Role of Antibiotics in the Management of Inflammatory Bowel Disease: A Review

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The current model of pathogenesis for inflammatory bowel disease (IBD) is a dysregulated immune system that is triggered by an environmental factor in a genetically susceptible individual. Although much about this model remains unproven, it is believed that bacteria are often the environmental factor driving the inflammatory response. This is supported by indirect evidence that antibiotics are of benefit in the treatment of Crohn's disease (CD) and pouchitis, and observations that enteric infections may result in activation of ulcerative colitis disease activity. In CD, limited studies have demonstrated that metronidazole, ciprofloxacin, and rifaximin improve clinical disease activity, and this is more pronounced in the treatment of colonic disease and for perianal fistulas (with metronidazole and ciprofloxacin). In addition, limited evidence supports the use of metronidazole in the prevention of recurrence after resection in CD. Antibiotics have not shown substantial benefit in the treatment of ulcerative colitis, but a variety of antimicrobial agents have a definite role in the treatment of acute and recurrent or chronic pouchitis. The absence of specifically identified organisms that are primarily responsible for the observed clinical picture remains the challenge to confirming the relationship between bacteria and IBD. A proposal for future therapies is provided that might include a combination therapy aimed at both a reduction in pathogenetic bacteria and immune modulation to achieve the most durable remission of disease.
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Inflammatory bowel disease (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are characterized by a waxing and waning chronic inflammation of the intestinal tract. Although the cause of these conditions remains incompletely understood, the prevailing etiologic hypothesis favors a dysregulated immune system that is triggered by an environmental factor in a genetically susceptible indi-

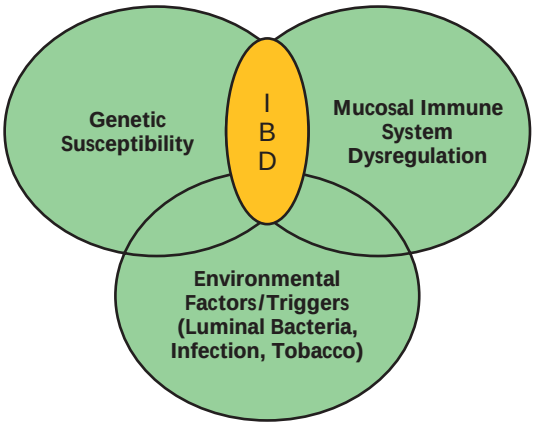

Figure 1. *A pathogenetic model of chronic inflammatory bowel disease (IBD).*

Table 1
Infectious Complications of IBD Requiring Antibiotics

- Intra-abdominal and perianal abscesses, inflammatory masses
- Perianal fistulas and fissures
- Small intestinal bacterial overgrowth
- Postoperative infections
- Toxic megacolon and peritonitis
- *Clostridium difficile* infection

IBD, inflammatory bowel disease.

vidual (see Figure 1). There is indirect evidence in humans that commensal or pathogenic bacteria play a significant role as the environmental trigger.

This evidence, in part, consists of case reports of the onset of IBD after infectious gastroenteritis, infectious models of chronic disease, and a growing understanding of the genetic underpinnings of some types of IBD. This includes the fact that in a subset of CD, CARD15 alleles on chromosome 16 code for a toll-like receptor protein that recognizes bacterial peptidoglycan-derived muramyl dipeptides and secretes peptides (defensins) related to protection of the host. Alterations in this gene may result in an abnormal host defense and subsequent disease.[1]

Proof of Bacteria Causing IBD: Koch's Postulates

Scientist Robert Koch outlined a set of postulates that need to be satisfied in order to document a microbial cause of disease. These include 1) the presence of the infecting organism in every case of the disease, 2) no other disease in which the infectious organism is found incidentally, and 3) inducement of disease by inoculating a healthy host with the purified infecting organism.[2] At this time, the evidence for the role of bacteria in the etiopathogenesis of IBD remains primarily indirect as Koch's postulates have not been fulfilled. This purported association is instead based on observational data related to enteric infections and the onset of disease, experiments in animal models, and improvement of disease activity with antibiotic therapy.

There is evidence that anaerobic bacteria and *Enterobacteriaceae* adhere to the bowel more in IBD patients than in controls, and a genetically-altered animal model has demonstrated a variety of intriguing behaviors when exposed to bacteria. The genetically-modified rodent does not develop colitis if maintained in a germ-free environment, but rapidly develops colitis when commensal bacteria are introduced. Antibiotics treat and prevent this model of disease in these animals.[3]

Goals of IBD Treatment: The Role of Antibiotics

Antibiotic therapy is used for the infectious complications of IBD that occur as a result of transmural inflammation, penetrating or fistulizing disease, or secondary bacterial infections (see Table 1). This review focuses on the use of antibiotics to treat active IBD. Treatment goals include induction of remission of active inflammation and subsequent maintenance of remission. Therapies for remission induction need to be of rapid onset and broadly effective, as well as safe. Maintenance therapies should be effective and safe for long-term use.

Crohn's Disease: Treatment of Active Disease

A number of studies were designed to examine the question of remission induction of active CD with antibiotics, but few have shown maintenance benefits of antibiotics. The greatest amount of controlled and open label evidence is for metronidazole, which has been shown to be effective for active CD as well as perianal disease. In a study by Sutherland and colleagues,[4] metronidazole (20 mg/kg or 10 mg/kg) was compared to placebo for active CD for 16 weeks. The primary endpoint was change in the Crohn's Disease Activity Index (CDAI). Although 105 patients entered the study, only 56 completed it, due to drop-out secondary to worsening disease activity or adverse side effects. Of those who completed this trial, the benefit was greatest if they had CD of the colon, compared to CD of the ileum. However, improvement in the CDAI was not substantial enough to be considered a state of remission (< 150) in most patients.

In 1982, Ursing and colleagues[5] studied 78 CD patients with

metronidazole or sulfasalazine for 4 months, and found no difference in outcomes between study groups.

In 1996, Prantera and colleagues[6] described the outcome of a study of a combination of metronidazole 250 mg 4 times daily plus ciprofloxacin 500 mg twice daily versus methylprednisolone 0.7-1 mg/kg/day and a steroid taper for active CD in 41 patients for 12 weeks. Although there was a numerical difference in efficacy between study arms, it was not statistically significant, but it did suggest that the combination of antibiotics may be as effective as steroids in these patients. (This study was not powered to show equivalence or noninferiority.) The clinical implication is that induction of remission of active CD with antibiotics may be as effective as corticosteroids and may obviate the need for their use along with their accompanying side effects in some of these patients.

Small, open label trials of metronidazole for maintenance of CD remission from 6 months to as long as 36 months have been uniformly positive.[7-10] However, use of metronidazole has been limited by side effects of nausea, dysgeusia, "furry" tongue, and a dose- and time-related peripheral neuropathy. Ciprofloxacin, although less studied than metronidazole for active CD, also has side effects limiting its safety, including spontaneous rupture of tendons.

Most recently, fluoroquinolones were reported to be associated with complicated *Clostridium difficile*-associated disease, including colectomy and death.[11] A new gut-specific nonabsorbed antibiotic, rifaximin, has been studied open label in 29 CD patients with a mean baseline CDAI of 278 ± 51. It was shown to induce remission (CDAI < 150) at the 4-month endpoint in 59% of patients. This antibiotic's nonsystemic nature also provided a favorable side-effect profile.[12]

In one of the few combination therapy trials, Steinhart and colleagues[13] performed a randomized, double-blind, parallel group study of metronidazole (500 mg bid) plus ciprofloxacin (500 mg bid) plus ileal-release budesonide (9 mg/d) or placebo plus budesonide in 130 patients with active CD for 8 weeks. There was no difference between groups in the overall outcome, but there was a small favorable benefit in some subgroups analyzed. Patients with colonic disease did show benefit with the antibiotics.No trials have compared antibiotics to infliximab or immune modulators.

Use of metronidazole for maintenance of Crohn's disease remission has been limited by side effects of nausea, dysgeusia, "furry" tongue, and a dose- and time-related peripheral neuropathy.

Whether an atypical mycobacterium may be the cause of some cases of CD has elicited interest. However, multiple studies with different combinations of antimycobacterial therapies have been negative. Larger placebo-controlled trials testing this hypothesis are ongoing.[3]

Crohn's Disease: Treatment of Perianal Disease

There are no controlled trials studying antibiotics for the treatment of perianal fistulas, yet this management strategy is supported by open-label data and is widely used and accepted. In the Bernstein open-label experience with metronidazole (20 mg/kg/d), 83% of 21 patients had fistula closure.[10] Turunen and colleagues[14] treated 8 patients with open-label ciprofloxacin (1-1.5 g/d) for 3 to 6 months and reported improvement of disease activity.[14] Open-label combination therapy with both metronidazole and ciprofloxacin also has been effective in small numbers of patients.

In all of these experiences, antibiotic cessation resulted in recurrence of the draining fistulas. Assessment of long-term outcomes of antibiotic therapy in perianal CD and controlled trials are still needed.

Table 2
Indications for Surgery in Crohn's Disease

- Failure of medical therapy, including steroid dependence
- Bowel obstruction resistant to medical therapy
- Fistula or abscess (including recto-vaginal)
- Hemorrhage
- Growth retardation (children)
- Perforation
- Cancer

Crohn's Disease: Prevention of Postoperative Recurrence

Unfortunately, up to 92% of CD patients will require surgery for a variety of reasons[15] (see Tables 2 and 3). A fascinating phenomenon is that it recurs at the site of the anastomosis, but in most patients, it remains in a durable remission if the fecal stream is diverted. In a study of the natural history of postoperative patients, Rutgeerts and colleagues[16] observed that the 1-year endoscopic recurrence was 73% and symptomatic occurrence was 20%, with an increase to 85% and 34%, respectively, at 3 years.

Therefore, it is important that we discover the mechanism of postoperative recurrence and develop effective strategies for its prevention. In an

Table 3
Factors Associated With Postoperative Recurrence in CD

- Smoking
- Perforating-type of disease behavior
- Small-bowel disease
- Ileocolonic disease
- Perianal fistulas
- Young age of first resection

CD, Crohn's disease.

elegant study, D'Haens and colleagues[17] describe their experience with patients who had surgical resection with a temporary loop ileostomy in order to study the effects of diversion as well as re-infusion of effluent into the efferent limbs. While diverted, the ileocolonic anastomosis remained free of endoscopic recurrence. Take-down of the ileostomy resulted in endoscopic recurrence in less than 1 month, and, perhaps most interesting, infusion of the contents from the afferent loop into the efferent small bowel resulted in evidence of inflammation within 1 week.

Studies of Antibiotics to Prevent or to Delay Postoperative Recurrence

Rutgeerts and colleagues[18] administered metronidazole (20 mg/kg/d) or placebo to 60 patients after ileocecal resection for 3 months and followed these patients subsequently for endoscopic and clinical recurrence. Although the study was limited by patient drop-out due to adverse side effects from the metronidazole, there was less endoscopic recurrence at 3 months (52% vs 75% $P = .09$) and significantly less clinical recurrence at 1 year in the metronidazole arm than in the control arm (4% vs 25%, $P \leq .044$). By 2 and 3 years, this clinical benefit was lost.

Recently, the same investigators published the results of a metronidazole relative, ornidazole (1 g/d) versus placebo for 1 year in CD patients postoperatively.[19] Again, at 1 year the endoscopic and clinical recurrence rates were significantly less in the antibiotic-treated group than in the placebo group (54% vs 79%, $P = .036$; 8% vs 37%, $P = .002$). The clinical benefit again was lost by 2 and 3 years.

These pivotal trials suggest that antibiotics are an effective short-term treatment strategy for the prevention of postoperative recurrence in CD, but that effective, longer-term therapy will be required. In order to consider long-term antibiotic use, safety, tolerability, and issues of selective resistance need to be considered.

Treatment of Ulcerative Colitis

One of the first effective therapies for UC was sulfasalazine, which is composed of an antibacterial sulfa moiety bound to the anti-inflammatory molecule 5-aminosalicylic acid (5-ASA). It was believed that the antibacterial action of sulfasalazine explained the observed benefit, until later studies demonstrated a stronger effect of the 5-ASA component alone. Nonetheless, the interest in treating UC with antibiotics remained.

Crohn's disease recurs at the site of anastomosis, and in most patients remains in a durable remission if the fecal stream is diverted.

There have been a number of clinical trials assessing antibiotic therapy for UC, with mixed short- and long-term results. Dickinson and colleagues[20] compared vancomycin 500 mg IV every 6 hours to placebo in 33 patients with severe UC and in 7 patients with severe Crohn's colitis, and did not see a significant difference in avoidance of colectomy between the groups, although post-hoc analysis showed the UC patients trending toward benefit with vancomycin ($P = .06$).

Chapman and colleagues[21] subsequently published their placebo-controlled experience with IV metronidazole 500 mg every 8 hours in 39 patients with severe UC, and did not see a significant difference in clinical improvement at 5 days. Mantzaris and colleagues[22] studied 39 patients with severe UC treated with metronidazole plus tobramycin versus placebo, and also found no significant difference at 10 days.

A subsequent study of 81 patients with mild, moderate, or severe UC receiving oral tobramycin 120 mg 3 times daily or placebo for 1 week demonstrated a significantly greater "clinical improvement" at 3 to 4 weeks compared to placebo (80% vs 40%, $P = .008$), but these differences were lost at 1-year follow-up.[23,24]

Both oral and IV ciprofloxacin have been studied compared to placebo for mild to moderate or severe UC, but there was no difference between drug and placebo in these trials.[25,26] Turunen[27] showed in a 6-month placebo-controlled trial of oral ciprofloxacin 500 mg twice daily or 750 mg twice daily that the ciprofloxacin-treated patients had a significantly better response, but the study was limited by a lack of control for other medications and variables.

In Italy, Gionchetti and colleagues[28] studied rifaximin 400 mg twice daily versus placebo for 10 days in UC, and did not see a significant difference. Given the available evidence, however, there does not appear to be a consistent response to antibiotics in UC, and the explanation for this difference compared to CD is not known.

Treatment of Pouchitis

The surgical treatment of UC usually involves creation of a continent reservoir of distal small bowel, and this procedure may result in a distinct inflammatory condition that has become known as pouchitis. It is characterized by symptoms of urgency and increased stool frequency and may be diagnosed clinically. Newer definitions, however, incorporate endoscopic and histologic findings because the differential diagnosis of pouchitis may include undiagnosed CD or even functional abnormalities.[29] Pouchitis has a cumulative risk in patients who have had surgery for UC of as high as 48% at 10 years follow-up, but only 10% to 15% will have chronic pouchitis characterized by recurrent active inflammation and a need for maintenance therapies.[30]

Although the pathogenesis of pouchitis remains incompletely understood, it has been shown to be quite responsive to antibiotic therapy in both open-label and more recent controlled trials. In addition, although there is some evidence for the role of probiotics in the treatment of pouchitis, the specific agents and doses and duration of therapies have not yet been adequately described.

Madden and colleagues[31] performed a double-blind crossover study of 13 patients with active pouchitis who received metronidazole 400 mg 3 times daily versus placebo and found a substantial response to metronidazole. Hurst and colleagues[32] described the results of an open label study of 52 patients with active pouchitis treated with metronidazole 250 mg 3 times daily for 7 days. Failure to respond was then treated with ciprofloxacin 500 mg twice daily. Among the patients, 41 out of 52 (79%) responded to the metronidazole alone, and 8 out of 11 (73%) for whom metronidazole failed responded to ciprofloxacin.

Gionchetti[28] studied 18 patients with chronic pouchitis who were no longer responsive to single antibiotics by treating them with rifaximin 1000 mg twice daily plus ciprofloxacin 500 mg twice daily for 15 days. It was noted that of the 16 patients who improved, 6 achieved remission. The patients showed significant decreases in fecal bacterial counts.

Shen and colleagues[33] performed a randomized, unblinded trial comparing ciprofloxacin 1000 mg/day and metronidazole 20 mg/kg/day for 2 weeks. Both groups had significant reductions in disease activity at 4 weeks, but 33% of the metronidazole group experienced dysgeusia, nausea, and transient neuropathy. Mimura[34] performed an open-label, uncontrolled study of metronidazole 400-500 mg twice daily plus ciprofloxacin 500 mg twice daily for 28 days in 44 patients with refractory or recurrent pouchitis. All patients improved, and 82% achieved remission.

Summary

Previous studies and emerging research support the current theory of IBD in which commensal or pathogenic bacteria are a driving force for the abnormal mucosal immune response. Antibiotics have been shown to improve disease activity in luminal CD, perianal fistulas, and in the prevention of postoperative recurrence in the short term. Although antibiotic therapy has not demonstrated a consistent effect for UC, antibiotics are extremely effective in treating acute, recurrent, and chronic pouchitis.

The ideal approach to long-term management of inflammatory bowel disease should involve a safe, well-tolerated therapy that maintains a balance of commensal organisms while decreasing mucosal inflammation.

Limits to current therapeutic strategies include the need for more controlled data and studies of longer duration. In addition, side effects of metronidazole and ciprofloxacin and

Main Points

- Although the cause of inflammatory bowel disease, Crohn's disease (CD), and ulcerative colitis (UC) is not completely understood, the current theory is that a dysregulated immune system is triggered by commensal or pathogenic bacteria.
- Studies have examined the induction of remission of active CD with antibiotics, although few have shown maintenance benefits. Metronidazole has been shown to be effective for active CD as well as perianal disease.
- Although antibiotic therapy has not demonstrated a consistent effect for UC, antibiotics are extremely effective for treatment of acute, recurrent, and chronic pouchitis.
- The side effects of metronidazole and ciprofloxacin and concerns about potential resistance limit their long-term use.
- A proposed approach for achieving disease remission is combination therapy with antimicrobials and immune modulators, with the possibility of supplementation with probiotics.

concerns about potential resistance limit their long-term use. The ideal approach to long-term management of IBD should involve a safe, well-tolerated therapy that maintains a balance of commensal organisms while decreasing mucosal inflammation. One proposed approach is combination therapy with antimicrobials to reduce the antigenic drive along with immune modulators to modify the dysregulated response, with the possibility of supplementation with probiotics. Further study with novel and existing agents is needed. ■

References

1. Abreu MT. Nod2 in normal and abnormal intestinal immune function. *Gastroenterology.* 2005;129:1302-1304.
2. Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin Microbiol Rev.* 1996;9:18-33.
3. Isaacs KL, Sartor RB. Treatment of inflammatory bowel disease with antibiotics. *Gastroenterol Clin North Am*. 2004;33:335-45, x.
4. Sutherland L, Singleton J, Sessions J, et al. Double blind, placebo controlled trial of metronidazole in Crohn's disease. *Gut*. 1991;32:1071-1075.
5. Ursing B, Alm T, Barany F, et al. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: the cooperative Crohn's disease study in Sweden. II. Result. *Gastroenterology.* 1982;83:550-562.
6. Prantera C, Zannoni F, Scribano ML, et al. An antibiotic regimen for the treatment of active Crohn's disease: a randomized, controlled clinical trial of metronidazole plus ciprofloxacin. *Am J Gastroenterol*. 1996;91:328-332.
7. Gugler R, Jensen JC, Schulte H, Vogel R. [The course of Crohn disease and side effect profile with long-term treatment using metronidazole]. *Z Gastroenterol.* 1989;27:676-682.
8. Jakobovits J, Schuster MM. Metronidazole therapy for Crohn's disease and associated fistulae. *Am J Gastroenterol.* 1984;79:533-540.
9. Brandt LJ, Bernstein LH, Boley SJ, Frank MS. Metronidazole therapy for perineal Crohn's disease: a follow-up study. *Gastroenterology.* 1982;83:383-387.
10. Bernstein LH, Frank MS, Brandt LJ, Boley SJ. Healing of perineal Crohn's disease with metronidazole. *Gastroenterology.* 1980;79:599.
11. Muto CA, Pokrywka M, Shutt K, et al. A large outbreak of *Clostridium difficile*-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. *Infect Control Hosp Epidemiol*. 2005;26:273-280.
12. Shafran I, Johnson LK. An open-label evaluation of rifaximin in the treatment of active Crohn's disease. *Curr Med Res Opin*. 2005;21:1165-1169.
13. Steinhart AH, Feagan BG, Wong CJ, et al. Combined budesonide and antibiotic therapy for active Crohn's disease: a randomized controlled trial. *Gastroenterology*. 2002;123:33-40.
14. Turunen UM, Farkkila MA, Seppala K. Long-term treatment of perianal or fistulous Crohn's disease with ciprofloxacin. *Scand J Gastroenterol*. 1989;24:144.
15. Farmer RG, Whelan G, Fazio VW. Long-term follow-up of patients with Crohn's disease. Relationship between the clinical pattern and prognosis. *Gastroenterology.* 1985;88:1818-1825.
16. Rutgeerts P, Geboes K, VanTrappen G, et al. Predictability of the postoperative course of Crohn's disease. *Gastroenterology.* 1990;99:956-963.
17. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology.* 1998;114:262-267.
18. Rutgeerts P, Hiele M, Geboes K, et al. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology*. 1995;108:1617-1621.
19. Rutgeerts P, Van Assche G, Vermeire S, et al. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology.* 2005;128:856-861.
20. Dickinson RJ, O'Connor HJ, Pinder I, et al. Double blind controlled trial of oral vancomycin as adjunctive treatment in acute exacerbations of idiopathic colitis. *Gut*. 1985; 26:1380-1384.
21. Chapman RW, Selby WS, Jewell DP. Controlled trial of intravenous metronidazole as an adjunct to corticosteroids in severe ulcerative colitis. *Gut*. 1986;27:1210-1212.
22. Mantzaris GJ, Hatzis A, Kontogiannis P, Triadaphyllou G. Intravenous tobramycin and metronidazole as an adjunct to corticosteroids in acute, severe ulcerative colitis. *Am J Gastroenterol*. 1994;89:43-46.
23. Burke DA, Axon AT, Clayden SA, et al. The efficacy of tobramycin in the treatment of ulcerative colitis. *Aliment Pharmacol Ther*. 1990;4:123-129.
24. Lobo AJ, Burke DA, Sobala GM, Axon AT. Oral tobramycin in ulcerative colitis: effect on maintenance of remission. *Aliment Pharmacol Ther.* 1993;7:155-158.
25. Mantzaris GJ, Petraki K, Archavlis E, et al. A prospective randomized controlled trial of intravenous ciprofloxacin as an adjunct to corticosteroids in acute, severe ulcerative colitis. *Scand J Gastroenterol.* 2001;36:971-974.
26. Mantzaris GJ, Archavlis E, Christoforidis P, et al. A prospective randomized controlled trial of oral ciprofloxacin in acute ulcerative colitis. *Am J Gastroenterol*. 1997;92:454-456.
27. Turunen UM, Farkkila MA, Hakala K, et al. Long-term treatment of ulcerative colitis with ciprofloxacin: a prospective, double-blind, placebo-controlled study. *Gastroenterology.* 1998;115:1072-1078.
28. Gionchetti P, Rizzello F, Ferrieri A, et al. Rifaximin in patients with moderate or severe ulcerative colitis refractory to steroid-treatment: a double-blind, placebo-controlled trial. *Dig Dis Sci*.1999;44:1220-1221.
29. Shen B, Achkar JP, Lashner BA, et al. Endoscopic and histologic evaluation together with symptom assessment are required to diagnose pouchitis. *Gastroenterology*. 2001;121:261-267.
30. Meagher AP, Farouk R, Dozois RR, et al. J ileal pouch-anal anastomosis for chronic ulcerative colitis: complications and long-term outcome in 1310 patients. *Br J Surg.* 1998;85:800-803.
31. Madden MV, McIntyre AS, Nicholls RJ. Double-blind crossover trial of metronidazole versus placebo in chronic unremitting pouchitis. *Dig Dis Sci*. 1994;39:1193-1196.
32. Hurst RD, Chung TP, Rubin M, Michelassi F. The implications of acute pouchitis on the long-term functional results after restorative proctocolectomy. *Inflamm Bowel Dis*. 1998;4:280-284.
33. Shen B, Achkar JP, Lasner BA, et al. A randomized clinical trial of ciprofloxacin and metronidazole to treat acute pouchitis. *Inflamm Bowel Dis*. 2001;7:301-305.
34. Mimura T, Rizzello F, Helwig U, et al. Four-week open-label trial of metronidazole and ciprofloxacin for the treatment of recurrent or refractory pouchitis. *Aliment Pharmacol Ther*. 2002;16:909-917.