired gas clearance in IBS patients is related to abnormal gut reflexes: the prokinetic effect of gut distension is impaired and the inhibitory effect of intestinal lipids is upregulated, further arguing for an impaired adaptation in IBS patients suffering from bloating and pain, possibly triggered by bacterial overgrowth of the small intestine. The cause of such an overgrowth remains obscure. Possibly, immunological impairments might be responsible.

CONCLUSIONS

According to our current knowledge, four major origins/mechanisms of intestinal gases, bloating, and discomfort must be anticipated, which can cause impairment of the GI barrier:

1. Impaired adaptation (intestinal muscle/nerve failure leading to an functional outlet obstruction),
2. Abnormal composition of the colonic flora,
3. Small intestinal bacterial overgrowth
4. Diet (beans etc.) and/or small bowel malabsorption (e.g. lactose intolerance).

A number of possible effects of intestinal gas must be considered. These include bloating and distension that may lead to discomfort and pain (although the correlations are weak), impairment of the mechanical barrier including epithelial injury, bacterial translocation, enhanced secretion, leaky gut syndrome and malabsorption, impairment of the mucosal immune system, and impairment of the ENS leading to pain, diarrhoea, and psychological effects such as depression.

The ultimate question whether intestinal gases truly matter must remain open. Likely, gas is not necessarily the offending element, but rather other intraluminal components that could trigger the abnormal responses and thus be responsible for the abdominal symptoms that patients misinterpret and attribute to intestinal gas.

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