Irritable bowel syndrome : the role of the intestinal microbiota, pathogenesis and therapeutic targets

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Abstract

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder that predominantly affects women and accounts for up to 40% of the gastroenterology unit outpatient visits. The pathophysiology is complex and multifactorial. In the present review we will focus on the role of intestinal dysbiosis in its pathogenesis and treatment.

Post-infectious IBS (PI-IBS) can put light on the mechanisms underlying IBS. Modified commensal gut flora may lead to mucosal inflammation. Several changes such as an increase in mucosal cellularity (enterochromaffin cells, lamina propria T lymphocytes and mast cells), modified pro-inflammatory/anti-inflammatory cytokine balance and disordered neurotransmission have been observed.

The normal microbiota is an essential factor in health. A modification of the flora, such as small intestinal bacterial overgrowth (SIBO) is thought to play a pathogenic role in IBS. Changes in the composition of the luminal and mucosal colonic flora have been linked to IBS. It is not clear however, whether these changes are a cause or a consequence of the syndrome. The comprehension of the interaction between the dysbiotic microbiota and the host will probably lead to the development of focused therapies.

Based on these assumptions, treatments modulating the microbiota have been investigated. On the one hand several probiotics have shown a reduction in IBS symptoms by an immunomodulatory and analgesic effects. On the other hand antibiotic treatment has proven efficacy in treating IBS with or without associated SIBO.

Due to its complex pathophysiology, treating IBS nowadays implies multiple approaches, one of which may be modulation of the intestinal flora. (Acta gastroenterol. belg., **2011**, 74, **375-380**).

Key words : microbiota, probiotics, irritable bowel syndrome.

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder that affects between 4 and 20% of the population in Europe and in North America, predominantly women. It accounts for up to 40% of the gastro - enterology unit outpatients (1). Although the diagnosis is well defined by the Rome criteria the pathogenesis of irritable bowel syndrome is not yet fully understood and is thought to be multifactorial.

Clinical recognition of the typical symptoms is the first step in the management of IBS patients. The Rome III classification categorizes the patients into three subtypes depending on the predominant bowel habit : IBS-D for diarrhoea-predominant ; IBS-C for constipation-predominant ; and IBS-M for a mixed pattern (2).

The pathophysiology is complex and may implicate various pathways. Classically, defective gastrointestinal motor function and/or altered visceral perceptions have been postulated and identified in many cases. More recently, research has focused on intestinal dysbiosis and low grade inflammation of the mucosa. Psychological factors also take part in the pathophysiology. This can be illustrated by the frequency of psychiatric comorbidities and non-psychiatric disorders associated with IBS such as depression, anxiety, fibromyalgia and chronic fatigue syndrome.

Six to 17% of the patients with IBS link the onset of their symptoms to an infectious illness. It suggests that an exposure to a pathogenic organism could lead to a disruption of intestinal function in specific patients. The unravelling of these post-infectious IBS (PI-IBS) cases could perhaps lead to a better understanding of the underlying mechanisms of the different subtypes of IBS.

The evolving knowledge of the physiological, molecular and psychological basis of the disease has led to the development of several therapeutic agents.

In this review we will focus the discussion on the advances made with PI-IBS on the characterization of the pathophysiology, the role of the microbiota and the therapies that affect the gut flora such as probiotics and antibiotics.

Post-Infectious IBS

PI-IBS is defined as the acute onset of IBS symptoms as per the Rome criteria, in a patient that has not previously presented such features, immediately after a gastrointestinal infection with two or more of the following features : vomiting, fever, diarrhoea or positive stool culture with an infectious agent (2,17).

The percentage of individuals who develop PI-IBS after an acute infectious gastro-enteritis ranges from 5 to 32% compared to 0.3 to 10% of controls who develop IBS during the same period (5, 17). The IBS symptoms occur during a 3 to 12 months follow-up period after the infection.

Although most of the patients develop acute diarrhoea while abroad and no positive stool culture is available, a

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wide variety of bacterial and viral pathogens have been implicated in the development of PI-IBS such as Campylobacter jejuni, Shigella, Salmonella and E. Coli (O157:H7). All pathogens are probably associated with the same risk of developing IBS. However, the viral gastroenteritis seems to cause a more transient form of PI-IBS.

The duration of the disease is a risk factor for developing PI-IBS indicating the importance of bacterial toxicity. For example, a study of Wang *et al*. studying Shigella enteritis showed a RR of 4,61 (CI 95%, 2,1-9,9) between diseases lasting more than 14 days compared with those lasting less than 8 days (3).

As in the other forms of IBS, psychosocial factors play an important role in the development of PI-IBS. Psychological factors associated with PI-IBS include hypochondriasis, somatisation, neuroticism, stress, negative perception of illness and depression. In a prospective study of 496 patients with acute Campylobacter infection, the risk of developing IBS (n = 49) was significantly associated with pre-existing levels of anxiety, stress, somatisation and negative illness perception. (The odds ratios were respectively 1,14 (95% CI 1,05 to 1,23) ; 1,10 (CI 95% 1,04 to 1,15) ; 1,17 5 CI 95% 1,02 to 1,35) ; 1,14 (CI 95% 1,03 to 1,27).) (6)

PI-IBS occurs more frequently in female with reported odds ratios between 1,47 and 2,86 (5). However, as showed by Gwee *et al*. when multivariate analyses were performed the effect of gender disappeared after adjustment for other covariates. This may be due to higher incidences of psychological disorders in female (7). In a different Chinese study (3) the risk of developing PI-IBS was equal in men and women after a Shigella infection.

Little is known about the pathophysiological mechanisms of PI-IBS. Changes in the commensal gut flora after an acute infectious gastroenteritis may lead to a break in the homeostasis.

Furthermore changes in intestinal motility and permeability, increased neuroendocrine mediators such as serotonin, expression of inflammatory cytokines and increased numbers in inflammation related cells have been implicated in the pathogenesis of PI-IBS. Is there a link between those mechanisms and a change in the microbiota ?

An acute gastroenteritis is followed by a loss of normal flora and fermentation products. An altered synthesis of short chain fatty acids (SCFA) such as butyrate, propionate and acetate by the colonic flora leads to an increase in luminal pH. This may induce overgrowth of unusual bacteria that are usually inhibited by those SCFA. It is well known that SCFA fuels the colonic mucosa and colonic sodium and water absorption (9). The gut motility is also influenced by SCFA. Acidification by SCFA increases colon motility whereas it reduces the motility of proximal gut. The latter could predispose to small intestinal bacterial overgrowth (SIBO) and therefore favour diarrhoea. Indeed, reflux of SCFA from the colon into the ileum liberates neuropeptides such as peptide YY, neurotensin and glucagon-like peptide-1 that are responsible for the ileal brake.

Rectal biopsies showed increase in mucosal cellularity including rectal enterochromaffin cells, lamina propria T lymphocytes (CD3, CD4 and CD8) and mast cells in patients with PI-IBS compared with healthy controls (5,11,12). The elevation in rectal enterochromaffin cells is also linked to a rise in post-prandial serotonin level, which may in turn influence gastrointestinal motility and sensitivity.

To emphasize the fact that inflammation plays an important role in the pathogenesis of PI-IBS the expression of pro-inflammatory cytokine interleukin 1β was shown to be increased in rectal biopsies of PI-IBS patients (11,13). It has been postulated that patients developing PI-IBS after an acute gastro-enteritis may have a defective down regulation of their normal inflammatory response. Some cytokine polymorphisms have been associated with this phenomenon (17,11,5).

Elevation in gut permeability was observed by Spiller *et al*. (14) as assessed by the lactulose/mannitol ratio at onset and 12 weeks after a Campylobacter jejuni infection. However there was no relation between permeability and lymphocyte numbers in the biopsies. A defect in epithelial barrier might promote intestinal inflammation.

The treatment of infectious gastroenteritis by antibiotics has been shown to be a risk factor to develop PI-IBS. The RR of developing IBS in patients with traveller's diarrhoea was 4,1 (CI 95% 1,1-15,3) (15). However an antibiotic therapy may simply be an indirect marker of the severity of the illness.

It would seem logic to treat PI-IBS according to the pathophysiological mechanisms. However, to date no specific therapy has proven any efficacy. As an example, in attempt to modulate the excessive inflammatory reaction after a gastro-enteritis, Spiller et al evaluated the role of prednisolone in patients with PI-IBS. Twenty-nine PI-IBS patients underwent a placebo-controlled, double-blind trial of 30 mg oral prednisolone per day vs. placebo. There was no significant reduction of the IBS symptoms neither with prednisolone nor with placebo. However there was a significant decrease in lamina propria T-lymphocyte count in the prednisolone group testifying an effect on the local inflammation. This inflammatory reaction may be the trigger for other mechanisms that cause the symptoms such as nerves and mucosal damages (43). Therefore, a conventional symptom-based approach similar to non PI-IBS should be adopted.

The role of the microbiota and its implications in IBS

The normal microbiota is an essential factor in health. This is a complex ecosystem composed by approximately 300 to 500 bacterial species including around three million genes, known as the microbioma. A person carries, on average, 540000 genes corresponding to some 160 species (44).

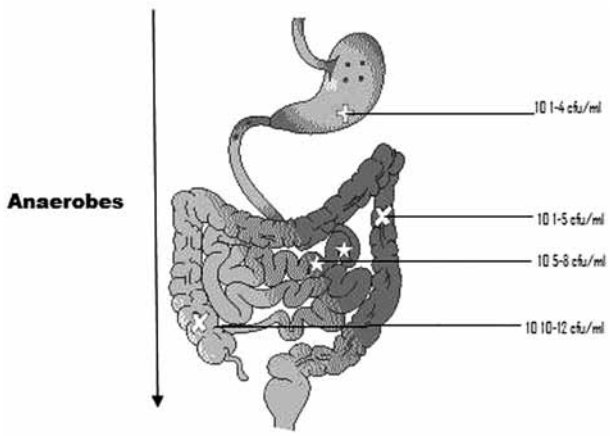

Fig. 1. — Concentration of bacteria in the intestinal tract (colony forming unit per milliliter (cfu/ml)).

The gut microbiota is divided into a luminal ecosystem linked with faeces and food particles and a mucosa-associated ecosystem where the bacteria are bound to the mucus of the intestinal epithelium (18,19).

The microbiota is known to play an important role in maintaining the epithelial barrier integrity and promoting mucosal immunity. A normal bacteria flora prevents colonization by pathogenic agents.

The stomach and proximal small bowel usually contain few bacteria. The concentration of bacteria increases from 10^{1-4} colony forming unit per ml (cfu/ml) in the duodenum to 10^{1-5}cfu/ml in the proximal ileum, 10^{5-8} cfu/ml in the terminal ileum and 10^{10-12} cfu/ml in the caecum. There is a progressive modification of the flora from the upper to the lower gut. The terminal ileum represents a transition zone between the jejunum containing predominantly facultative anaerobes and the colon with a dense population of anaerobes (Fig. 1).

The traditional cultures permit to isolate only 30% of all microorganisms. The other microbes are recognized only by their 16S rDNA sequences. Molecular techniques have permitted an expanded understanding of the microbial ecology of the gastrointestinal tract. The composition of the dominant bacterial species observed in the faecal microbiota of healthy adults seems to be subject-specific and stable through time.

Disturbances in the microbiota are thought to take part in the pathogenesis of several diseases including IBS and IBD, although it is not clear if this could be merely a consequence of these disorders (20).

The small intestinal bacterial overgrowth is usually defined as a total growth of $\geq 10^5$ cfu/ml of intestinal fluid. The clinical diagnosis is based on indirect tests such as hydrogen breath tests using lactulose or glucose and ^{14}C-xylose breath test. Small intestinal bacterial overgrowth (SIBO) has been proposed to be an important factor in the pathogenesis of IBS. Initial studies found overgrowth in 38 to 84% (21-23) of patients with IBS. In contrast, Posserud *et al*. (21) found SIBO only in 4% of patients with IBS which was not different from controls. In this last study, however, the diagnosis was based on jejuna cultures and not on lactulose breath test. Alterations in intestinal motility could be responsible for SIBO. In line with a role of SIBO in IBS, several trials by Pimentel *et al*. showed an improvement in IBS symptoms after antibiotic treatment with neomycin (23) or rifaximin.

Another factor that could contribute to the pathogenesis of IBS is a dysbiosis in the colonic microbiota. A study of Codling *et al*. has shown more variation in the gut microbiota of healthy volunteers than that of IBS patients (24). This abnormal variation is likely to reflect a loss of homeostasis with a lack of diversity and outgrowth of certain phylogenic groups (25).

A study analysing enteric pathogens in faecal samples of 96 patients with IBS (26) showed Staphylococcus aureus in 17% of the samples whereas none of the healthy controls were positive ($p < 0,05$). Clostridium perfringens was found significantly more frequently in IBS patients (17 vs. 13%). These results may suggest a role for a potential intestinal pathogen in the genesis of IBS.

A study of the microbiota in IBS patients compared the 3 main IBS subgroups, IBS-D, IBS-C and IBS-M and in healthy subjects. An overall view of the microbiota of IBS patients permitted to show a lowering in Clostridium coccoides and Bifidobacterium catenulatum populations compared to healthy patients. When comparing IBS-D and IBS-C, lower amounts of Lactobacillus spp. were present in the samples of the first group whereas an increased amount of Veillonella spp. was seen in the second (28).

A recent review on gastrointestinal microbiota (25) showed that most of the studies identified a more pronounced difference between IBS-D patients and healthy subjects than IBS-C. The IBS-D patients have an enriched pattern in Streptococci and a distinctive Clostridia set. The studies using qPCR and microarray to compare microbiota of IBS-patients with healthy controls observe differences mainly in the Firmicutes phylum. Variability in other taxa such as Bacteroidetes, Proteobacteria and Actinobacteria suggests a specific pattern and an association of these with IBS.

All the above mentioned studies focus on luminal microbiota. Very few studies focused on the variability of the mucosa-associated bacteria in IBS patients. Comparing mucosa-associated and luminal ecosystems in IBS-patients and healthy controls as proposed by Kerckhoffs *et al*. showed an increased Pseudomonas population (45,8% of the clones) in the first group. q-PCR analysis revealed higher levels of P. aeruginosa in the small intestine and in the faeces of IBS patients in opposition to healthy subjects suggesting a potential pathogenic role of this bacteria in IBS (29). The study of Codling *et al*. did not show significance differences in the variability of faecal and mucosal microbiota.

The dysbiosis may lead to altered organic acids production and IBS symptoms. Indeed, a gut flora richer in Lactobacillus and Veillonella, as observed in IBS patients, is known to produce acetic and propionic acids that may affect visceral sensation and cause abdominal symptoms and subsequently an impaired quality of life. This is the hypothesis made by Tana *et al*. in their study based on bacterial cultures of the faeces (27).

The comprehension of the interaction between the dysbiotic microbiota and the host needs further researches to elucidate the communication processes between the immune and the nervous system. The Toll Like Receptors (TLR) and inflammatory cytokines seem to be key components of the pathogenesis of IBS. Brint *et al*. reported a 5-fold up-regulation of TLR4 in women with IBS compared to healthy controls, while reporting a reduced expression of TLR7 and TLR8 in the same patients (30,31). Mast cell hyperplasia is a consequence of the immune activation and may be implicated in the pathophysiology by releasing algesic mediators causing visceral hypersensitivity (30,45).

A better understanding of the gut microbiota and the impact of the dysbiosis on patient's homeostasis would open new therapeutic perspectives not only for IBS patients but also in other diseases such as inflammatory bowel diseases, celiac disease, sepsis shock, obesity,…

Probiotics in the treatment of IBS

Probiotics are defined by World Health Organization (WHO) as live microorganisms which when administered in adequate amounts confer a health benefit on the host.

The term is very generic and includes a large number of species of microorganisms in particular lactobacilli and bifidobacteria.

As discussed above changes in the microbiota may play an important role in the pathogenesis of irritable bowel syndrome. Probiotic treatment is therefore an interesting approach for in an attempt to modulate the composition of the gut microbiota (32).

The probiotics are part of the commensal flora but when consumed in important quantities they can have a beneficial effect. The most commonly used bacterial probiotics include Streptococcus species, Lactobacillus species and Escherichia coli. The only probiotic yeast is Saccharomyces boulardii and cerevisiae. The strains are used alone or in mixtures.

Immunomodulation by probiotics seems to be the cornerstone of their mechanism of action. Some probiotics act in the gut lumen by elaborating bacteriocins, toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains. Others increase the production of innate immune molecules like defensins produced by the intestinal Paneth cells or mucins derived from goblet cells to enhance the intestinal barrier (33, 34,37).

Some probiotics have shown an effect in pain modulation in animal models. A specific strain of Lactobacilli induced the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells of rats and mice that could help pain relief in visceral hyperalgesia similar to the effect of morphine (36).

A recent meta-analysis of eighteen randomized controlled trials evaluating 1650 participants was carried out. There was a statistically significant improvement in pain scores, in flatulence and urgency. The effect on bloating was less clear (35). However, there was an important heterogeneity in all the analysed studies in terms of size, the strains and the doses used, precluding any conclusions on the value of probiotics as a group.

The strains that seem to offer the greatest efficacy data seem to be the Bifidobacteria. For example Bifidobacteria infantis 35624 effectively reduces the symptoms of patients with IBS-D (38). Improvement of objective parameters can also be observed with Bifidobacteria therapy, for example, Agrawal *et al*. observed improvement of the abdominal girth in IBS-C patients treated with B lactis DN-173 010 (51).

Fifty four patients were included in a randomized controlled trial comparing Streptococcus faecium treatment with placebo. After four weeks of treatment 81% of the Streptococcus treated group and 41% of the placebo group showed clinical improvement (47).

Lactobacillus strains were also evaluated. Lactobacillus acidophilus-SDC 2012, 2013 a particular strain isolated from Korean infant's faeces was able to reduce abdominal pain and discomfort (48). Another study of O'Mahony *et al*. compared Lactobacillus salivarius UCC4331 with Bifidobacterium infantis. Only the latter was effective on IBS symptoms (49). In parallel, these authors were able to show an improvement in the anti-inflammatory/pro-inflammatory cytokine pattern.

Multispecies probiotics (Lactobacillus rhamnosus GG, L. rhamnosus Lc705, Propionibacterium freudenreichii ssp. shermanii JS and Bifidobacterium animalis ssp. Lactis Bb12) have also shown efficacy in reducing the IBS symptom score. Although most of the studies showed an effect of the probiotic on the gut flora only when taken, the study of Kajander *et al*. reported durable changes of the microbiota composition (50).

Adverse events with probiotics are very rare. The only reported side effects occurred in non IBS-patients such as bacteraemia, fungaemia, endocarditis and the most severe was a fatal bowel ischemia. Those events were described in high risk patients like prematurely born neonates and immunodeficient patients (19,32).

Given their relative low cost and their exceptional safety profile probiotics are considered an interesting therapy for patients with IBS. One has to keep in mind that the results obtained with one strain cannot be extrapolated to another and that probiotics may not beneficial to all patients.

Another possible way to influence the intestinal microbiota is the use of prebiotics. They are defined as fermentable substances that selectively stimulate the growth and activity of specific species of bacteria

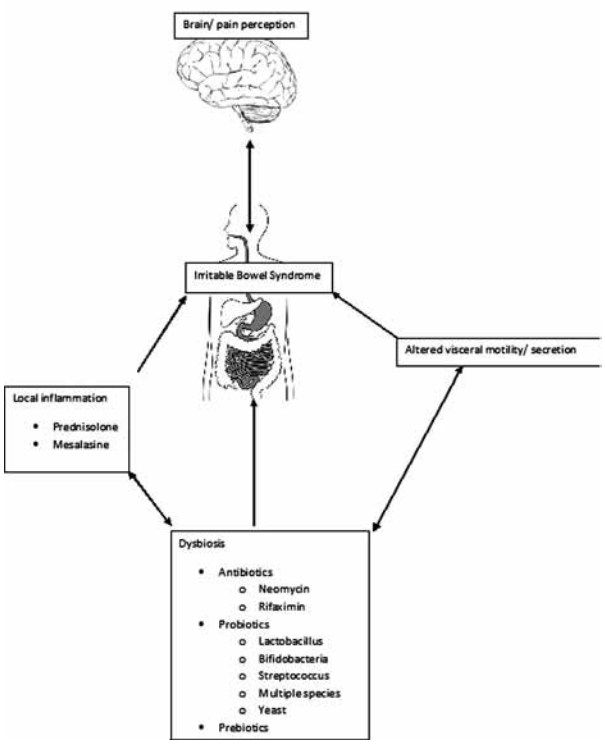

Fig. 2. — Irritable bowel syndrome : Pathophysiology and therapeutic targets.

already settled in the colon. Galacto-oligosaccharide was used in a randomized controlled trial on 44 IBS patients and showed efficacy in reducing the IBS symptoms and selectively stimulated the Bifidobacteria (46).

Antibiotics in the treatment of IBS

The treatment of IBS with antibiotics (39-42) was based on the fact that some patients with IBS might suffer from SIBO. The treatment of SIBO aims at reducing the bacterial colonization of the small intestine using antibiotics. In a study by Pimentel *et al*. treating 111 IBS patients with neomycin resulted in a response (≥ 50% improvement of composite symptom score) rate of 40% compared to 23% with placebo (39).

Rifaximin, an oral, nonsystemic, broad-spectrum antibiotic that targets the gut, has shown efficacy in treating SIBO (40).

A better understanding of the role of gut flora in the pathogenesis of the disease led to consider antibiotics in the treatment of IBS patients even in the absence of SIBO.

Recently, in two identically designed, phase 3, double-blind placebo-controlled trials (TARGET 1 and TARGET 2), 1260 patients with IBS without constipation were randomly assigned to either rifaximin at a dose of 550 mg or placebo three times daily for 2 weeks. Patients were then regularly evaluated during a 10-week post-treatment follow-up period.

The subjects treated with rifaximin had a better relief of global IBS symptoms (40.7% vs. 31.7%, $P < 0.001$), in the two studies combined. Interestingly, the improvement of the global symptoms persisted after the end of the treatment and during the total 3 months study period (41). This sustained effect can be explained by a modification in the microbiota, which in turn may modify its interaction with the host and the underlying mechanisms of IBS symptoms leading to clinical improvement. It is not known yet, which patients are specifically suited for antibiotic treatment.

As for SIBO a relapse can be observed. While an additional antibiotic treatment would probably be successful to treat such recurrence, this has not been demonstrated so far.

Conclusion

IBS pathogenesis is multifactorial. It is unlikely that a treatment focusing on a single abnormality will suit all patients. A better understanding of the underlying mechanisms and an accurate characterization of each individual patient would probably be helpful (Fig. 2).

The study of the microbiota and its interaction with the human host via the mucosal immune system is one of the clues for the unravelling and treatment of several diseases. In the case of IBS, additional pathophysiological mechanisms such as psycho-social factors, defective neurotransmitters, visceral hypersensitivity and low-grade inflammation have also to be considered. All these aspects interact in different proportions in every patient in the genesis of the IBS symptoms.

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