Novel Therapies for IBD

Digestive Diseases

Dig Dis 2015;33(suppl 1):105–112
DOI: 10.1159/000437104

Targeting the Microbiome in Inflammatory Bowel Disease: Critical Evaluation of Current Concepts and Moving to New Horizons

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Key Words
Inflammatory bowel disease · Microbiota · Antibiotics · Probiotics · Fecal transplantation · Prebiotics

Abstract
Microorganisms present in the intestine possess proinflammatory or anti-inflammatory activities which may modulate inflammatory bowel disease (IBD). The concepts followed by researchers in trying to target the microbiota in IBD were to decrease pathogens or pathobionts, or only the microbial load, and more recently, to favor growth and persistence of favorable microorganisms. We review, here, those concepts and critically analyze the clinical data (especially randomized controlled trials) obtained using antibiotics and probiotics. We eventually present and criticize the rational and data obtained so far following new research strategies including the use of new probiotics, genetically modified organisms and fecal transplantation.

Introduction: Why Targeting the Gut Microbiota in IBD?

Genomic analysis have shown that inflammatory bowel disease (IBD) patients exhibit polymorphisms in human genes involved in response to microorganisms [1–3] and an abnormal composition of their fecal and mucosa-associated microbiota (called 'dysbiosis') [4–7]. The ecosystem is thus disturbed on its 2 different and connected sides: the human cells and the microbiota. The concept that controlling the microbiota would improve disease appeared very early but currently has only limited applications. We will review, here, the current knowledge on efficacy of antibiotics and probiotics and discuss the new concepts and approaches.

Critical Evaluation of Concepts and Current Data

The first idea was to use antibiotics to decrease the load of endogenous (anaerobic) microorganisms. Similarities between Crohn's disease (CD) and tuberculosis (especially granuloma formation) pushed to try anti-tuberculous combinations. Later on, antibiotics targeting *Escherichia coli* within macrophages (macrolides) were (and are still) evaluated. The concept supporting the use of probiotics was to influence the ecosystem by a safer method and, later on, to use bacteria or yeast with anti-inflammatory properties (often studied in animal models before being tested in humans). The first trials used lactic bacteria, especially lactobacilli and bifidobacteria. The rational for this choice was that (1) they were found to be often lower in patients than in healthy controls by using culture methods, (2) they had already been used in food products for

0257–2753/15/0335–0105$39.50/0

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years and were generally recognized as safe (GRAS status) and (3) food companies helped the research in this field. The rational of trying *Saccharomyces boulardii* was that it was also recognized as safe by many clinical trials and that of trying *E. coli* Nissle 1917 was based not only on several effects of this strain on human defenses but also on its theoretical ability to occupy the niche of *E. coli* pathobionts (competitive exclusion) [8].

Many studies have thus tested the hypothesis that antibiotics or probiotics might influence some of the various aspects of IBD, especially in improving or preventing symptoms or endoscopic lesions. Randomized controlled trials (RCTs) have been reviewed recently [9, 10], and we summarize here the consensus expressed in guidelines on their clinical applications (ECCO) [11, 12]. Positive trials and those including more than 80 subjects are few and are presented in table 1.

Antibiotics

Crohn's Disease

The most studied antibiotics have been metronidazole, ciprofloxacin, rifaximin and anti-mycobacterial regimens [13–21]. Three RCTs, respectively, using ciprofloxacin [16], an anti-mycobacterial regimen [13] (clarithromycin + rifabutin + clofazimine) and rifaximin [18] have shown a significantly higher remission rate in the patients treated with antibiotics than in the control group (table 1). Prantera et al. [18] performed a multicenter, randomized, double-blind trial of the efficacy and safety of 400, 800 and 1,200 mg rifaximin, given twice daily to 402 patients with moderately active CD for 12 weeks. At the end of the 12-week treatment period, 62% of patients who received the 800 mg dosage of rifaximin were in remission, compared with 43% of patients who received placebo ($p = 0.005$). Remission was achieved by 54 and 47% of the patients given the 400 mg and 1,200 mg dosages of rifaximin, respectively (difference NS vs. placebo). A meta-analysis concluded for a statistically significant effect of antibiotics to induce remission in active CD compared with placebo (relative risk (RR) of active CD not in remission 0.85; 95% CI 0.73–0.99, $p = 0.03$) [22]. The experts involved in guidelines considered heterogeneity of the drugs used and effectiveness of only an intermediate dose of rifaximin, and as listed in table 2, they gave a very limited place to those agents in active CD [11].

The few studies that tested antibiotics to maintain remission of CD have been negative. The meta-analysis of those considered to have the highest quality (table 1) [13, 23, 24] suggested a beneficial effect of antibiotics [22] but again one must consider the heterogeneity of the drugs used, and the guidelines do not propose an antibiotic therapy in this situation (table 2) [11].

Four RCTs investigated the effect of antibiotics (3 nitroimidazole and 1 ciprofloxacin) in the prevention of the postoperative recurrence of CD. Nitroimidazoles were shown to be effective in the prevention of relapse (PR) in the first 2 years after surgery but side effects prevented their use for more than 3 months, and this probably explains their absence of efficacy on longer term [20, 21]. The RCT [25] testing of ciprofloxacin (500 mg; twice daily) versus placebo for 6 months did not show prevention of CD recurrence but a higher rate of drug-associated side effects ($p = 0.043$). In a pilot study, 50 patients with CD undergoing intestinal resection with ileocolic anastomosis were treated with azathioprine for 1 year and randomized to receive either metronidazole or placebo also for the first 3 months. Overall, the addition of metronidazole to azathioprine did not significantly reduce the risk of endoscopic recurrence beyond azathioprine alone and did not worsen its safety profile [26].

Ulcerative Colitis

Several RCTs [27–30] tested antibiotics in the treatment of active ulcerative colitis (UC) but only few had positive results and/or a sufficient statistical power (table 1) [31]. Ciprofloxacin and metronidazole have been the most studied. A meta-analysis in 2011 concluded that patients treated with antibiotics reached a remission state more frequently than those in the control group (RR of active UC not in remission 0.64; 95% CI 0.43–0.96, $p = 0.03$) [22]. As for CD, the heterogeneity of antibiotics used led the ECCO group to consider evidence too low for any recommendation (table 2) [12]. Two RCTs from Japan showed that an association of amoxicillin, tetracycline and metronidazole for 2 weeks [27, 28] was associated with a higher clinical response and remission rate as well as a higher mucosal healing rate at 3 and 12 months than a placebo administration. The evidence for efficacy of this association is thus good for Japanese patients, and one should now study other populations. Access to oral tetracycline may be limited in some countries.

Metronidazole and/or ciprofloxacin are recommended to treat acute pouchitis (table 2) [32]. This is based on few RCTs performed on lesser number of subjects, but the results were conclusive and confirmed in the daily practice. Other studies suggested the efficacy of antibiotics in relapsing pouchitis but the low number of subjects studied limits the evidence [33].

Table 1. Results of RCTs testing antibiotics or probiotics in CD or UC with either n >80 or a positive result

Situation	Product	Control	n	Duration[a], weeks	Effect		p	Ref.
					Product, %	control, %		
CD	Clarithromycin + rifabutin + clofazimine	Placebo	213	16 (104)/156	Remission at week 16: 66 Remission at week 104: 30.4 Remission at week 156: 13.7	50 21.6 9	* NS NS	[13]
CD	Ciprofloxacin + metronidazole	Placebo	130	8/8	Remission at week 8: 33	38	NS	[14]
CD	Metronidazole	Placebo	99	16/16	Remission at week 16: 31.7	25	NS	[15]
CD	Ciprofloxacin	Placebo	47	24/24	Remission at week 24: 76	25	*	[16]
CD	Rifaximin	Placebo	83	12/12	Remission at week 12: 42.3	33	NS	[17]
CD	Rifaximin	Placebo	402	12/24	Remission (800 mg/day): 62	43	*	[18]
CD–PR	*Saccharomyces boulardii*	Placebo	165	52/52	Relapse at week 52: 47.5	53.2	NS	[35]
CD–PR	Ethambutol + rifampicine + isoniazide	Placebo	126	104/104	Corticosteroid requirement: 85.7	85.7	NS	[19]
CD–PPOR	Metronidazole	Placebo	60	12/156	Relapse at week 52: 7 Relapse at week 156: 31	25 50	*	[20]
CD–PPOR	Ornidazole	Placebo	80	54/156	Relapse at week 54: 7.9 Relapse at week 156: 45.9	37.5 47.5	* NS	[21]
CD–PPOR	*Lactobacillus johnsonii* LA1	Placebo	98	24/24	Relapse at week 24: 49	64	NS	[41]
UC	Amoxicillin + tetracycline + metronidazole	Placebo	20	2/52–60	Remission: 90	50	*	[27]
UC	Amoxicillin + tetracycline + metronidazole	Placebo	210	2/52	Response at week 52: 49.5 Remission at week 52: 27.6	21.8 14.9	* *	[28]
UC	Ciprofloxacin	Placebo	83	24/52	Response at week 24: 79 Response at week 52: 55	56 40	* NS	[29]
UC	Tobramycin	Placebo	84	1/3	Remission at week 3: 74	43	*	[30]
UC	VSL#3	Placebo	144	8/8	Response at week 8: 63.1	40.8	*	[43]
UC	VSL#3	Placebo	147	12/12	Remission at week 12: 42.9	15.7	*	[44]
UC	VSL#3	Placebo	29	52/52	Remission at week 4: 92.8 Relapse at week 52: 21.4	36.4 73.3	* *	[45]
UC–PR	*E. coli* Nissle 1917	5-ASA	120	12/12	Relapse 16	11.3	NS	[46]
UC–PR	*E. coli* Nissle 1917	5-ASA	120	52/52	Relapse 67	73	NS	[47]
UC–PR	*E. coli* Nissle 1917	5-ASA	327	52/52	Relapse 36.4	33.9	NS	[48]
CD fistulas	Infliximab + ciprofloxacin	Infliximab	24	4/18	Fistula response at week 18: 73	39	NS	[68]
CD fistulas	Adalimumab + ciprofloxacin	Adalimumab	76	12/24	Fistula response at week 18: 71 Fistula response at week 18: 62	47 47	* NS	[69]

[a] Product administration/total study duration. * Statistically equivalent, $p < 0.05$.

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Table 2. Place of antibiotics and probiotics in the treatment of UC and CD in the last ECCO guidelines

CD (ECCO guidelines 2010) [11]
- Antibiotics cannot be recommended in mildly active localized ileocecal CD (EL1b, RG A).
- However, they can be added if septic complications are suspected (temperature or focal tenderness or imaging indication of an abscess) (EL5, RG D).
- Adding ciprofloxacin and metronidazole to budesonide has shown no advantage over budesonide alone in active CD.
- At present, antibiotics are only considered appropriate for septic complications, symptoms attributable to bacterial overgrowth or perineal disease.
- Anti-mycobacterial therapy cannot be recommended on the evidence from controlled trials.
- *E. coli* Nissle is an effective alternative to 5-ASA for maintenance (EL1b, RG A).

UC (ECCO guidelines 2012) [12]
- Antibiotic therapy may induce remission in active UC, but the diverse number of antibiotics tested means the data are difficult to interpret.
- Data are regarded as insufficient by the consensus to recommend antibiotics for maintenance of remission in UC.
- There is insufficient evidence for the use of *T. suis* ova, *Saccharomyces boulardii* or bifidobacteria in the treatment of UC (EL5, RG D).
- *E. coli* Nissle is an effective alternative to 5-ASA for maintenance (EL1b, RG A).

Pouchitis (ECCO guidelines 2008) [32]
- The majority of patients respond to metronidazole or ciprofloxacin, although the optimum modality of treatment is not clearly defined (EL1b, RG B).
- In chronic pouchitis, combined antibiotic treatment is effective (EL1b, RG B).
- VSL#3 has shown efficacy for maintaining antibiotic-induced remission (EL1b, RG B).
- VSL#3 has shown efficacy for preventing pouchitis (EL2b, RG C).

Probiotics

Crohn's Disease

The RCTs that tested probiotics for the PR of CD after drug-induced remission (table 1) [34–38] were negative [39]. Those testing the effects of probiotics in prevention of postoperative relapse (PPOR) [40–42] were also negative (table 1) [39].

Ulcerative Colitis

Among 7 RCTs, 5 reported a beneficial effect of probiotics in mild-to-moderate UC (table 1). Three of them [43–45] used VSL#3 (a combination of 8 bacterial strains). VSL#3 has been shown to be superior to placebo. *E. coli* Nissle 1917 has been shown to be as effective as 5-aminosalicylic acid (5-ASA) in 3 large studies [46–48] and is recommended by the German guidelines for UC in patients intolerant to mesalazine [49]; its efficacy is also recognized by the ECCO guidelines (table 2) [12].

Summary

Guidelines give a limited place to antibiotics and a very limited one for probiotics in the treatment algorithms for IBD (table 2) [11, 12]. This is so despite some positive RCTs and meta-analyses. Good quality studies are somewhat hidden by less convincing ones with low number of subjects, absence of confirmation study, sometimes limited tolerance (especially on the long-term basis for imidazole derivatives), cost or potential side effects. Opponents express that meta-analysis of probiotics and even antibiotics may not be scientifically wise when their mechanisms of action differ. There was also a limited confidence of opinion leaders in the concepts, and this may be a reason for the difficulties to include patients in RCTs [50]. There is also a degree of fear of charlatanism especially as some probiotics do not have any proof of action in humans, with branded names suggesting usefulness in IBD. Finally, and obviously, research efforts have been more limited than in other fields such as biologics.

New Concepts and Directions

The recent progress in the understanding of the interplay between the gut microbiota and the host still strongly support the strategy to search for disease modifiers among microbiota-targeting agents. Some bacterial genera or bacterial metabolites are drivers of immune tolerance, particularly due to their ability to influence regula-

tory T cells [51–53]. The microbiota and the host are considered as an ecosystem with both parts influencing the other one. IBD can thus be seen as an ecological disorder and studied as such.

Genetically Modified Organisms

The concept is to use recombinant bacteria as vectors to deliver therapeutic molecules at target sites in the gut. Vector selection considers safety and confinement (to avoid dissemination of genetically modified organisms in the environment), but pharmacokinetics (i.e. capacity of the vector to reach targets in the gastrointestinal tract and deliver its biological activity) has not been considered until now. Lactococci have been the most studied because of their ease to be genetically modified; however, they exhibit a low survival till they reach the ileum [54]. *Lactococcus lactis* that secretes IL-10 has shown positive effects in murine colitis mode [55]. Unfortunately, the results in humans were disappointing. A genetically modified strain of *Lactobacillus casei*, BL23 that was engineered to produce superoxide dismutase, had preventive efficacy in TNBS-induced colitis in a murine model [56]. *Streptococcus thermophilus* CRL 807, also engineered to produce superoxide dismutase, has anti-inflammatory properties too [57]. Recently, genetically modified *Lactococcus lactis* that secretes elafin (a human protease inhibitor) [58], or IL-27 (a cytokine with immunosuppressive effects) [59], was also shown to have anti-inflammatory effects in colitis models and is, therefore, a candidate for clinical trials. The present limits of this research strategy are that the pharmacokinetics of currently developed vectors are not optimal, especially their survival in the gastrointestinal tract [54]. Considering the role of defending abnormalities in IBD, genetically modified organisms that vehiculate defensins would be very interesting to test [60].

New Generation of Probiotic Strains or Combinations

Faecalibacterium prausnitzii, a major bacterium of the normal core and dominant microbiota, has been shown to have anti-inflammatory effects in vitro on intestinal epithelial cells and in vivo in colitis models [51]. Moreover, it is able to stimulate a recently identified human regulatory T cell subset (CD4CD8αα) [61]. These data pave the way to use anti-inflammatory commensals or their active metabolites as treatment for IBD. The main candidates are presently *Faecalibacterium prausnitzii*, *Butyricicoccus pullicaecorum*, *Akkermansia muciniphila* and *Roseburia*. Of note, many of them are butyrate producers and belong to the core members of the human colonic microbiota. Developing therapies using them either alone or in combination requires more effort (and time) to establish pharmacokinetics, conditions of activity, efficacy and safety.

Fecal Transplantation

Fecal microbiota transplantation (FMT) has a high therapeutic efficacy in recurrent *Clostridium difficile* infection [62] and is now recommended in guidelines in this indication [63]. It is thus attractive to try this treatment strategy in IBD, although the pathogenesis is different. By transferring a healthy microbiota in an IBD patient, it is hoped to restore the appropriate host-microbiota crosstalk. To achieve this goal, many parameters should be taken into account and, notably, the selection of the donor as well as the precise indication and timing. Several controlled clinical trials are ongoing worldwide, but currently, only uncontrolled observations such as case reports and open-label studies are available. A recently published meta-analysis of 9 cohort studies, 8 case studies and 1 RCT including 122 IBD patients showed, overall, a remission rate of 45% during follow-up with a better result in CD than in UC [64]. An open-label study, not included in this meta-analysis, in active pediatric CD has been recently published and showed a 78% remission rate at 2 weeks (7 of 9) [65]. Although these results are promising, we still miss RCTs. Moreover, although the procedure seems safe (no serious adverse event have been reported until now), it is theoretically associated with a risk of transmitting not only classical infectious diseases but also still unknown infectious or non-infectious transmissible diseases. For example, transferring a microbiota from an obese mouse to a lean one can lead to obesity in the receiver [66]. Researchers are actually already thinking of the post-FMT era and are developing defined consortia with a limited number of microorganisms. This strategy has the advantage to be better defined and more easily controlled. However, it is based on a 'one size fits all' theory which remains to be proven in IBD. To reach the goal of building artificial microbiota, we probably first need to perform appropriately sized RCTs including mechanistic analysis, particularly regarding the donor microbiota.

Prebiotics and Other Abiotic Factors

So far, the trials with prebiotics or synbiotics in IBD were disappointing and sometimes showed poor tolerance, probably due to their fermentation [67]. Finding substrates (fibers?) favoring protective ecological conditions remains very important.

Treatment Combinations to Target Both the Microbiota and the Inflammatory Process

A double-blind placebo-controlled study [68] compared combination therapy with infliximab and ciprofloxacin (18 weeks) versus infliximab alone in 24 subjects with perianal fistulas. Response during week 18 was 73% in the ciprofloxacin group versus 39% in the placebo group, but this did not reach statistical significance as the statistical power of the first pilot trial was quite low ($p = 0.12$). Logistic regression analysis showed a tendency toward a better clinical response with ciprofloxacin than with placebo (OR 2.37; 95% CI 0.94–5.98, $p = 0.07$). Moreover, the PDAI score was significantly lower in the ciprofloxacin group compared with the placebo group (3.5; range 0–7 vs. 8.5, range 2–11, $p = 0.03$). A multicenter double-blind placebo-controlled study compared the efficacy of adalimumab monotherapy to a combination therapy of adalimumab and ciprofloxacin in 76 patients with perianal CD and showed that combination therapy was more effective at week 12 in terms of clinical response (71 vs. 47%, respectively; $p = 0.047$) and remission (65 vs. 33%, respectively; $p = 0.009$). At week 24 (12 weeks after cessation of ciprofloxacin), no difference existed between the 2 treatment groups [69]. Altogether, these data suggest that combining antibiotics to anti-TNF induction therapy offers the advantage to anti-TNF alone for fistulizing CD, but this needs to be studied further, with longer periods of antibiotic administration.

Conclusion

The microbiota has a role in experimental IBD, and dysbiosis is present in humans suffering from IBD. Microbial markers will probably soon be introduced to improve diagnosis. However, targeting the microbiota presently has only a limited value in IBD except for superinfections and pouchitis. Novel approaches using anti-inflammatory commensals, active metabolites from the microbiota, recombinant bacteria or FMT are currently under investigation. Combo therapy with other disease modifiers and using long-term ecologic pressure (smoking and fibers) are also rational.

Disclosure Statement

M. Bejaoui: has no conflict of interest.

P. Marteau: lecture fees and/or consultancy from Abbvie, Biocodex, Danone, Hospira, Merck medication familiale, MSD, Nestlé.

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