Intestinal Bacteria and Ulcerative Colitis


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Abstract

Convincing evidence from both animal models and the study of patients with ulcerative colitis (UC) implicates the intestinal microflora in the initiation and maintenance of the inflammatory processes in this condition. Despite this, no specific pathogen has been identified as causal and the disease is widely believed to occur as the result of a genetically determined, but abnormal immune response to commensal bacteria. When compared with healthy people, UC patients have increased levels of mucosal IgG directed against the normal microflora. Studies of mucosal bacterial populations in UC indicate that there may be increased numbers of organisms, but reduced counts of “protective” bacteria such as lactobacilli and bifidobacteria. In animal models of colitis, antibiotics, particularly metronidazole, clindamycin, ciprofloxacin and the combination of vancomycin/impinemem protect against UC, especially if given before the onset of inflammation. These antibiotics target anaerobes and some Gram-positive organisms such as enterococci. However, antibiotic use in more than a dozen randomised control trials has been very disappointing, probably because we do not know which species to target, when to give the antibiotics, for how long and in what combinations. Surprisingly, therefore, there is a consistent benefit in the small number of studies reported of probiotics to manage UC and pouchitis. There is scope for more work in this area focussing on the mucosal microflora, its interactions with the gut immune system, its metabolic properties and the potential ways of modifying it.

Introduction

Microorganisms cover the surface of the large bowel mucosa and bacterial cell densities in adjacent lumenal contents are around $10^{12}$ per gram. Most of us tolerate this complex metabolically active and antigenic microflora, but in approximately two per 1000 of adults living in industrialised western countries (Mayberry et al., 1989; Montgomery et al., 1998; Loftus et al., 2000) an intense inflammation develops in the mucosa, associated with bloody diarrhoea, urgency to defecate and general malaise, that is not associated with known pathogens.

Ulcerative colitis (UC) is one of the two major forms of idiopathic inflammatory bowel disease (IBD). It is an acute and chronic disabling condition treated primarily with drugs that suppress inflammation. Antibiotics are of limited use, despite overwhelming evidence that bacteria are involved in a process whereby genetically determined but abnormal mucosal immune responses occur to the microflora (Campieri and Gionchetti, 2001; Linskens et al., 2001; Farrell and Peppercom, 2002; Farrell and La Mont, 2002). UC, unlike Crohn’s disease (CD), occurs only in the large bowel, where bacterial numbers are several orders of magnitude greater than in the rest of the gut, and where the rate of passage of material is characterised by the slow movement of digestive materials (Eve, 1966; Metcalf et al., 1987; Cummings et al., 1992). However, the human large intestine contains only about 200 grams of contents (Cummings et al., 1990), and in such a large organ with a surface area of between 1 and 2 m², that is often distended with gas, this means that parts of the mucosal surface will not be in contact with the luminal microflora for some hours each day. This is especially so for the sigmoid and rectal regions, which are emptied after defecation, yet it is these areas where UC always commences. This points to an adherent mucosal microflora rather than luminal bacteria as the causal agents.

Other circumstantial evidence for the microflora being essential in UC comes from animal models (Elson et al., 1995; Fiocchi, 1998), the study of serum and mucosal antibodies (Macpherson et al., 1996; Hooper et al., 2001), characterisation of the mucosal bacterial communities in patients with active disease, and recently, the use of probiotic bacteria in treatment (Kruis, et al., 1999; Rembacken et al., 1999).

Bacteria Associated with UC

Many bacteria evoke an acute inflammatory response in the host gut mucosa. The principal organisms involved are toxigenic, adherent or invasive to the gut epithelium, and include pathogenic *Escherichia coli*, as well as species belonging to the genera *Yersinia*, *Shigella*, *Salmonella*, *Campylobacter*, *Clostridium* and *Aeromonas* (Cohen and Giannella, 1991; Macfarlane and Gibson, 1995). The clinical effects of these infections are usually acute rather than chronic, and the pathogenic mechanisms involved and host responses have been well studied. However, the role of bacteria in other, more chronic forms of gut disease such as antibiotic-associated colitis, or UC and CD is less clear.

Species that are part of the colonic microflora are believed to be involved in both the initiation and maintenance stages of UC. In early microbiological studies, a variety of bacteria including *Streptococcus mobilis*, fusobacteria, and shigellas received attention as being involved in IBD (Onderdonk, 1983), largely because these organisms are able either to penetrate the gut epithelium, or cause a similar spectrum of pathology in experimental
animals. Salmonella and yersinia have also been linked to UC (Sartor et al., 1996), while experiments on transgenic rats showed that colonic inflammation was related to numbers of bacteroides in the gut (Rath et al., 1996; 1999).

Certain strains of E. coli isolated from the colitic bowel have increased adhesive properties (Chadwick, 1991), although this may be an adaptation to the disease state in the host. Escherichia coli has been implicated in UC in a number of studies, and isolates from faeces and rectal biopsies in UC patients have been reported to have greater adherent abilities than those obtained from healthy people or individuals with infectious diarrhoea (Burke and Axon, 1988). However, other investigators have not found this to be the case (Hartley et al., 1993; Schultsz et al., 1997), while some workers observed that E. coli occurred in only a small number of tissue samples taken from UC patients (Walmsey et al., 1998).

Bacterial L-forms, or cell wall deficient organisms have been found in both UC and CD. These organisms lack cell wall antigens, and so are less visible to host immune surveillance and can survive intracellularly. L-forms, mainly Enterococcus faecalis and E. coli, were detected in 42% of patients with UC (n=121) and in 34% of CD patients (n=71), but in only 1% of healthy controls (n=140). It is not clear, however, whether these cell forms actually play a role in the aetiology of UC, or whether they are organisms adapted to the different nutritional and environmental conditions that occur in the inflamed bowel. However, if L-forms are aetiologic agents in UC, it would partly explain why pathogenic bacteria have not been visualised in, or isolated from diseased mucosa in IBD patients.

In general, there is no real evidence for a specific transmissible agent in UC in humans, as indicated by the work of Victor et al., (1950), who injected cell-free filtrates of stool and rectal mucosa from IBD patients into the monkey colon, and failed to induce inflammation. Furthermore, the majority of bacteria, which have been implicated in various studies, are not found in all patients with the disease. Nevertheless, there is a good case for bacteria growing on the gut wall playing a major role in IBD, either as pathogenic organisms proliferating on the epithelial surface and invading the underlying mucosa, or alternatively, by non-pathogenic commensals occupying adhesion sites on the mucosa and preventing colonisation by harmful species. This was indicated to occur when non-pathogenic Escherichia coli were used to displace resident strains in UC patients (Rembacken et al., 1999).

**Dissimilatory Sulphate-Reducing Bacteria (SRB) and UC**

SRB are normal inhabitants of the human large intestine. However, an intracellular Gram-negative bacterium, known as ileal symbiont intracellularis (ISI) related to desulfovibrios, has been associated with bloody diarrhoea, weight loss and anorexia in ferrets, hamsters and pigs (Gebhardt et al., 1993; Fox et al., 1994). This condition is infectious, and histology shows epithelial hyperplasia, goblet cell depletion, crypt abscesses and inflammatory cell infiltrates. Cultivation of these bacteria has been unsuccessful, but when grown in tissue culture and inoculated into hamsters, they induce characteristic inflammatory changes. However, ISI does not seem to play a similar role in human IBD. When rectal biopsies from 19 UC patients were studied using an immunofluorescent assay, with mouse monoclonal antibody IG4 targeted to ISI (Pitcher, 1996), none of these bacteria were observed, although fluorescent curved bacilli were demonstrated to be present in the apical cytoplasm of biopsies from a pig with porcine proliferative enteropathy. These experiments also established that there was no reactivity against eight SRB isolates cultured from UC patient faeces.

In a study of 87 healthy subjects in the UK, SRB were isolated from faeces from 66 individuals (76%), with the predominant species being members of the genus Desulfovibrio (Gibson et al., 1991, 1993). Growth of mucosal-associated SRB was detected in 92% of UC samples as compared to 52% in controls (Zinkevich and Beech, 2000). In these studies, PCR analysis further indicated that SRB were ubiquitous, and were present in all of these biopsies studied. However, a recent investigation, in France, involving 151 subjects (Loubinoux et al., 2002) found a lower prevalence of SRB in faeces in healthy subjects (12%), while the incidence of SRB (desulfovibrios) was significantly higher in IBD (UC and CD) patients (55%).

Gibson et al., (1991) observed that although SRB occurred in lower numbers in UC faeces, compared to stools from healthy subjects, their metabolic activities, as assessed by specific rates of sulphate reduction, were considerably higher than in the controls. It was also shown that SRB isolated from UC patients were particularly adapted to grow under low sulphate concentrations, at high specific growth rates, which was related to ecological selection by environmental conditions in the colitic bowel.

The significance of SRB in the large intestine is that sulphate is used by these bacteria as a terminal electron acceptor in metabolism, where it is ultimately reduced to sulphide, a strong cellular toxin (Pitcher et al., 1998). Hydrogen sulphide has been shown to inhibit butyrate oxidation in colonocytes (Roediger et al., 1993), evoke cellular hyperproliferation in the colonic mucosa, and to induce colonocyte metabolic abnormalities similar to those that occur in UC (Christl et al., 1996). In rats, perfusion of the colon with physiological concentrations of sulphide leads to superficial ulceration of the bowel (Aslam et al., 1992), while its immunomodulatory effects inhibit the abilities of polymorphonuclear leucocytes to phagocytose and kill bacteria, which may facilitate their translocation in the gut (Gardiner et al., 1995). Pitcher et al., (2000) showed that untreated UC patients had significantly higher concentrations of sulphide in faeces, when compared to healthy controls, and that 5-ASA, the main drug used to treat UC, strongly inhibited sulphide production in vivo and in vitro. However, despite the undoubted toxicological effects of sulphide in in vitro model systems, and in animal studies, evidence for this bacterial metabolite acting as a specific cellular toxin in UC remains circumstantial (Cummings and Macfarlane, 1999). A number of investigations have now linked SRB to UC, but whether these bacteria are aetiologic agents, or are simply taking advantage of changes in the gut...
ecosystem resulting from mucosal inflammation, tissue destruction and diarrhoea is unclear. Sulphide is produced by amino acid fermenting anaerobes in the large bowel, as well as by SRB. Thus, even if this metabolite is an important mucosal toxin in UC, the presence or absence of SRB, as well as their metabolic propensities, does not necessarily explain the prevalence or severity of the disease.

**Antibody Responses to the Normal Microflora in UC Patients**

Compared to healthy people, UC patients have greatly increased levels of mucosal IgG directed against members of the normal colonic microbiota, which have been suggested to contribute to relapse (Macpherson et al., 1996). These workers tested a range of bacterial proteins prepared from *Bacteroides fragilis*, *E. coli*, *Clostridium perfringens*, *Enterobacter faecalis*, *Staphylococcus epidermidis*, *Haemophilus influenzae* and *Klebsiella aerogenes* against mucosal IgG obtained from intestinal washings in UC and CD patients. Binding was observed to occur with *B. fragilis*, *C. perfringens* and *E. coli*. In contrast, there was little binding of mucosal IgG to the nonintestinal species, although serum IgG titres were high to these organisms.

Monteiro et al., (1971) found increased antibody production against strictly anaerobic species, and UC patients are known to have increased antibody titres to bacteroides (Tvede et al., 1983), particularly *B. vulgatus* (Bamba et al., 1995), where the response was to a low molecular mass protein. Similarly, a recent study has suggested that *Bacteroides ovatus* was one of the principal colonic microorganisms eliciting the systemic antibody response (IgG and IgA) in UC and CD patients (Saitoh et al., 2002). This was attributed to a 19.5 kDa protein, though its role in disease aetiology was unclear. Other evidence linking bacterial proteins to the host immune response in UC indicated that *E. coli* outer membrane protein ompC, and a 100 kDa protein of *Bacteroides caccae* were immunoreactive to a pANCA monoclonal antibody (Cohavi et al., 2000), which is produced in response to a neutrophil protein in the majority of UC patients (Satsangi et al., 1998).

Matsuda et al., (2000) reported high serum antibody responses against *Bacteroides vulgatus*, *B. fragilis* and *Clostridium ramosum* in UC patients. With respect to *B. vulgatus*, 54% of UC patients had IgG antibodies active against a 26 kDa outer membrane protein, compared to 9% in healthy subjects. These antibodies were suggested to play a role in UC aetiology. This investigation also showed that 29% of UC patients produced IgG antibodies against a 50 kDa *E. coli* protein, against only 6% of controls.

**Mucosal bacterial populations**

Since bacteria growing on the mucosal surface in the large intestine exist in close juxtaposition to host tissues, it might intuitively be expected that these organisms interact to a greater extent with the host immune and neuroendocrine systems than their lumenal counterparts. It is therefore surprising that few investigations have been made on the bacteria that inhabit the colonic mucosa surface compared to those present in the gut lumen. There are several reasons for this: firstly, faeces and material from the lumen of the bowel are readily available for study, while in healthy individuals, there are practical and ethical problems in obtaining fresh mucosal biopsy tissue.

Nevertheless, there is now sufficient evidence to suggest that bacterial populations colonising the large bowel mucosa are different in composition to those in the gut lumen (Macfarlane et al., 1999, 2000). Using colonial biopsies taken from healthy subjects, Hartley et al., (1979) found that while bacteria adhered directly to the bowel wall, they were present in higher numbers in the mucus layer. These studies indicated that one *E. coli* strain predominated in each tissue sample, and that this strain occurred throughout the length of the gut. Croucher et al., (1983) studied colonic tissue from different regions of the bowel in four sudden death victims, none of whom had UC, and noted the existence of distinct bacterial communities in each individual. It was also reported that anaerobe : aerobe ratios were much lower than in the gut lumen, at about 10^4 : 1. However, bacteroides, fusobacteria and bifidobacteria predominated. Microscopic analysis suggested that the majority of organisms occurred under the top surface of the mucus layer.

Poixton et al., (1997) reported that viable counts on mucosal tissue taken during colonoscopy were comparable in the rectum and proximal colon, and that numbers of anaerobic species were 10 to 100-fold higher than those of aerobes and facultative anaerobes. Six patients with UC were studied, together with six individuals with noninflammatory bowel conditions. Few consistent or significant differences in mucosal bacterial populations were evident in the two groups, however, *Bacteroides thetaiotaomicron* seemed to have a higher prevalence in the UC patients. These studies further showed that bacteroides predominated on mucosal surfaces, accounting for up to 69% of total anaerobe counts, with *B. vulgatus* being the dominant species.

Comparison of bacterial communities in biopsies from normal and inflamed colonic mucosa in patients with acute UC showed significant reductions in lactobacilli in the IBD group, as well as lesser numbers of bifidobacteria, while *B. thetaiotaomicron* increased in frequency (Pathmakanthan et al., 1999). The authors concluded that reduced numbers of mucosal lactobacilli in UC enabled the bacteroides, as well as other putatively pathogenic species to colonise the bowel wall.

More recently, Matsuda et al., (2000) reported that counts of both aerobic and anaerobic bacteria increased on the rectal mucosa of UC patients, and that *Bacteroides vulgatus* predominated. These experiments indicated that the frequency of isolation of bacteroides, peptostreptococci, *Clostridium ramosum* and *Bifidobacterium breve* was higher in UC, and that fusobacteria were only present in healthy controls.

Other studies, using fluorescein labelled 16S rRNA oligonucleotide probes, have indicated that the mucus layer in rectal biopsies is more heavily colonised by bacteria in IBD patients, including those with UC (Schultz et al., 1999). These investigations found no bacteria in 71% of the control subjects and 32% of the IBD patients, which is surprising.
since, as related above, culturing studies consistently show the rectal mucosa to harbour large numbers of bacteria in both health and disease.

**Antibiotics**

*Animal Models*

Since Marcus and Watt described the first reliable animal model of colitis, feeding carrageenan to guinea pigs, there have been a number of descriptions of colitis induced in other conventional species (rat, mouse, hamster, rabbit and monkeys, including the Cotton Top Tamarin), at least twelve knockout or transgenic animals and using a dozen or more different agents, such as dextran sulphate, amylopectin sulphate, TNBS (trinitrobenzene sulphonic acid) etc (Watt and Marcus, 1973; Marcus and Watt, 1969; Elson et al., 1995; Fiocchi, 1998). It is almost invariable in these widely differing models that the presence of a microflora is needed to induce colitis, and as Table 1 shows, that the severity of the inflammatory process can be ameliorated by certain antibiotics. Can we learn anything about the role of bacteria in UC from these models?

Firstly, not all antibiotic regimes prevent or improve colitis in animals. Eleven antibiotics are used in the papers described in Table 1, of which metronidazole, first reported by Bartlett’s group (Onderdonk et al., 1977) is most consistently beneficial. Metronidazole is an absorbed antibiotic and antiprotozoal drug active against anaerobic microorganisms, and it is one of the most widely used antibiotics for anaerobic infections in current clinical practice. The lack of effect of metronidazole in the study by Videla et al., (1994) may be because it was given by enema, although enema and suppository are usually successful routes of delivery when treating human infections. Perhaps antibiotics just do not work with the TNBS model. Moreover, the timing of drug administration may be important, since in most of the studies in Table 1, the benefit of metronidazole, or other antibiotics, is seen only when the drug is given before colitis is induced.

None of the other antibiotics used in these animal studies have such good effects against intestinal anaerobes. Several, notably gentamycin, tobramycin, neomycin and streptomycin have been chosen because of their activity against Gram-negative facultative anaerobes, especially *E. coli*. This gut commensal and common pathogen is readily cultured and was one of the first bacteria to be specifically implicated in UC (Monteiro et al., 1971; Van der Waaij et al., 1974; Burke and Axon, 1988). However, these aminoglycoside antibiotics are singularly lacking in effect in preventing or curing animal colitis, and as mentioned earlier, the role of *E. coli* as a primary pathogen in UC has never been established. The combination of trimethoprim-sulphamethoxazole, used only in specific human infections such as *Pneumocystis carinii* and toxoplasmosis, because of its toxicity, is also active against enterobacteria, and it produced modest benefit in reducing disease severity in two studies, although it by no means prevented ulceration in the colon (Van der Waaij et al., 1974; Onderdonk and Bartlett, 1979).

Vancomycin, a glycopeptide antibacterial agent that is not absorbed from the gut, is active particularly against Gram-positive cocci and some anaerobes. Although there are increasing reports of resistant enterococci in humans, it has been a popular choice of antibiotic in animal studies. Used alone it is not effective, nor in combination with tobramycin, but it was consistently beneficial in three different models, when combined with imipenem. In the study by Rath et al., (2001), this combination was best at both preventing and treating colitis in both HLA-B27 transgenic rats and dextran sulphate treated mice. Imipenem given alone is without benefit (Videla et al., 1994). Imipenem is another non-absorbable antibiotic active against Gram-positive cocci, like vancomycin, but while it is also active against Gram-negative bacilli and some anaerobes, this drug is not currently used in human medicine.

Clindamycin is similarly active against Gram-positive cocci, but also against many anaerobes. It has very limited and specific uses in human infection because it is the antibiotic most likely to cause antibiotic associated diarrhoea. Thus it might be predicted that clindamycin has a profound effect on the microflora, and in the only study reported of its use in animals, it was found to be very effective in preventing ulceration in guinea pigs (Onderdonk and Bartlett, 1979).

Penicillins are virtually never used in animal models, but one study (Videla et al., 1994) has reported the use of amoxicillin with clavulanic acid by rectal enema in the rat TNBS model. This treatment was beneficial both acutely (7 days) and in the longer term (21 days). Amoxicillin is a broad spectrum penicillin that is well absorbed, diffuses readily through tissues and is active against both Gram-negative and Gram-positive species. It is inactivated by penicillinases, but when combined with the β-lactamase inhibitor clavulanic acid, the combination is active against β-lactamase producing species including *E. coli*, many bacteroides and klebsiella.

A currently popular antibiotic for gastrointestinal infection is ciprofloxacin, which is employed successfully in CD (Vermeire and Rutgeerts, 2001). This drug has been used in three animal models of colitis, and was clearly beneficial, especially in preventing disease (Madsen et al., 2000; Hans et al., 2000; Rath et al., 2001), but it was less effective in managing established colitis. Combination with metronidazole was also beneficial. Ciprofloxacin is a fluoroquinolone that is well absorbed and is active against Gram-negative facultative anaerobes and microaerophiles, such as salmonella, shigella and campylobacter, and it also has modest activity against Gram-positive organisms such as *Enterococcus faecalis*.

Is there any common theme or lesson to be learnt from these studies? To be effective an antibiotic for animal colitis should have activity against gut anaerobes. Those antibiotics that specifically target Gram-negative facultative species such as *E. coli* are not successful in IBD. Some combination of antibiotics, e.g. vancomycin and imipenem, seem better than the drugs given separately. This may simply reflect better combined anaerobe activity. Thus the most consistently effective drugs are metronidazole, clindamycin and ciprofloxacin, if given before onset of colitis, and the vancomycin/imipenem combination. Apart from their activity against anaerobes,
<table>
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<th>Antibiotic Use</th>
<th>Results</th>
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<tr>
<td>Guinea pig</td>
<td>Carrageenan 5%</td>
<td>Metronidazole prevented ulceration</td>
<td>Vanden Waaij et al., 1978</td>
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<tr>
<td>Mouse</td>
<td>IL-10 deficient</td>
<td>Ciprofloxacin both regimes prevented colitis when given early</td>
<td>Madsen et al., 2000</td>
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<tr>
<td>Mouse</td>
<td>DSS 5%</td>
<td>Metronidazole and neomycin and improved established colitis</td>
<td>Madsen et al., 2000</td>
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<tr>
<td>Rat</td>
<td>Caecal self-filling blind loop in SPF</td>
<td>Metronidazole attenuated inflammation and eliminated bacteroides</td>
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<tr>
<td>Mouse</td>
<td>BALB/c with DSS 5%</td>
<td>Ciprofloxacin partially beneficial in HLA-B27 prevention only</td>
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</tr>
<tr>
<td>Mouse</td>
<td>DSS 5%</td>
<td>Metronidazole</td>
<td>Hans et al., 2000</td>
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<tr>
<td>Mouse</td>
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<td>Hans et al., 2000</td>
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<tr>
<td>Rat</td>
<td>HLA-B27 transgenic</td>
<td>Metronidazole prevented colitis in both models but not when established</td>
<td>Rath et al., 2001</td>
</tr>
<tr>
<td>Mouse</td>
<td>SPF or populated with assorted species</td>
<td>All developed colitis</td>
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these antibiotics also target Gram-positive bacteria such as enterococci. Are these findings reflected in human studies of antibiotic use in UC?

**Human Studies in UC**

More than a dozen randomised controlled trials (RCT) of antibiotic use in UC have been published in recent years. Six antibiotics have been used, given orally in all but three studies, and singly, apart from one study (Table 2). Metronidazole has been the subject of four trials and given its value in animal models of UC, it might be hoped that it would be equally successful in humans. However, in only one of the four reported studies was metronidazole shown to be beneficial. Gilat et al., (1987, 1989) first treated 42 UC patients in an acute attack for 28 days with 1.35 grams metronidazole per day by mouth, and found no benefit over treatment with sulfasalazine in an RCT. Only 26% improved with antibiotic, versus 66% with sulfasalazine. They then followed the patients for one year, giving 0.6 grams metronidazole, or 2 grams sulfasalazine per day. Metronidazole was found to be “slightly more effective” after 12 months of treatment. No side effects were reported, which given the propensity for metronidazole to cause peripheral neuropathy in long term use, was reassuring. In addition to these studies by Gilat and co-workers, two other investigations with metronidazole have been reported, one of which was combined with tobramycin. Neither showed benefit. However, three of the four reported studies were short term, 5–28 days, treating acute attacks of colitis. In two investigations (Chapman et al., 1986; Mantzaris et al., 1994), the antibiotics were given intravenously. The use of intravenous antibiotic therapy in acute severe UC was first reported by Truelove and Jewell (1974), when tetracycline was incorporated into a bag containing glucose/saline, a parenteral feed, steroids and vitamins. Thirty six out of 49 patients improved rapidly, but this was not an RCT, and no conclusions regarding the efficacy of tetracycline can be drawn from it. As far as metronidazole is concerned, it would not appear to be of benefit in acute colitis, but the drug is worth testing in more long term studies.

Tobramycin appears to be more successful in acute attacks. Axon’s group (Burke et al., 1990) found 74% of patients in remission at 28 days, following a 7 day course of oral antibiotic, compared with 43% on placebo. The same group found no benefit for tobramycin at 12 and 24 months in 81 patients (Lobo et al., 1993). Overall therefore, tobramycin chosen principally for its effect against Gram-negative species such as *E. coli*, showed no consistent benefit.

The first RCT of antibiotics to treat UC was also done by the Leeds group (Dickinson et al., 1985) using vancomycin (2 grams given orally per day), which was chosen because of its benefits in antibiotic associated diarrhoea. In 33 cases of acute UC, there was no clear benefit although fewer came to surgery in the antibiotic group (2/18) compared to the placebo group (7/15).

Whilst the objective of using antibiotics in UC is to change the microflora beneficially, it is surprising that very few studies include reports of any bacteriology done on the patients. One of two studies in which ciprofloxacin has been used (Turunen et al., 1998), included semi-quantitative estimates of mucosal bacteria from endoscopic biopsies. They reported no enteric pathogens at the start of the study, and the disappearance of Gram-negative facultative anaerobes in the antibiotic group. After six months of ciprofloxacin treatment, the failure rate was 21% in the treated group, and 44% with the placebo (p = 0.02). However, endoscopic and histological changes were no different between these groups at six months, and there was no clinical benefit at one year. Serum IgG, IgM and IgA antibodies to *E. coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* were all higher at entry to the study in the UC patients. During treatment with ciprofloxacin, IgG antibodies to *E. coli* fell in the treated group and these bacteria disappeared from stools (Turunen et al., 1999).

The other two investigations with ciprofloxacin (Mantzaris et al., 1997, 2001) were in acute colitis of varying degree of severity, and they showed no benefit for the antibiotic.

One study with amoxicillin/clavulanic acid has been published (Casellas et al., 1998). The drug was given orally for 5 days to patients who had an acute attack without apparent benefit, except in the release of inflammatory mediators, quantitated by mucosal release of eicosanoids using rectal dialysis. A report published only in abstract some years ago (Danzi, 1989) suggests trimethoprim-sulphamethoxazole is of benefit in severe total UC, possibly because of its anti-folate (immunosuppressive) effect.

Rifaximin is a new generation antibiotic targeted at the gut, that is derived from rifamycin. It is non-absorbable (Gionchetti et al., 1997) and has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, as well as colonic anaerobes. Because of its activity against pathogens such as *E. coli*, salmonella and shigella, it is being used for traveller’s diarrhoea and appears to be as good as ciprofloxacin (DuPont et al., 1998; Steffen, 2001). Rifaximin is poorly absorbed, even in UC and pouchitis patients with inflamed mucosae (Rizzello et al., 1998; Gionchetti et al., 1999), and high concentrations can be found in stools (Jiang et al., 2000). This drug has been used successfully to treat pouchitis, in combination with ciprofloxacin (Gionchetti et al., 1999), and there are now two reports of its use in UC (Rizzello et al., 1997; Lukas et al., 2002). In an open label study, 31 patients with mild to moderate, predominately left sided UC, took 400 mg twice daily for 10 days. At 28 days, the clinical activity index and sigmoidoscopy scores were significantly better, and only two patients were worse (Lukas et al., 2002). In the Rizzello et al., (1997) RCT, the same dose was used in moderate to severely affected, steroid resistant UC patients. Sixty four percent of treated patients were substantially better versus 42% of the placebo group, and significant improvement was seen in stool frequency, rectal bleeding and sigmoidoscopy scores. In a separate study using this same group of patients, 1.8 grams of rifaximin were given daily for three treatment periods of 10 days, to 12 UC patients and the effects on the faecal microflora were characterised (Brigidi et al., 2002). The antibiotics suppressed lactobacilli, bifidobacteria, bacteroides and *Clostridium perfringens*, but the numbers returned to their initial values during the 25 day washout period. At the
<table>
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<th>Dose/day</th>
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<tr>
<td>Metronidazole</td>
<td>1.5 g</td>
<td>I-V</td>
<td>5 days</td>
<td>39</td>
<td>RCT severe UC</td>
<td>No benefit</td>
<td>Chapman et al., 1986</td>
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<tr>
<td>Metronidazole</td>
<td>1.35 g</td>
<td>Oral</td>
<td>28 days</td>
<td>42</td>
<td>RCT v. salazopyrine</td>
<td>No benefit</td>
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<td>4.5 g, Acute attack</td>
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<tr>
<td>Metronidazole</td>
<td>0.6 g</td>
<td>Oral</td>
<td>12 months</td>
<td>33</td>
<td>RCT v. salazopyrine 2 g, Maintenance of remission</td>
<td>Benefit at 12 months, also longer remission</td>
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</tr>
<tr>
<td>Tobramycin and metronidazole</td>
<td>12 mg/kg</td>
<td>I-V</td>
<td>10 days</td>
<td>39</td>
<td>RCT Acute severe UC</td>
<td>No benefit</td>
<td>Mantzaris et al., 1994</td>
</tr>
<tr>
<td></td>
<td>1.5 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td></td>
<td>Oral</td>
<td>7 days</td>
<td>84</td>
<td>RCT Acute UC At 28 days tobramycin better</td>
<td>Burke et al., 1990</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2 g</td>
<td>Oral</td>
<td>7 days</td>
<td>81</td>
<td>RCT Long-term follow up</td>
<td>No benefit at 12 or 24 months</td>
<td>Dickinson et al., 1985</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1-1.5 g</td>
<td>Oral</td>
<td>6 months</td>
<td>83</td>
<td>RCT</td>
<td>Benefit at 6 months, but not at 12</td>
<td>Turunen et al., 1998</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>800 mg</td>
<td>I-V</td>
<td>10 days</td>
<td>39</td>
<td>RCT Acute severe UC</td>
<td>No benefit</td>
<td>Mantzaris et al., 2001</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>500 mg</td>
<td>Oral</td>
<td>14 days</td>
<td>70</td>
<td>RCT Mild or moderate acute UC</td>
<td>No benefit</td>
<td>Mantzaris et al., 1997</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>2.25 g</td>
<td>Oral</td>
<td>5 days</td>
<td>30</td>
<td>RCT Acute attack</td>
<td>Release of inflammatory mediators reduced</td>
<td>Casellas et al., 1998</td>
</tr>
<tr>
<td>Rifamixin</td>
<td>800 mg</td>
<td>Oral</td>
<td>10 days</td>
<td>28</td>
<td>RCT Moderate to severe steroid resistant UC</td>
<td>Substantial clinical and endoscopic benefit</td>
<td>Rizzello et al., 1997</td>
</tr>
<tr>
<td>Rifamixin</td>
<td>800 mg</td>
<td>Oral</td>
<td>10 days</td>
<td>31</td>
<td>Open label Active UC</td>
<td>Effective in patients with mild to moderate left-sided UC</td>
<td>Lukas et al., 2002</td>
</tr>
</tbody>
</table>

**Abbreviations**

IV Intravenous
RCT Randomised controlled trial
UC Ulcerative colitis
Table 3. Problems associated with antibiotic use in ulcerative colitis. Modified from Cummings and Macfarlane (2001)

<table>
<thead>
<tr>
<th>Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria involved not known, therefore no antibiotic sensitivities</td>
</tr>
<tr>
<td>Bacteria may be part of a biofilm consortium on the mucosal surface</td>
</tr>
<tr>
<td>Therapeutic levels of antibiotics may not be reached at target site</td>
</tr>
<tr>
<td>Resistance developing, especially with long term use</td>
</tr>
<tr>
<td>Effect of antibiotic on microflora and barrier resistance to pathogens</td>
</tr>
<tr>
<td>Timing of use. Animal studies suggest antibiotics need to be given</td>
</tr>
<tr>
<td>before onset of inflammation</td>
</tr>
<tr>
<td>Systemic side-effects</td>
</tr>
</tbody>
</table>

The present time, rifamixin has the best record for treating UC, but long term studies are clearly needed to fully evaluate its worth.

Antibiotics are not used routinely in clinical practice for either the acute attack or maintenance of remission in UC. Evidence for their effectiveness in both animal studies and human RCT’s is less than compelling. Why should this be the case when so much evidence points to the gut microflora playing an essential role as a causative agents in the inflammatory process in UC, and when antibiotics are used so successfully in CD and pouchitis?

Firstly, we do not as yet know precisely what bacteria, or groups of bacteria are principally involved in UC (see Table 3). Over the years many different species have been implicated, but none have stood the test of time (Cummings and Macfarlane, 2001). Studies of faecal bacteria in UC have been largely unhelpful, possibly because of the many hours of work required to characterise these microfloras, in even a simple way. The investigator is faced with finding a needle in a haystack, although new techniques for identifying bacteria by chemotaxonomy and molecular typing may be more productive. What physician would treat an infection, especially long term, without knowing the causative agent and its antibiotic sensitivities?

Another important reason why antibiotics do not apparently work in UC is that the causative bacteria may well be present on the epithelial surface, as part of a biofilm community. In this state they are more resistant to antibiotics (Anwar et al., 1990). Furthermore, we do not know if therapeutic levels of antibiotics reach the mucosal interface with biofilm bacteria. Many antibiotics used, such as tobramycin, rifamixin and vancomycin are not absorbed. Conversely, well absorbed antibiotics such as metronidazole achieve only very low concentrations in lumenal contents in the large bowel.

Other important problems concern the development of bacterial antibiotic resistance, with prolonged use, and systemic side effects which are more likely long-term. Timing is another important factor to take into consideration. Animal studies suggest that most benefit is achieved if antibiotics are given before the onset of colitis. This is clearly not possible in UC, but it may be an argument for maintenance antibiotic therapy in patients who are in remission.

Finally, most current antibiotics are active against several bacterial species, and their capacity to breakdown the normal barrier function of the microflora in the gut is well known. Antibiotics, unless very specifically targeted, could just get rid of one problem species, while facilitating the introduction of others. A more effective long-term treatment may lie in the use of probiotics.

Probiotics in UC and Pouchitis

Surprisingly few studies have been reported on the effects of probiotics in UC. However, a probiotic mixture comprising three bifidobacteria, four lactobacilli and a streptococcus has been used in an uncontrolled investigation with 20 UC patients in remission, over a 12 month period (Venturi et al., 1999). Large doses of probiotic were involved (six grams were given per day, with bacterial counts corresponding to 5 x 10^11 per gram). Microbiological analysis of faeces showed high numbers of probiotic bacteria during feeding, although total anaerobic and aerobic counts, together with clostridia, bacteroides, enterobacteria were not significantly affected. Fifteen of 20 patients were maintained in remission during probiotic feeding, while four relapsed. This probiotic was subsequently used in an RCT where 17 out of 20 pouchitis patients remained in remission for nine months while taking the probiotic, all of whom relapsed within a few months after the treatment was stopped (Campieri and Gionchetti, 1999).

Lactobacilli have been reported to prevent colitis in IL-10 deficient mice (Madsen et al., 1999) and to reduce colitis in rats induced with either acetic acid (Fabia et al., 1993a) or methotrexate (Mao et al., 1996). In studies with Lactobacillus plantarum, the bacterium was shown to stabilise the gut mucosal barrier in IL-10 deficient mice with colitis (Kennedy et al., 2000), whereas when Lactobacillus plantarum 299V was used to prevent and treat spontaneous colitis in the IL-10 mouse model of colitis (Schultz et al., 2002), the probiotic reduced mucosal IgG, interferon-γ and IL-12, and attenuated established inflammatory processes.

Reduced numbers of lactobacilli in colonic biopsies were found in pouchitis patients (Fabia et al., 1993b), while pouchitis patients were reported to have low numbers of lactobacilli and bifidobacteria in gut contents by Ruseler van Embden et al., (1994), suggesting that they may a protective role in UC.

In two RCT, a non-pathogenic E. coli was compared with mesalazine in patients either in remission, over 12 weeks, or after relapse, over 12 months. Both studies found the probiotic equally as effective as mesalazine in preventing relapse.

To date, probiotic studies in UC have provided little insight into the microbiological and immunological mechanisms involved in inflammatory bowel diseases. While probiotics and possibly prebiotics do show some promise as therapies in IBD, until more is known about the mucosa-associated microflora and the mechanisms of inflammation, their employment will remain largely empirical.
References


Fox, J.G., Dewhirst, F.E., Fraser, G.J., Paster, B.J., Shames, B. and Murphy, J.C. 1994. Intracellular Campylobacter-like organism from ferrets and hamsters.


