

Probiotics, Prebiotics and Antibiotics in the Treatment of Inflammatory Bowel Disease

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ABSTRACT

The rationale for using probiotics, prebiotics, and antibiotics in inflammatory bowel diseases (IBD) is based on convincing evidence that intestinal bacteria are implicated in the pathogenesis of these diseases. Probiotics are “living organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition. Several mechanisms have been proposed to account for the action of probiotics. VSL#3, a highly concentrated cocktail of probiotics has been shown to be effective in the prevention of pouchitis onset and relapses. Results on the use of probiotics in UC are promising, both in terms of the prevention of relapses and the treatment of mild-to-moderate attacks. Results in Crohn’s disease are not yet clear because of conflicting data and the limited number of well-performed studies. Prebiotics are dietary substances, usually nondigestible carbohydrates, which beneficially affect the host by selectively stimulating the growth and activity of protective commensal enteric bacteria. Evidence supporting the use of these nutraceuticals in IBD is still limited. Antibiotics have an essential role in treating the septic complications of Crohn’s disease, including intrabdominal and perianal abscesses and perianal fistulae. The use of antibiotics in UC is not supported by the available studies while their use in pouchitis is largely justified although proper controlled trials have not been conducted.

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Key words: Probiotics; Prebiotics; Antibiotics; Pouchitis; Crohn’s disease; Ulcerative colitis

INTRODUCTION

The rationale for using probiotics, prebiotics and antibiotics in IBD is based on convincing evidence that implicates intestinal bacteria in the pathogenesis of the disease. The distal ileum and the colon are the areas with the highest bacterial concentrations and represent the sites of inflammation in IBD. Similarly, pouchitis, the nonspecific inflammation of the ileal reservoir after ileo-anal anastomosis, appears to be associated with bacterial overgrowth and dysbiosis. Enteric bacteria and their products have been found within the inflamed mucosa of patients with Crohn’s disease (CD)^[1]. The composition of the enteric flora is altered in patients with IBD, increased numbers of aggressive bacteria, such as *Bacteroides*, adherent/invasive *Escherichia coli*, *enterococci*, and decreased numbers of protective *lactobacilli* and *bifidobacteria* have been observed^[2]. Manichanh *et al*^[3] reported a restriction of biodiversity in the fecal microbiota of CD patients. The phylum Firmicutes and particularly the species *F. prausnitzii* are under-represented in active CD and UC compared with healthy subjects^[4], and reduction of *F. prausnitzii* is associated with higher risk of postoperative recurrence of ileal CD^[5]. Moreover there is evidence of a loss of immunological tolerance to commensal bacteria in patients with IBD^[6]; patients with CD consistently respond to diversion of fecal stream, with immediate recurrence of inflammation after restoration of intestinal continuity or infusion of luminal content into the bypassed ileum^[7,8]. Furthermore, pouchitis does not occur prior to closure of the ileostomy^[9].

The most compelling evidence that intestinal bacteria play a role in IBD is derived from animal models. Despite great diversity in genetic defects and immunopathology, a consistent feature of many transgenic and knockout mutant murine models of colitis is that the presence of normal enteric flora is required for full expression of inflammation^[10].

All of these observations suggest that IBD may be prevented or treated by the manipulation of intestinal microflora, and increasing evidence supports a therapeutic role for probiotics, prebiotics and

antibiotics in IBD^[11].

PROBIOTICS

The potential benefit of probiotics in health maintenance and disease prevention has long been acknowledged. At the turn of the last century, the Russian Nobel Prize winner Elie Metchnikoff suggested that high concentrations of lactobacilli in the intestinal flora were important for health and longevity in humans^[12]. The bacteria most commonly associated with probiotic activity are *Lactobacilli*, *Bifidobacteria*, and *Streptococci*, but other, non-pathogenic bacteria (e.g. some strains of *E. coli*) and nonbacterial organisms (e.g. the yeast *Saccharomyces boulardii*) have been used. It is believed that in order to be clinically useful probiotics should be resistant to acid and bile, metabolically active within the luminal flora, where they should survive but not persist in the long term, antagonistic against pathogenic bacteria, safe for human use, and viable during manufacturing processes^[13].

Several mechanisms have been proposed to account for the action of probiotics (Table 1). These may include modulation of microbiota, enhancement of barrier function, and immunomodulation through direct effects of probiotic bacteria on different immune and epithelial cell types^[14].

Table 1 Mechanisms of action of probiotics.

Action	Mechanism
Inhibit pathogenic enteric bacteria	Decrease luminal pH Secrete bacteriocidal proteins Colonization resistance Block epithelial binding
Improve epithelial and mucosal barrier function	Produce short-chain fatty acids Enhance mucus production Increase barrier integrity
Alter immunoregulation	Increase IL-10 and TGF-β and decrease TNF-α Increase immunoglobulin A production

IL-1-: Interleukin-10; TGF-β: Transforming growth factor-β; TNF: Tumor necrosis factor-α.

Encouraging results have been obtained with probiotic therapy in experimental colitis: administration of *Lactobacillus reuteri* has been shown to significantly reduce inflammation in acetic acid- and methotrexate-induced colitis in rats^[15,16]. More recently, a mixture of species of *Lactobacilli* was shown to prevent the development of spontaneous colitis in interleukin-10 (IL-10)-deficient mice^[17], and continuous feeding with *Lactobacillus plantarum* was shown to attenuate established colitis in the same knockout model^[18]. A strain of *Lactobacillus*, *Lactobacillus salivarius subsp salivarius UCC18*, has been reported to reduce the rate of progression from inflammation through dysplasia and colon cancer in IL-10-deficient mice^[19]. Furthermore, certain strains of *Bifidobacterium infantis* and *L. salivarius* have been shown to attenuate inflammation by reducing T helper type 1 cytokine production in the IL-10 knockout model^[20]. Shibolet and colleagues demonstrated that VSL#3 (VSL Pharmaceuticals, Inc, Ft. Lauderdale, FL, USA), a cocktail of probiotic bacteria, significantly attenuates inflammation by decreasing myeloperoxidase and nitric oxide synthase activity in iodoacetamide-induced colitis in rats^[21]. Using the same probiotic mixture, Madsen and colleagues reported a significant improvement in inflammation, a reduction in mucosal levels of proinflammatory cytokines, and normalization of colonic barrier integrity in IL-10 knockout mice^[22]. More recently Pagnini *et al*^[23] have shown that VSL#3 was able to promote gut health through stimulation of the innate immune system in a model of chronic CD-like ileitis.

Ulcerative colitis

Tables 2 and 3 summarize results of clinical trials carried-out with

probiotics in UC. Three double-blind, controlled trials evaluated the efficacy of the probiotic preparation *Escherichia coli Nissle 1917 (ECN)* in the prevention of relapses of ulcerative colitis (UC). In the first study 120 patients with UC were treated for 12 weeks with either 5×10¹⁰ colony forming units (cfu) of ECN or 1.5 g/d mesalazine. After 12 weeks 16% of the patients in ECN group and 11.3% in the mesalazine group relapsed. The statistical power of this study was low and duration of treatment too short, and therefore the equivalence was not demonstrated^[24]. In the second study 116 patients were treated with ECN or mesalazine at lower dose (1.2 g/d) for 1 year. Surprisingly high relapse rate occurred in both the ECN and mesalazine group (67% versus 73%)^[25]. In the third study 327 patients were treated with either ECN or mesalazine (1.5 g/d) for 1 year. The relapse rate were respectively of 36% and 34% in the probiotic group and mesalazine, showing equivalence of the two treatment in an appropriate way^[26]. Recently the same preparation has been used as enemas in patients with mild to moderate distal UC in a double-blind study. Ninety patients were randomly assigned to receive either 40, 20 or 10 mL containing ECN or placebo for 8 weeks. In the per-protocol analysis ECN rectal application was significantly superior to placebo and well tolerated, in contrast to intention-to-treat analysis^[27].

Table 2 Probiotics in UC: induction of remission.

Study	N	Duration	Probiotic	Control	Remission Probiotic/Cont	p-value
Rembacken 1999	116	4 mo	Prednisone/ Gentamicin + <i>E coli</i>	Prednisone/ Gentamicin + 5ASA	68%; 75%	equal to 5-ASA? Pred effect
Guslandi 2003	25	1 mo	Nissle	open label	63%	
Bibiloni 2005	34	6 wk	S boulardii	open label	32.5%; 10%	
Sood 2009	147	12 wk	VSL#3	placebo	Improvement in UCDAI	<0.001
Tursi 2010	144	8 wk	VSL#3	placebo	60.5% 92%; 36.4%	<0.017
Miele 2009	29	1 mo	VSL#3	placebo	56%	<0.001
Huynh <i>et al</i> 2009	13	8 wk	VSL#3	open label		

Table 3 Probiotics in UC: maintenance of remission

Study	N	Duration	Probiotic	Control	Remission Probiotic ; Cont	p-value
Rembacken 1999	116	12 mo	E coli Nissle	5ASA	26%; 25%	Relapse rates near placebo
Kruis 1997	120	3 mo	E coli Nissle	5ASA	84%; 89%	Equivalence to 5ASA
Kruis 2004	327	12 mo	E coli Nissle	5ASA	64%; 66%	Equivalence to 5ASA
Venturi 1999	20	12 mo	VSL#3	open label	75%	
Ishikawa 2003	21	12 mo	Bifidobacterium fermented milk	placebo	73%; 10%	0.018
Miele 2009 [pediatric patients]	29	12 mo	VSL#3	Mesalamine	79.6%; 26.7%	0.014

In another small randomized controlled trial, Ishikawa *et al* evaluated the efficacy of a *Bifidobacterium* fermented milk as a dietary adjunct in maintaining remission of UC. Twenty-one patients were included in the study; in the group treated with *Bifidobacterium* fermented milk 3 of 11 (27%) patients had a relapse of UC compared with 10 of 11 (90%) of patients in the control group^[28]. Similarly, in a 4-week, open-label study, 25 patients with mild to moderate clinical flare-up were treated with the yeast *S. boulardii* at the dose of 250 mg three times/d for 4 weeks; 17 patients (68%) achieved clinical remission^[29].

Even VSL#3 has been investigated in the treatment of UC. This product contains cells of four strains of *Lactobacilli* (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii subsp. bulgaricus*), three strains of *Bifidobacteria* (*B. longum*, *B. breve*, *B. infantis*), and one

strain of *Streptococcus salivarius subsp thermophilus*. Each packet of VSL#3 contains 450 billion viable lyophilized bacteria. A pilot study was performed using VSL#3 as a maintenance treatment in UC patients in remission who were either allergic or intolerant to sulphasalazine and mesalazine. Patients ($n=20$) received, 1.8×10^{12} CFU VSL#3 for 12 months and were assessed clinically and endoscopically at baseline, at 6 and 12 months, or if relapse occurred. Fecal concentrations of *lactobacilli*, *bifidobacteria*, and *S. thermophilus* were significantly increased by VSL#3. In total, 15 of the 20 patients (75%) remained in remission during the study^[30]. In an open-label study, high-dose VSL#3 (3.6×10^{12} CFU) induced remission, after 6 weeks, in 63% of patients with active mild-to-moderate disease, who failed to respond to mesalazine or corticosteroids, and was associated with a positive response in a further 23%^[31]. In a multicenter, double-blind, placebo-controlled trial 147 patients with mild to moderate UC were randomized to receive either 3.6×10^{12} CFU VSL#3 or placebo for 12 weeks. At 6 weeks the rate of patients with >50% reduction in UCDAI (primary end-point) were respectively 32.5 and 10% for VSL#3 and placebo ($p=0.001$). At 12 weeks the rate of remission were 42.9% for VSL#3 and 15.7% for placebo ($p<0.001$). The VSL#3 group had significantly greater decreases in UCDAI scores and individual symptoms at weeks 6 and 12 weeks compared with placebo group^[32].

More recently, in a multicenter, double-blind, randomized, placebo-controlled study, a total of 144 patients with relapsing UC, while on treatment with salicylates or immunosuppressants, were treated with either VSL#3 (71 patients) at the dose of 3.6×10^{12} CFU/d or placebo (73 patients) for 8 weeks. The decrease of UC activity index (UCDAI) scores of 50% or more and improvement in rectal bleeding were significantly higher in the VSL#3 treated group, while endoscopic improvement and remission rate did not reach statistical significance. Only few patients reported mild side-effects with placebo and VSL#3^[33].

In 2 small recent studies, VSL#3 has been reported to achieve remission/response in children with mild to moderate UC. In the first double-blind, placebo-controlled study, 29 patients with newly diagnosed UC were randomized to receive either VSL#3 (weight-based dose, range 0.45×10^{12} CFU - 1.8×10^{12} CFU) or placebo both in induction and maintenance of remission in adjunct to standard therapy. Remission was achieved in 13 (92.8%) treated with VSL#3 and in 4 (36.4%) treated with placebo ($p<0.001$). VSL#3 was also significantly superior in maintenance of remission^[34]. In the second, open-label trial, 18 patients with mild to moderate active UC were treated with VSL#3 in 2 divided doses (the dose was based on the age of children) for 8 weeks; 10 (56%) children achieved remission after 8 weeks, and post-VSL#3 treatment demonstrated a bacterial taxonomy change in rectal biopsy. VSL#3 was well tolerated^[35].

Pouchitis

Table 4 summarizes the results of trials carried-out with probiotics in pouchitis. Total proctocolectomy with ileal-pouch anal anastomosis (IPAA) represents nowadays the surgical treatment of choice for the management of patients with familial adenomatous polyposis (FAP) and ulcerative colitis (UC), and pouchitis, a non specific (idiopathic) inflammation of the ileal reservoir, is the most common long-term complication after pouch surgery for UC^[36]. The etiology of pouchitis is still unknown, and is likely to be multifactorial; however the immediate response to antibiotic treatment suggests a pathogenic role for the microflora; recently pouchitis was associated with a decreased ratio of anaerobic to aerobic bacteria and reduced fecal concentrations of *lactobacilli* and *bifidobacteria*^[37]. Antibiotics are the mainstay of treatment, and metronidazole and ciprofloxacin are the common initial

therapeutic approach and most patients have a dramatic response within few days. A double-blind study to compare the efficacy of VSL#3 with placebo in the maintenance treatment of chronic pouchitis was carried-out. Patients ($n=40$) who were in clinical and endoscopic remission after 1 month of combined antibiotic treatment (2 g/d of rifaximin plus 1 g/d of ciprofloxacin) were randomized to receive either VSL#3 (1.8×10^{12} CFU) or placebo for 9 months. Patients were assessed clinically every month, and assessed endoscopically and histologically at entry and every 2 months thereafter. Stool culture was performed before and after antibiotic treatment, and monthly during maintenance treatment. Relapse was defined as an increase of at least 2 points in the clinical section of the Pouchitis Disease Activity Index (PDAI) and was confirmed endoscopically and histologically. All 20 patients treated with placebo relapsed during the follow-up period. In contrast, 17 of the 20 (85%) patients treated with VSL#3 were still in remission after 9 months. Interestingly, all these 17 patients relapsed within 4 months of suspension of the active treatment. Fecal concentrations of *lactobacilli*, *bifidobacteria*, and *S. thermophilus* were significantly increased within 1 month of treatment initiation and remained stable throughout the study only in the group treated with VSL#3^[38]. A subsequent double-blind, placebo-controlled study on the effectiveness of VSL#3 (at a daily dose of 1.8×10^{12} CFU) in the maintenance of antibiotic-induced remission in patients with refractory or recurrent pouchitis reported similar results^[39]. After 1 year of treatment, 85% of those in the VSL#3 group were in remission versus only 6% of those in the placebo group. With regard to the mechanism of action of VSL#3 in these patients, it has been shown that continuous administration of VSL#3 decreases matrix metalloproteinase activity, significantly increases tissue levels of IL-10, and significantly decreases tissue levels of the proinflammatory cytokines IL-1, tumor necrosis factor- α , and interferon γ ^[40]. In contrast, in a 3-month double-blind, placebo-controlled trial *Lactobacillus rhamnosus strain GG* (two gelatin capsules/d of $0.5-1 \times 10^{10}$ CFU/capsule) in patients with a previous history of pouchitis showed that this probiotic was not effective in preventing relapses^[41].

Table 4 Probiotics in Pouchitis.

Study	N	Duration	Probiotic	Control	Remission Probiotic ;Cont	p-value
Gionchetti 2002 [Maintenance]	40	9 mo	VSL#3	placebo	85%; 0%	<0.001
Mimura 2004 [Maintenance]	36	12 mo	VSL#3	placebo	85%; 6%	<0.001
Gionchetti 2003 [Post-op prevention]	40	12 mo	VSL#3	placebo	90%; 60%	<0.05
Shen 2005 [Maintenance]	31	8 mo	VSL#3	open label	19.4%	ns
Kuisma 2003 [Acute pouchitis]	20	3 mo	Lactobacillus GG	placebo	0%; 0%	ns
Gionchetti 2007 [Acute pouchitis]	29	4 wk	VSL#3	open label	69%	<0.01

In an open study Shen and colleagues treated 31 patients with antibiotic-dependent pouchitis with VSL#3 at the dose of 1.8×10^{10} CFU/d after having had a clinical improvement with 2 weeks treatment with ciprofloxacin 500 mg PO BID. At 8-month follow-up, six patients were still on VSL#3, while 25 had discontinued therapy due to either recurrence of symptoms or adverse effects. This study has numerous limitations. Firstly patients had to purchase VSL#3 which was obtained from the company's web site; VSL#3 is not covered by insurance and therefore patient's adherence to therapy was a problem; Secondly, because VSL#3 was self-administered by patients medicine counts and prescription records were impossible. Further, fecal bacteriology, as in previous study was not done and this further raise the issue of adherence

to therapy. Another important issue is that patients were not assessed endoscopically before starting VSL#3 treatment (differently from previous placebo-controlled studies); this would have missed residual inflammation in the pouch and may explain the high relapse rate. Finally patients' response was only based on symptoms assessment, and recurrence of symptoms, during VSL#3 assumption, does not necessarily indicates the presence of pouchitis^[42].

A double-blind, placebo-controlled trial has evaluated the efficacy of VSL#3 in the prevention of pouchitis onset in patients following ileal-pouch anal anastomosis for UC^[43]. Within 1 week after ileostomy closure, 40 patients were randomized to receive either VSL#3 (0.9×10^{12} CFU) or placebo for 12 months. Patients were assessed clinically, endoscopically, and histologically at 1, 3, 6, 9, and 12 months according to PDAI score. During the first year after ileostomy closure, patients treated with VSL#3 had a significantly lower incidence of acute pouchitis compared with those treated with placebo (10% vs 40%; $p < 0.05$). Moreover, IBD questionnaire score was significantly improved only in the group treated with VSL#3 and among those who did not develop pouchitis, the median stool frequency was significantly lower in the VSL#3 group. More recently, an open-label study evaluated the efficacy of high-dose of VSL#3 (3.6×10^{12} CFU/d) in the treatment of mild pouchitis, defined as a score between 7 and 12 in the PDAI. Sixteen of 29 patients (69%) were in remission after 4 weeks^[44].

Recently ECCO guidelines suggested the use of VSL#3 both for maintenance of antibiotic-induced remission and for prevention of pouchitis^[45].

Crohn's disease

Tables 5 and 6 summarize the results of clinical trials carried-out in Crohn's disease (CD). In a small pilot study, *E. coli Nissle 1917* was compared with placebo in the maintenance of steroid-induced remission of colonic CD^[46]. Twelve patients were treated with *E. coli Nissle 1917* and 11 were treated with placebo. At the end of the 12-week treatment period, relapse rates were 33% in the *E. coli* group and 63% in the placebo group; unfortunately, due to the very small number of patients treated, this difference did not reach statistical significance. In a small, comparative, 6-month, open-label study, 32 patients with CD in clinical remission were randomised to receive either combination therapy with the yeast *S. boulardii* (1 g/d) plus mesalamine (2 g/d) or mesalamine (3 g/d). Relapse rates were 37.5% and 6.25% respectively in the mesalamine monotherapy group and in the combination group^[47]. In a 1-year, double-blind, placebo-controlled trial, *Lactobacillus GG* was not effective in the prevention of post-operative recurrence^[48]. Similarly in a double-blind trial *Lactobacillus GG* was shown not be superior than placebo in prolonging remission in children with CD when given as an adjunct to standard therapy^[49]. Two randomised double-blind, placebo-controlled study showed *Lactobacillus johnsonii LA1* (4×10^9 CFU/d) was not superior to placebo to prevent endoscopic recurrence of CD^[50,51].

Table 5 Probiotics in CD: maintenance of remission.

Study	N	Duration	Probiotic	Control	Remission Probiotic; Cont	p-value
Malchow 1997	28	12 mo	<i>E coli</i> Nissle 1917	placebo	70%; 30%	ns
Guslandi 2000	32	6 mo	<i>S boulardii</i>	5ASA	62.5%; 93.75%	0.04
Bousvaros 2005	75	24 mo	<i>L.rhamnosus GG</i> +standard therapy	placebo+standard therapy	71%; 83%	ns
Willert 2010	38	12 mo	VSL#3+standard therapy	Placebo+standard therapy	43%; 11%	ns

We performed a single-blind study to compare a sequential antibiotic-probiotic treatment with mesalazine in the prevention of post-operative

recurrence of CD. Within 1 week after curative surgery, 40 patients were randomized to receive either high-dose rifaximin (a nonabsorbable wide-spectrum antibiotic) for 3 months followed by VSL#3 (1.8×10^{12} CFU/d) for 9 months, or mesalazine (4 g/d) for 12 months. Patients were assessed clinically and endoscopically at 3 and 12 months. Compared with placebo, the combined antibiotic-probiotic treatment was associated with a significantly lower incidence of severe endoscopic recurrence, both at 3 months (10% vs. 40%; $p < 0.01$) and 12 months (20% vs. 40%; $p < 0.01$)^[52]. More recently, VSL#3 at the dose of 1.8×10^{12} CFU/d, was shown not to be superior than placebo in maintaining remission in colonic CD, in a 12-month, randomized, double-blind trial^[53].

Table 6 Probiotics in CD: prevention of postoperative recurrence.

Study	N	Duration	Probiotic	Control	Remission Probiotic; Cont	p-value
Campieri 2000	40	12 mo	Rifaximin 3 mo followed by VSL#3	5ASA	endoscopic 80%; 60%	benefit probiotic
Prantera 2002	45	12 mo	<i>L rhamnosus GG</i>	placebo	clinical 83%; 89% endoscopic 40%; 65%	ns
Marteau 2006	98	6 mo	<i>L johnsonii LA1</i>	placebo	endoscopic 51%; 36%	ns
Van Gossum 2006	70	3 mo	<i>L johnsonii LA1</i>	placebo	endoscopic 21%; 15%	ns

PREBIOTICS

Prebiotics are dietary substances, usually nondigestible carbohydrates, which beneficially affect the host by selectively stimulating the growth and activity of protective commensal enteric bacteria^[54]. Fructo-oligosaccharides (FOS), inulin, bran, psyllium, and germinated barley foodstuff (GBF) stimulate the growth of bifidobacteria and lactobacilli, which in turn antagonize pathogenic bacteria by decreasing the luminal pH, inducing colonization resistance, and inhibiting epithelial adhesion and translocation. In addition, these substances increase bacterial fermentation, which produces SCFAs (especially butyrate) that improve epithelial barrier function^[55]. These findings suggest that prebiotics are functionally equivalent to probiotic bacteria.

Studies in animal models

A variety of different prebiotic preparations have been tested in animal models of colitis. Lactulose has been shown to attenuate inflammation and to stimulate the growth of lactobacilli in IL-10 knockout mice^[56], while administration of inulin and GBF has been shown to inhibit dextran sodium sulphate (DSS)-induced colitis in rats by increasing the luminal concentration of SCFAs, *lactobacilli*, and *bifidobacteria*^[57,58]. Experiments on FOS have produced conflicting results. Cherbut *et al*^[59] reported that FOS attenuates the trinitrobenzene sulphonic acid-induced colitis in rats, while Moreau *et al*^[60] reported no benefit of FOS in the DSS rat model of colitis. Furthermore, a combination of inulin and FOS significantly decreased inflammation in HLA-B27 transgenic rats^[61]. Taken together, these findings suggest that combination therapy with different prebiotics may be more effective than monotherapy, due to the fact that each agent has specific biological properties.

Human IBD studies

A few small, controlled studies have investigated the use of prebiotics in UC, whereas there have been no studies on prebiotics in CD or pouchitis. In a small group of UC patients in remission, *psyllium* (also known as *ispaghula* or *Plantago ovata*) was shown to be superior to

placebo in decreasing symptom severity, and produced a significant increase in the fecal concentration of *bifidobacteria*^[62].

In an open-label, randomized trial, *Plantago ovata* seeds, which have previously been shown to stimulate the production of SCFAs, were tested as a maintenance treatment in UC patients in remission. In this 12-month study, 105 patients were randomized to receive either *Plantago ovata* seeds alone (10 g twice daily), mesalamine alone (500 mg three times daily), or a combination of *Plantago ovata* seeds plus mesalamine at the same doses administered for monotherapy. Rates of remission were similar for the three groups, and a significant increase in the fecal concentration of butyrate was observed after *Plantago ovata* seed administration^[63].

GBF is comprised of the glutamine- and hemicellulose-rich extracts of spent beer-brewing constituents. Use of this probiotic in patients with mild-to-moderate UC has been investigated in a small pilot study and a placebo-controlled trial^[64,65]. At a dose of 25-30 mg/d, GBF decreased clinical and endoscopic activity in these patients and significantly increased fecal concentrations of bifidobacteria. Similar results were reported by a 24-week, open-label trial^[66].

Lindsay *et al* performed a small, open-label study in 10 patients with active ileo-colonic CD using a combination of 15 g/d of oligofructose and inulin (ratio 70:30%). They found a significant reduction in disease activity, concomitant with a significant increase in mucosal *bifidobacteria*. Interestingly prebiotic treatment increased colonic dendritic cells expressing IL-10, Toll-like receptor (TLR)-2 and TLR-4, indicating that these prebiotics affected the innate mucosal immune response^[67]. In a small placebo-controlled study oligofructose-enriched inulin was administered as adjunctive treatment to mesalazine 3 g/d for 2 weeks in mild to moderate, active UC. This study showed a significant reduction of the fecal calprotectin in prebiotic treated patients compared to placebo^[68].

ANTIBIOTICS

Animal models

In several rodent models the use of broad-spectrum antibiotics can both prevent onset and treat experimental colitis, whereas metronidazole and ciprofloxacin can only prevent experimental colitis but not reverse established disease^[69,70]. Broad-spectrum antibiotics are effective in almost all models of acute and chronic colitis^[71-76], however have only a transient efficacy in HLA-B27 transgenic rats^[77]. Interestingly ciprofloxacin and metronidazole had selective efficacy in different colonic regions in IL-10 knock-out mice, suggesting that different bacteria cause inflammation in different colonic segments. These studies suggest that most clinical forms of IBD may respond if a proper combination of broad-spectrum antibiotics are used.

Ulcerative Colitis

Only few trials of antibacterial agents have been carried out in ulcerative colitis (UC) and results are controversial. Most clinicians have used antibiotics as adjuvant therapy in severe UC. Dickinson *et al*^[78] have carried out a double-blind controlled trial on the use of oral vancomycin as adjuvant therapy in acute exacerbations of idiopathic colitis. No significant difference was found between the two treatment groups with only a trend towards a reduction in the need for surgery in patients treated with vancomycin.

Intravenous metronidazole, used as adjunctive treatment to corticosteroids, was similarly effective than placebo to induce remission in patients with severe UC^[79].

In a double blind, placebo controlled trial in patients with acute relapse of UC, 84 patients were randomized to receive corticosteroids

plus oral tobramycin or placebo. After 1 week of treatment, 74% of patient in the tobramycin treatment group vs 43% in the placebo group ($p < 0.003$) achieved a complete symptomatic remission^[80]. Subsequently the combination of tobramycin and metronidazole did not show any beneficial effect when associated to a standard steroid treatment in severely acute UC^[81]. Ciprofloxacin has been tested in a randomized, placebo controlled study; 70 patients with mild to moderate active UC were randomized to receive ciprofloxacin 250 mg b.i.d or placebo for 14 d. At the end of the study, 70.5% of patients in the ciprofloxacin group vs 72% in the placebo group achieved remission^[82]. Similarly a short course of intravenous ciprofloxacin was not effective as adjunctive treatment to corticosteroids in severe UC in a prospective, randomised, double-blind, placebo-controlled trial^[83]. Nevertheless, in a more recent randomized, placebo controlled trial, ciprofloxacin was administered for 6 months to patients with active UC poorly responding to conventional therapy with steroids and mesalamine. At the end of the study, the treatment-failure rate was 21% in the ciprofloxacin-treated group and 44% in the placebo group ($p < 0.002$). This difference was detected using clinical criteria; while endoscopic and histological findings showed differences only at 3 months but not at 6 months^[84].

The nonabsorbable, broad-spectrum antibiotic, rifaximin was tested in a small controlled study to evaluate its efficacy and systemic absorption in patients with moderate to severe active UC refractory to steroid treatment. Twenty-eight patients were randomized to receive rifaximin 400 mg b.i.d. or placebo for 10 d as an adjunct to standard steroid treatment. Although there was no significant difference in clinical efficacy between the two treatments, only rifaximin determined a significant improvement of stool frequency, rectal bleeding and sigmoidoscopic score^[85].

Crohn's disease

There are several studies looking at the use of antibiotics as primary therapy for luminal CD. Unfortunately, the majority of these are observational, uncontrolled studies or lack sufficient power to truly detect important differences. Metronidazole has been the mostly investigated agent. In 1978, Blichfeldt *et al*^[86] in a placebo-controlled, double-blind, crossover trial did not find difference between metronidazole and placebo-treated patients, but a positive trend in favour of metronidazole was observed when only the colon was involved. In the National Cooperative Swedish study, metronidazole was compared to sulfasalazine as primary treatment for Crohn's disease; no significant difference was found between the two groups, but, interestingly, in the cross-over section of the study, metronidazole was effective in patients not responders to sulfasalazine^[87]. Metronidazole was used as single therapy or associated to cotrimoxazole and compared to cotrimoxazole alone and placebo in patients with a symptomatic relapse of Crohn's Disease. At the end of the four weeks of treatment there was no difference in response among the three groups^[88]. In a Canadian randomized, placebo-controlled trial, Sutherland *et al*^[89] have shown that treatment with metronidazole for 16 weeks significantly decreased the Crohn's Disease Activity Index (CDAI), but no difference was found in the rates of remission compared with placebo; benefit was dose-dependent with 20 mg/kg having a greater benefit than 10 mg/kg. As in the case of the Swedish study, in the Canadian study metronidazole was effective for colonic and ileocolonic Crohn's disease but not for ileitis. Metronidazole has important side-effects that includes nausea, anorexia, dysgeusia, dyspepsia, and peripheral neuropathy that limit its use in approximately 20% of patients. An antibiotic association was used in an Italian randomized controlled study in which metronidazole 250 mg four times daily plus ciprofloxacin 500 mg twice daily were compared to a standard steroid treatment for 12 weeks. No differences

were reported in the rates of remission between treatments (46% with ciprofloxacin plus metronidazole vs 63% with methylprednisolone) suggesting that this antibiotic association could be an alternative to steroid treatment in acute phases of Crohn's disease^[90]. Combination of metronidazole and ciprofloxacin was associated with budesonide 9 mg/d in active Crohn's disease; no difference was registered compared to placebo, but surprisingly the overall response in the two groups was lower than the previous studies on budesonide. Also in this study antibiotic treatment was more effective when the colon was involved than for isolated small bowel disease^[91].

Ciprofloxacin 1g/d was compared to mesalamine 4 g/d in a controlled study in mild-to-moderate active CD. After 6 weeks an equivalence in efficacy was registered (remission observed in 56% and 55% of patients respectively with ciprofloxacin and mesalamine), offering an alternative treatment in active CD^[92]. In a small study ciprofloxacin was shown to be effective in association to standard treatment in patients with resistant disease^[93]. Other antibiotics have been tested. Shafran *et al*^[94] carried out an open-label study on the efficacy and safety of rifaximin 600 mg/d for 16 weeks in the treatment of mild-to-moderate active CD. At the end of the study, 59% of patients were in remission (CDAI<150) with a significant reduction of the mean CDAI score compared to baseline ($p<0.0001$). In an open-label trial, Leiper *et al*^[95] reported an impressive positive response (64% patients improved or were in remission after 4 weeks) of clarithromycin in a group of 25 patients with active Crohn's disease, many of whom were unresponsive to other treatments. As stated by European Crohn's Colitis Organisation (ECCO), 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^[96].

Antibiotics have been also tested in prevention of post-operative recurrence. Metronidazole at the dose of 20 mg/kg/d was compared with placebo in double-blind, controlled trial by Rutgeerts *et al*^[97]. Sixty patients were randomized to receive metronidazole or placebo for 12 weeks. At the end of the treatment, endoscopic relapse was evaluated by Rutgeerts score. Metronidazole significantly decreased the incidence of severe endoscopic relapse (grade 3 or 4) in the neoterminal ileum 6 months after surgery and the clinical recurrence rates at 1 year, with a trend towards a protective effect after 3 years. More recently, the similar antibiotic ornidazole, used continuously for 1 year was significantly more effective than placebo in the prevention of severe endoscopic recurrence in the neoterminal ileum both at 3 and 12 months^[98]. Imidazole antibiotics, as suggested by the ECCO Consensus on CD management, may be a therapeutic option after ileocolic resection but are poorly tolerated^[96].

Campieri *et al* performed a randomized trial to evaluate the efficacy in the prevention of post-operative recurrence of rifaximin 1.8 g daily for 3 months followed by a probiotic preparation (VSL#3) 6 g daily for 9 months versus mesalamine 4 g daily for 12 months in 40 patients after curative resection for CD. After 3 months of treatment, rifaximin determined a significant lower incidence of severe endoscopic recurrence compared to mesalamine [2/20 (10%) vs 8/20 (40%)]. This difference was maintained since the end of the study using probiotics [4/20 (20%) vs 8/20 (40%)]^[99].

Many studies have tried to evaluate the efficacy of antimycobacterial drugs in patients with CD, pursuing the possibility that a strain of Mycobacterium might be an aetiological agent in CD. Borgaonkar *et al*^[100] evaluated all randomized controlled trials where antimycobacterial therapy was compared with placebo, suggesting the efficacy of antimycobacterial therapy only as a maintenance treatment in patients who obtained remission after a combined treatment with

corticosteroids and antimycobacterial agents. However, the investigator emphasized the high incidence of side-effects, and that, because the small number of studies included in the meta-analysis, the data were not conclusive and should be taken with caution.

The same antibiotics used to treat luminal Crohn's disease have been reported to be beneficial in the treatment of perianal Crohn's disease, but no controlled trial have been performed^[101]. Metronidazole 20 mg/kg has shown rates of fistulae closure from 62% to 83%^[102,103]. The combination of metronidazole and ciprofloxacin determined an improvement in 64% of patients and fistulae closure in 21%^[104]. Unfortunately fistulae tend to recur in most patients after stopping treatment. Although the results of these uncontrolled studies are not conclusive, metronidazole, ciprofloxacin or their combination are used by most clinicians as first line treatment in patients with perianal disease, in combination with surgical drainage of abscesses.

Pouchitis

The awareness of the crucial importance that faecal stasis and the bacterial overgrowth may represent in the pathogenesis of acute pouchitis has led the clinicians to treat patients with antibiotics, which have become the mainstay of treatment, in absence of controlled trials. Usually metronidazole represents the most common first therapeutic approach, and most patients with acute pouchitis respond quickly to administration of 1-1.5 g/d^[105,106]. A double-blind, randomised, placebo-controlled, crossover trial was carried out by Madden *et al* to assess the efficacy of 400 mg three times a day of metronidazole per os in 13 patients (11 completed both arms of the study) with chronic, unremitting pouchitis. Patients were treated for two weeks, and metronidazole was significantly more effective than placebo in reducing the stool frequency (73% vs 9%), even without improvement of endoscopic appearance and histologic grade of activity. Some patients (55%) experienced side effects of metronidazole including nausea, vomiting, abdominal discomfort, headache, skin rash and metallic taste^[107].

More recently Shen *et al* have compared the effectiveness and side effects of ciprofloxacin and metronidazole for treating acute pouchitis in a randomised clinical trial. Seven patients received ciprofloxacin 1 g/d and nine patients metronidazole 20 mg/Kg/d for a period of 2 weeks. The results of this study have shown that both ciprofloxacin and metronidazole are efficacious as treatment of acute pouchitis: they reduced the total Pouchitis Disease Activity Index (PDAI) scores and led to a significant improvement of symptoms and endoscopic and histologic scores. However ciprofloxacin led to a greater degree of reduction in total PDAI score, to a greater improvement in symptoms and endoscopic scores; furthermore ciprofloxacin was better tolerated than metronidazole (33% of metronidazole-treated patients reported adverse-effects, none of ciprofloxacin-treated)^[108].

Medical treatment of patients with chronic refractory pouchitis is particularly difficult and disappointing. A possible therapeutic alternative for chronic refractory pouchitis is the use of a combined antibiotic treatment. In an open trial, 18 patients with active pouchitis not responding to the standard therapy (metronidazole or ciprofloxacin) for 4 weeks, were treated orally with rifaximin 2 g/d+ciprofloxacin 1g/d for 15 days; symptoms assessment, endoscopic and histological evaluations were performed at screening and after 15 days according with PDAI. Sixteen out of 18 patients (88.8%) either improved ($n=10$) or went into remission ($n=6$); the median PDAI scores before and after therapy were 11 and 4 respectively ($p<0.002$)^[109].

More recently, 44 patients with refractory pouchitis received metronidazole 800 mg-1g/d and ciprofloxacin 1 g/d for 28 days. Thirty-six patients (82%) went into remission; the median PDAI scores before and after therapy were 12 and 3 respectively ($p<0.0001$). Patients'

quality of life significantly improved with the treatment (median IBDQ increased from 96.5 to 175)^[110].

CONCLUSIONS

Many clinical and experimental observations indicate that the intestinal microflora are involved in the pathogenesis of IBD. Probiotics may provide a simple and attractive way of preventing or treating IBD, and patients find the probiotic concept appealing because it is safe, nontoxic, and natural. VSL#3, a highly concentrated cocktail of probiotics has been shown to be effective in the prevention of pouchitis onset and relapses. Results on the use of this probiotic in UC are promising, both in terms of the prevention of relapses and the treatment of mild-to-moderate attacks. Results with probiotics in CD are poor and there is the need of well-performed studies.

It is important to select a well-characterized probiotic preparation, considering that the viability and survival of bacteria in many of the currently available preparations are unproven. It should be noted that the beneficial effect of one probiotic preparation does not imply efficacy of other preparations containing different bacterial strains, because each individual probiotic strain has unique biological properties. Prebiotics are an exciting potential treatment for IBD patients. They offer a safe and cost-effective approach and may be considered for long-term treatment. However, experimental evidence supporting the use of these nutraceuticals is still limited. We need to improve our knowledge on the composition of enteric flora or “the neglected organ” and on the intestinal physiology and its relationship with the luminal ecosystem. The use of antibiotics in UC is not supported by the available studies, although large studies with broad-spectrum agents are required. Antibiotics have an essential role in treating the septic complications of Crohn’s disease, including intrabdominal and perianal abscesses and perianal fistulae. There is evidence that ciprofloxacin, metronidazole or their combination are effective in Crohn’s colitis and ileocolitis, but not in isolated ileal disease, however use of antibiotics as primary therapy in Crohn’s disease is poorly documented, and large, controlled trials are needed for defining the optimal antibiotic regimens. The use of antibiotics in pouchitis is largely justified although proper controlled trials have not been conducted.

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