GUT MICROBIOTA: MODULATE ITS COMPLEXITY TO RESTORE THE BALANCE

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MEETING SUMMARY

The importance of the gut microbiota to health is becoming more widely appreciated. The range of commensal microorganisms in healthy individuals and in patients with a variety of digestive diseases is under active investigation, and evidence is accumulating to suggest that both the diversity and balance of bacterial species are important for health. Disturbance of the balance of microorganisms – dysbiosis – is associated with obesity and a variety of diseases. Restoring the balance by modulating the microbiota through diet, probiotics, or drugs is now being developed as a potential treatment for digestive diseases. Rifaximin has been shown to increase levels of beneficial bacterial species without perturbing the overall composition of the microbiota in patients with a variety of digestive diseases, making it a 'eubiotic' rather than an antibiotic. Rifaximin has demonstrated clinical benefit in the treatment of symptomatic uncomplicated diverticular disease, where changes in the colonic microbiota contribute to the pathogenesis of this disease. Modulating the microbiota is also a promising treatment for some types of irritable bowel syndrome (IBS) that have been linked to an overgrowth of coliform and *Aeromonas* species in the small intestine. Rifaximin has demonstrated efficacy in relieving symptoms and reducing relapses in diarrhoeal IBS in the TARGET-1, 2, and 3 trials, without reducing microbial diversity or increasing antimicrobial resistance. While many aspects of the balance of gut microbiota in disease are not yet fully understood, the new understanding of rifaximin as a modulator of gut microbiota may open up new treatment options in digestive disease.

Introduction

Professor Fermín Mearin

Although the hypothesis that human beings can live in symbiosis with some bacteria dates back over a century, the idea that the gut microbiota might play a beneficial role in the health of the host has only recently arisen. The human gut is home to trillions of commensal bacteria, some of which may be beneficial and some of which may be harmful.

It is becoming apparent that both a diversity of bacterial types and a balance of different bacterial species are necessary for health. Modulating the complexity of the gut microbiota to restore the balance of bacterial species is a promising approach for treating gut diseases.

Composition and Function of the Fourth Organ of the Gastrointestinal Tract

Professor Antonio Gasbarrini

The gut microbiota, comprising a 95% gene identity of 9 phyla, over 1,000 species, and more than 15,000 strains, can be considered a metabolic organ – the gut metabolome rather than the gut microbiome. The gut microbiota consists mainly of bacteria. Firmicutes and Bacteroidetes constitute the majority of present phyla,[1] alongside characteristic viruses and yeasts,[2,3] and sometimes protozoa (e.g. helminths and other parasites). Many of these commensal organisms are either beneficial or harmless, while others can be harmful.[3,4] The variety of microorganisms within a person's gut, known as their enterotype, is unique to the individual, and is determined by many life events from birth onwards.[5,6] Factors that influence enterotype include: whether one is bottle or breast-fed and the types of solid food consumed as an infant, antibiotic treatments, malnutrition as a toddler, obesity as an adult, and old age.[6] The commensal gut microbiota contributes to gastrointestinal (GI) homeostasis in several ways. For example, the gut microbiota contributes to the barrier function of the intestinal lining,[7] although the mucus of the intestinal lining is the main constituent.[8,9] More importantly, the gut microbiota plays a role in the education of the innate and acquired immune systems.[10] In addition, the metabolic effects of the gut microbiota are considerable – without it, it would not be possible to metabolise the complex polysaccharides of dietary fibre.[11,12] The precise balance of the gut microbiota can influence persisting metabolic traits, and evidence from animal models suggests that the overgrowth of certain strains of gut bacteria may have a causal role in obesity.[13-15]

Maintaining the balance of gut bacteria species (eubiosis) is important for health. For instance, *Clostridium difficile*, especially toxin-producing species, remain in spore form in the gut due to the actions of *Clostridium scindens* on bile acids.

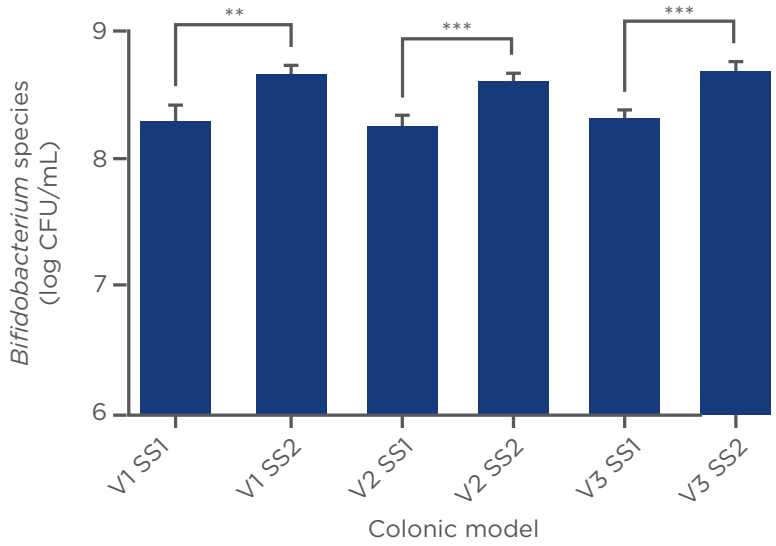

Figure 1: Rifaximin treatment increases the abundance of *Bifidobacterium* species in faecal microbiota of Crohn's disease patients.

Bifidobacteria detected by fluorescent *in situ* hybridisation in culture broths recovered from three different culture vessels (V1, V2, and V3) in an *in vitro* colonic model system before (SS1) and after (SS2) rifaximin treatment. Results are reported as the means of data of four colonic models ± standard error of mean. For each colonic model, measurements were performed in triplicate.

**p<0.01; ***p<0.001.

CFU: colony forming unit.

Beta-lactam antibiotics kill *C. scindens*, which may allow *C. difficile* to become vegetative and potentially toxic. Dysbiosis – the failure of the host–microbiota balance – and breakdown of the gut barrier are implicated in a variety of digestive diseases, such as irritable bowel syndrome (IBS) or diverticular disease. The modulation of the gut microbiota is therefore a promising target for treating these diseases. The balance of gut microbiota can be modulated by diet, by correcting predisposing conditions, or with antibiotics. Whereas systemic antibiotics, such as vancomycin, may kill beneficial commensal bacterial species as well as pathogenic species,[16] gut-specific topical, non-absorbable antibiotics, such as rifaximin, can have a beneficial effect on the overall balance of the gut microbiota.[17,18]

Rifaximin does not have a traditional antibiotic effect, but acts through inhibition of bacterial adherence to the gut mucosa.[17] Animal models suggested potential eubiotic effects of rifaximin favourably affecting the balance of gut bacteria, primarily by increasing *Bifidobacterium* (Figure 1), *Faecalibacterium prausnitzii*, and lactobacilli, without perturbing the overall composition of the microbiota.[19-22] Despite these promising experimental findings, it was not known if the eubiotic effects of rifaximin would translate into humans. A recent observational prospective study sought to answer this question in patients with a variety of digestive diseases, including ulcerative colitis, Crohn's disease, IBS, diverticular disease, and liver cirrhosis with hepatic encephalopathy. Patients were treated with 1,200 mg of rifaximin per day over a 10-day period. Levels of gut microbiota were measured at baseline, after the 10-day treatment period, and 1 month later. Principal coordinate analysis demonstrated that rifaximin did not change the overall composition of the gut microbiota. However, differential abundance analysis revealed a significant increase in lactobacilli at the end of treatment, which persisted 1 month after treatment ($p<0.0001$).[23]

It is thought that the increase in lactobacilli may mediate the anti-inflammatory effects of rifaximin.[24,25] Rifaximin may thus be viewed as a 'eubiotic' rather than an 'antibiotic', and is an important contribution to the armamentarium for modulating the microbiota to treat digestive diseases.

Microbiota Modulation in Diverticular Disease

Professor Peter Malfertheiner

Diverticulae in the colon are highly prevalent and age-dependent.[26] They are an important cause of morbidity and a significant health economic burden.[27,28] Diverticulae occur when the mucosal and submucosal gut lining extrovert through the muscular intestinal wall, generally at sites where the vascular system penetrates. The formation of faecaliths in the diverticulae can lead to inflammation and diverticulitis with potential complications, such as perforation, bleeding, stenosis, and fistula.[26] The majority (80%) of patients with diverticulae are asymptomatic and the remaining 20% of symptomatic patients have chronic relapsing symptoms, recently defined as symptomatic uncomplicated diverticular disease (SUDD), or may develop diverticulitis with or without complications such as bleeding (Figure 2).

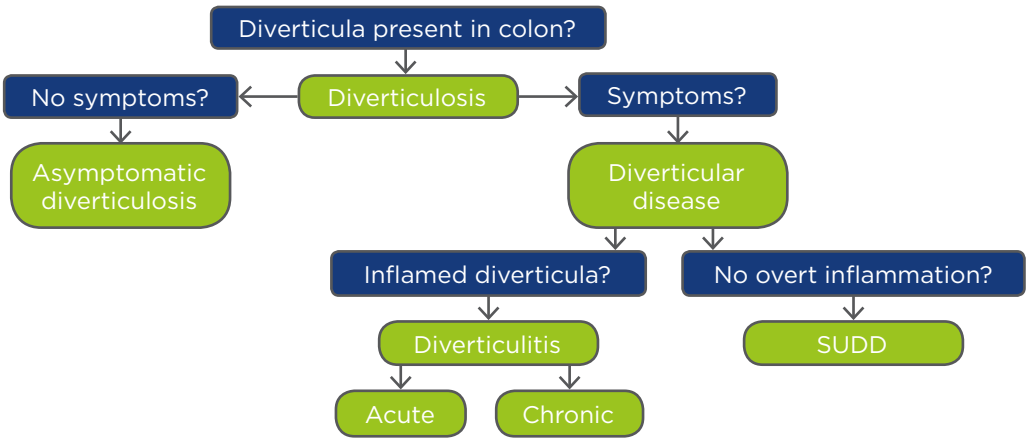

Figure 2: Proposed taxonomy of diverticular-related terms – basis for therapeutic decisions.[26]
SUDD: symptomatic uncomplicated diverticular disease.

Patients with SUDD are at an increased risk of developing acute diverticulitis.

Dysbiosis of gut bacteria has been linked to SUDD and diverticulitis.[29] This appreciation of the role of gut microbiota in diverticular disease has influenced treatment, following demonstrations that systemic antibiotics are not necessary to treat acute non-complicated diverticulitis.[30-32] SUDD is thought to develop from the weakening of the colonic wall and degenerative changes in the enteric nerves, combined with changes in the colonic microbiota and an inflammatory response. There are similarities between the symptoms of SUDD and IBS, and it has been proposed that left lower quadrant pain for >24 hours combined with increased faecal calprotectin should characterise SUDD.[33]

Treatment of SUDD aims to decrease symptom intensity and prevent the recurrence of acute diverticulitis by targeting the pathophysiological mechanisms of the disease.[26] As well as affecting the gut microbiota, fibre acts to normalise colon motility, and has a level 1 recommendation from the National Diverticula Study Group.[34] Although a clinical benefit has been demonstrated for 5-aminosalicylic acid drugs in the treatment of low-grade inflammation,[35,36] the PREVENT 1 and 2 trials failed to demonstrate a benefit for mesalazine over placebo in preventing relapses.[37] The gut microbiota is therefore a promising target to treat SUDD. Rifaximin has been proven to modulate the gut microbiota to beneficial effect in SUDD in several randomised trials.[38] In these trials, disease symptoms were improved and patients experienced a reduction in relapses of acute diverticulitis, with few adverse events.[39] Long-term cyclic administration of rifaximin is effective in reducing the symptoms, complications, severity, and frequency of diverticular disease. Most patients can readily benefit from symptom relief, and the number needed to treat for one patient to benefit from complete symptom relief was three, according to a recent meta-analysis.[38] However, more data are needed to better address the prevention of relapsing symptoms, as well as acute diverticulitis.

New Evidence in IBS: The Role of Gut Microbiota

Professor Mark Pimentel

Recent evidence indicates that the gut microbiota is important in IBS pathophysiology. At the same time, evidence is poor for psychological causes, such as stress. Notably, a recent study on the role of stress in the development of IBS identified only exposure to acute gastroenteritis as being associated with IBS.[40] Thus, a new hypothesis has emerged for the pathophysiology of IBS: IBS is a disease triggered, at least in part, by a change in the gut microbiota caused by gastroenteritis. The role of the gut microbiota suggests that IBS could therefore be an antibiotic-sensitive disease.

In a variety of trials, characterisation of the microbiota in patients with IBS has demonstrated changes in the gut microbiota, most notably an associated overgrowth of coliforms and *Aeromonas* species (Figure 3).[41-43]

The aforementioned hypothesis was the basis for the TARGET trials of the antibiotic rifaximin as a treatment for IBS. In TARGET 1 and 2, rifaximin not only demonstrated efficacy in symptom relief over placebo 4 weeks after treatment, but also showed a durable response 3 months after treatment.[44] It can therefore be suggested that rifaximin treats the cause of the disease rather than the symptoms alone. This contrasts with treatments such as the anti-diarrhoeal alosetron, where the benefits cease as soon as the treatment ends.[45] The TARGET 3 trial included a more stringent design to investigate the safety and efficacy of repeated use of rifaximin in patients who have had multiple relapses, and the durability of its effect. The trial had an initial open-label phase to screen out placebo responders, and a composite endpoint of simultaneous improvement of abdominal pain and stool consistency in 2 out of 4 weeks, with 18 weeks' follow-up for relapses. In the initial open-label phase, 72% of patients had an improvement in at least one component of the composite endpoint. Of the patients who met the composite outcome in the open-label phase, more than 1 out of 3 (35%) did not have a relapse of symptoms in the following 6 months. Those who did relapse were entered in the main double-blind phase of the trial.[46] Significantly more patients treated with rifaximin were responders in the first repeat treatment phase compared with the placebo group, which was a consistent treatment effect. Notably, patients did not return to baseline symptom severity after treatment with rifaximin.[46] TARGET 3 was the largest deep sequencing trial to date, and showed that rifaximin did not alter 98% of the organisms in the gut or the stool. Furthermore, microbial resistance did not change in the remaining organisms.[46]

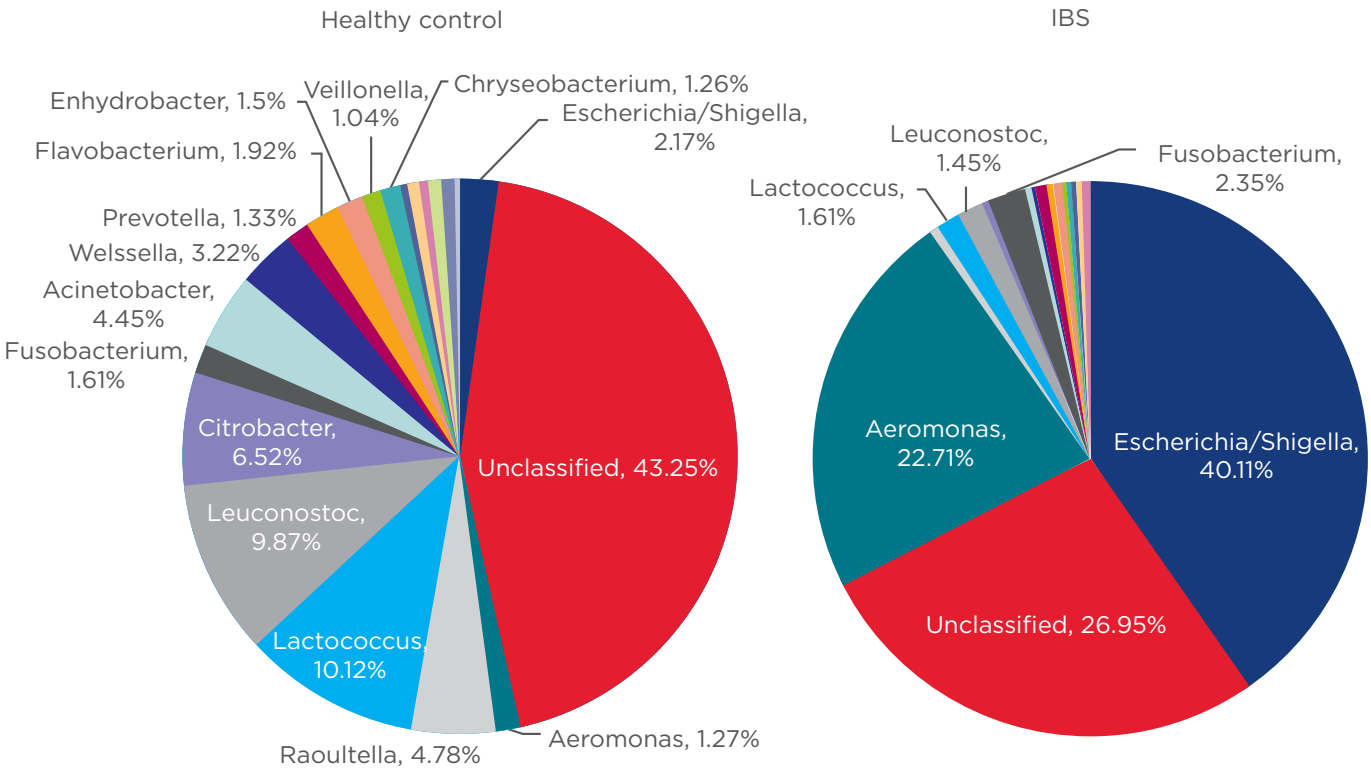

Figure 3: Microbial distribution on 16S amplicon sequencing in IBS patients and healthy controls demonstrates reduced gut microbiome diversity in IBS patients.[41]

The charts show the average relative abundance of bacterial genera identified in five irritable bowel syndrome (IBS) patients and five controls; genera with a relative abundance >1% are labelled by name.

The efficacy of rifaximin in IBS may be linked to the hypothesis that perturbations of gut microbiota contributes to the development of IBS. Studies have shown that acute gastroenteritis increases the risk of developing IBS.[47,48] An animal model of post-infectious IBS following *Campylobacter* infection tested the hypothesis that cytolethal distending toxin B (CdtB) was the toxin involved in development of IBS following gastroenteritis, and showed that *Campylobacter* strains lacking CdtB did not induce IBS.[49] Antibodies to CdtB were found to cross-react with vinculin. Blood tests for antibodies to CdtB and vinculin in patients from the TARGET 3 trial and in patients with other gut disorders were able to identify IBS patients;[50] this supports the hypothesised pathological sequence.

At least one subset of IBS has shown to be an organic disease because small intestine bacterial changes are seen in at least 60% of IBS patients, and diarrhoeal IBS can be effectively treated with the microbiome-balancing therapy rifaximin. Gastroenteritis is involved in the development of IBS, most likely through autoimmunity to vinculin triggered by immune responses to CdtB, and serum anti-CdtB and anti-vinculin can distinguish IBS from inflammatory bowel disease.

Conclusion

Many aspects of the balance of gut microbiota in disease are yet to be clarified, and improvements in the understanding of how rifaximin acts on the balance of microbiota in GI diseases are anticipated. The new definition of rifaximin as a modulator of gut microbiota may open up potential new indications for this therapy.

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