Newly Developed Antibiotic Combination Therapy for Ulcerative Colitis: A Double-Blind Placebo-Controlled Multicenter Trial

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OBJECTIVES: Fusobacterium varium may contribute to ulcerative colitis (UC). We conducted a double-blind placebo-controlled multicenter trial to determine whether antibiotic combination therapy induces and/or maintains remission of active UC.

METHODS: Patients with chronic mild-to-severe relapsing UC were randomly assigned to oral amoxicillin 1500 mg/day, tetracycline 1500 mg/day, and metronidazole 750 mg/day, vs. placebo, for 2 weeks, and then followed up. The primary study end point was clinical response (Mayo score at 3 months after treatment completion) and secondary end points were clinical and endoscopic score improvements at 12 months. Anti-F. varium antibodies were measured by enzyme-linked immunosorbent assay.

RESULTS: Treatment and placebo groups each had 105 subjects. At the primary end point, response rates were significantly greater with antibiotics than with placebo (44.8 vs. 22.8%, \( P = 0.0011 \)). Endoscopic scores significantly improved at 3 months (\( P = 0.002 \) vs. placebo). Remission rates were 19.0% (antibiotics) vs. 15.8% (placebo) at 3 months (\( P = 0.59 \)). At the secondary end point, response rates were significantly greater with antibiotics than with placebo (49.5 vs. 21.8%, respectively, \( P < 0.0001 \)). Endoscopic scores were significantly improved at 12 months after antibiotic treatment (\( P = 0.002 \) vs. placebo). Remission rates had improved to 26.7% with antibiotics vs. 14.9% for placebo, at 12 months (\( P = 0.041 \)). \( F. \) varium antibody titers decreased in responders but not in nonresponders, and more in the antibiotic than in the placebo group. More pretreatment steroid-dependent UC patients discontinued corticosteroids after treatment completion (6 months: 28.6 vs. 11.8%, respectively, \( P = 0.046 \); 9 months: 34.7 vs. 13.7%, respectively, \( P = 0.019 \); and 12 months: 34.7 vs. 13.7%, respectively, \( P = 0.019 \)). These effects were greater in the subanalysis of the active group (Mayo scores of 6–12) than in that of total cases (0–12). No serious drug-related toxicities occurred.

CONCLUSIONS: The 2-week triple antibiotic therapy produced improvement, remission, and steroid withdrawal in active UC more effectively than a placebo.

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INTRODUCTION

Evidence supporting a pathophysiological role for luminal bacteria in mucosal inflammation has been obtained from models such as non-germ-free, cytokine (interleukin-2 or -10)-knockout and other related gene-knockout mice that developed colitis (1–8). Recently, it was reported that a novel mouse line with defects in both transforming growth factor-β type II receptor and interleukin-10 receptor 2 signaling, as well as the T-bet−/−/RAG2−/− mouse model, rapidly and reproducibly developed a disease resembling severe fulminant human ulcerative colitis (UC) (9,10). These disease processes were completely inhibited by a combination of broad-spectrum antibiotics. Microbial agents have long been implicated in the initiation and/or exacerbation of UC in humans, suggesting a possible rationale for antibiotic treatment of UC. Although antibiotic therapy is supported by both clinical and experimental evidence, antibacterial therapy trials have produced conflicting results (11–20).

In 2002, Fusobacterium varium was reported to be present in the colonic mucosa of a high proportion (84%) of UC patients (21). Butyric acid, a product of F. varium culture supernatants, was also shown to cause UC-like lesions in mice (22). On the basis of these observations, we used an antibiotic combination regimen to which F. varium was susceptible (amoxicillin, tetracycline, and metronidazole (ATM)) and showed in a randomized controlled multicenter trial of this combination antibiotic treatment of UC. Although combination antibiotic therapy is supported by both clinical and experimental evidence, antibacterial therapy trials have produced conflicting results (11–20).

Study design

Patients were randomly assigned to either the ATM or the control group. The study used central randomization with a treatment allocation produced by a computer-generated randomization list. Randomization was carried out using numbered containers. The ATM group received a 2-week combination therapy consisting of amoxicillin 500 mg t.i.d., tetracycline 500 mg t.i.d, and metronidazole 250 mg t.i.d. These antibiotics were selected on the basis of F. varium susceptibility test results (23). The controls received three placebo preparations consisting of identical-appearing capsules and tablets containing sugar. Patients received identical drugs in identical bottles and remained blinded throughout the study. All clinical evaluations and endoscopies were carried out by clinicians who were blinded to the patients’ therapy. Compliance and adverse events were checked after 2 weeks of combination therapy. Doses of any concomitant oral or rectal medication for UC remained constant, except for corticosteroids, which were tapered after week 8 by 5 mg/week until it reached a dose of 20 mg/day. Thereafter, the dose was reduced by 2.5 mg/week until discontinuation. Corticosteroid discontinuation for more than 3 months in steroid-dependent UC was defined as steroid withdrawal. Steroid withdrawal rates at 6, 9, and 12 months after treatment completion were calculated.

Patients came to the hospital weekly or monthly, depending on their personal schedules and severity of their symptoms, and were assessed by clinical examination and total colonoscopy at trial start, and again at 3 and 12 months after treatment completion. Stool specimens were cultured for ordinary pathogens (C. difficile, Salmonellae, pathogenic E. coli, C. jejuni, Shigellae, etc.) at the start of the trial, 2 weeks after starting ATM or placebo, and 3 months after treatment completion.

The study was originally approved for 12 months and patients continued to be followed up for up to 12 months after completion of treatment. The blinding was not broken until the entire study was completed. Patients who stopped participating in the study included those with relapse, lack of efficacy, loss of responsiveness to ATM, and those without relapse who elected to discontinue the study. For patients who did not complete the 12-month follow-up, the data at 12 months were imputed by the last-observation-carried-forward method.

METHODS

Study subjects

A multicenter, randomized, double-blind, placebo-controlled study was conducted between January 2004 and July 2006 at 11 hospitals in Japan. This trial was registered at http://www.umin.ac.jp/ctr/index-j.htm:UMIN000000078. The institutional review board or ethics committee at each facility approved the protocol. All subjects gave written informed consent. All eligible patients had an established diagnosis of UC. Study subjects were selected from among patients with chronic relapsing UC (at least one relapse per year) with more than 1 year of follow-up and who regularly visited outpatient clinics. Eligibility criteria for study entry were mild-to-severe disease (11) with a colonoscopy score of at least 1 (erythema, decreased vascular pattern, and mild friability) based on a scale of 0 (normal or inactive) to 3 (spontaneous bleeding and ulceration) (25), and/or watery diarrhea at least 5 times/day with visible blood in stools. Patients with toxic megacolon or penicillin allergy, who were pregnant, or who had serious liver or renal disease or any psychological illness were excluded. Patients who had taken antibiotics within 4 weeks before study entry or had Clostridium difficile or other stool pathogens (Salmonellae, pathogenic Escherichia coli, Campylobacter jejuni, Shigellae, etc.) at entry were also excluded.

Clinical scoring, endoscopic scoring, and definitions of response, remission, and relapse

At each assessment, patients completed a symptom questionnaire and underwent clinical examinations to determine Mayo scores (25). Mayo scores provide an assessment of disease activity on the basis of a combination of symptoms, signs, and sigmoidoscopic findings, with scores ranging from 0 to 12. The initial endoscopy was performed no more than 2 weeks before starting therapy, with follow-up, including total colonoscopy, and biopsy at 3 and 12 months after treatment completion. After total colonoscopy, the endoscopic findings of the sigmoid colon and rectum were evaluated according to the Mayo system, with scores of 0–3. Zero represents normal findings or an appearance consistent with inactive UC. In all three scoring systems, a higher score indicates more severe disease.
For the Mayo system, clinical response was defined as a decrease from baseline in the total score of at least 3 points (26) if the baseline score was greater than 3 points, or a decrease of at least 2 points if the baseline score was 3 points or less. The decrease in the activity index was considered a clinical improvement if the baseline disease activity index was greater than 1 point. Clinical remission was defined as a total score of 2 points or lower with no individual subscore exceeding 1 point.

Clinical relapse was defined as reappearance of visible blood in stools for two consecutive days and/or recurrence of frequent diarrhea (five or more bowel movements/day), nocturnal diarrhea, or abdominal cramps. If a patient relapsed or showed exacerbation with severe or fulminant UC symptoms, study participation was stopped and the patient was treated appropriately.

**Bacterial assessment**

At study entry, and at 3 and 12 months, serum titers of immunoglobulins (IgG, IgM, and IgA) to *F. varium* were measured by enzyme-linked immunosorbent assay, performed blinded according to previously described methods (21,23). The primary antibodies (Protein Purify, Maebashi, Japan) for the standard solution were from rabbits immunized with a whole-cell preparation of *F. varium*. Dilutions of 6.25, 12.5, 25, 50, and 100 were performed to calculate the standard curve, and the optical density of dilution was defined as 1 unit/ml. Thus, 5 units/ml was equivalent to the optical density cutoff value of 0.25 (21), such that ≥5 units/ml was defined as positive for *F. varium* infection.

**Study end points**

The primary study end point was a clinical response documented by the Mayo score at 3 months after treatment completion; secondary end points were clinical and endoscopic score improvements at 12 months.

**Subset analysis**

Subset analyses with respect to the severity of disease at entry were carried out at the primary and secondary end points.

**Statistical analysis**

In our randomized controlled pilot trial (23), clinical responses were observed in 50% of the ATM and 30% of the placebo group. These results were used for sample size calculations. To detect a significant difference in these proportions at \( P < 0.05 \) with 80% power, 93 patients would be required in each treatment group. Assuming a maximal dropout rate of 10%, the sample size in each group was determined to be 103.

We recruited 210 patients, 105 in each group (Figure 1). After random allocation, four patients in the placebo group did not meet the inclusion criteria (inactive UC, no mucosal inflammatory findings, and no symptoms) and were excluded because further improvement in inactive UC would be unlikely, (i.e., inclusion could have biased the placebo group in the no-effect direction). Therefore, 206 patients were included in the full analysis set for an intention-to-treat analysis. For full analysis set analysis, the last-observation-carried-forward method was used to impute incomplete data, including those of patients who dropped out, relapsed, and/or were lost to follow-up. The proportions showing a clinical response at 3 months (primary end point) in the ATM and placebo groups were calculated, and the difference was statistically tested using Fisher’s exact test. For analyses of secondary end points, changes in clinical endoscopic scores at 12 months \( (x_{i,j}) \) from the baseline score \( (x_{i}) \) were calculated as \( d_{i,j} = x_{i} - x_{j} \). The data for \( d_{i,j} \) are summarized as mean and percentile values, and the \( d_{i,j} \) difference between the ATM and placebo groups was tested using the Mann–Whitney U-test.

Differences with two-sided \( P < 0.05 \) were considered statistically significant. SAS version 9.1 and STAT VIEW software version J 5.1 (SAS Institute, Cary, NC) were used for all analyses.

**RESULTS**

**Baseline characteristics**

The distributions of age, sex, disease duration, disease severity, extent of disease, concomitant medication, rates of corticosteroid-refractory and -dependent disease, and Mayo scores were well balanced between the two groups (Table 1). Out of 101 controls, 10 (9.9%) and out of 105 ATM patients, 12 (11.4%) were receiving long-term sulfasalazine or steroid therapy by enema at the time of recruitment.

**Primary end point**

Mayo score improvements were significantly greater in the ATM (Δ mean; \( -2.10 \)) than in the placebo group (\( -0.52 \)), at 3 months after treatment (Table 2) and Figure 2, \( P < 0.0001 \). Using the Mayo system, the subscores for stool frequency, rectal bleeding, physician’s global assessment, and endoscopic score (Table 2) in the ATM group were \(-0.50, -0.35, -0.67, \) and \(-0.58\), respectively, and those in the placebo group were \(-0.07, -0.16, -0.14, \) and \(-0.16\), respectively, at 3 months. The Δ means of all scores except rectal bleeding differed significantly between the ATM and placebo groups.

The clinical response rate was significantly higher in the ATM (47 of 105, 44.8%; 95% confidence interval (CI) 35.1–54.8) than in the placebo (23 of 101, 22.8%; 95% CI 15.0–32.2%) group at 3 months after treatment completion (Figure 3a, \( P = 0.0011 \)). Remission was seen in 20 of 105 ATM patients (19.0%, 95% CI 12.0–27.9%) and in 16 of 101 controls (15.8%, 95% CI 9.3–24.9%) at 3 months (Figure 3b, \( P = 0.59 \)).

**Secondary end points**

At 12 months after treatment completion, Mayo scores remained significantly lower in the ATM than in the placebo group (Table 2 and Figure 2, \( P < 0.0001 \)).

The subscores for stool frequency, physician’s global assessment, and endoscopic score (Table 2) showed significant improvement at 12 months in the ATM group than in the placebo group (stool frequency, \( P < 0.0001 \); global assessment, \( P < 0.0001 \); and endoscopic score, \( P = 0.0002 \)).

The clinical response rate was significantly higher in the ATM (49.5%, 95% CI 39.6–59.5%) than in the placebo group (21.8%, 95% CI 14.2–31.1%) at 12 months after treatment completion.
Remission was achieved in 28 of 105 ATM patients (26.7%, 95% CI 18.5–36.2%) but in only 15 of 101 controls (14.9%, 95% CI 8.6–23.3%) at 12 months (Table 2 and Figure 3b; \( P = 0.041 \)). Pretreatment steroid-dependent UC was present in 51 placebo and 49 ATM group patients. The proportions experiencing remission and corticosteroid discontinuation were higher in the ATM than in the placebo group at 6, 9, and 12 months after treatment completion (Table 2 and Figure 4; \( P = 0.0251 \) by Fisher’s exact test). Therefore, at the secondary end point, 50 ATM and 61 placebo patients were lost to follow-up. At 6, 9, and 12 months, more patients in the ATM group, which had a higher rate of clinical remission, were available for follow-up. The rate of long-term follow-up was thus higher in the ATM than in the placebo group (Table 2).

**Subset analysis**

According to disease severity at entry, patients were divided into active and inactive groups with Mayo scores of 6–12 and <6, respectively. At the primary end point in those with active disease (51 placebo and 65 ATM patients), the clinical response rate was significantly higher in the ATM than in the placebo group (Figure 5a, 34 of 65 vs. 11 of 51, 52.3% (95% CI 39.5–64.9) vs. 21.6% (CI 11.3–35.3), \( P = 0.0010 \)). When mucosal healing was defined as an absolute subscore for endoscopy of 0 or 1, the

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### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Complete follow-up at 3 months</th>
<th>Discontinued follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>81</td>
<td>30 (30.7%)</td>
</tr>
<tr>
<td>ATM</td>
<td>105</td>
<td>22 (21.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>52 (27.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Complete follow-up at 12 months</th>
<th>Discontinued follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>55 (54.5%)</td>
<td>22 (21.0%)</td>
</tr>
<tr>
<td>ATM</td>
<td>69 (65.7%)</td>
<td>27 (25.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>49 (42.7%)</td>
</tr>
</tbody>
</table>
The mucosal healing rate was significantly higher in the ATM than in the placebo group at 3 months after treatment completion (Figure 5b), 32 of 65 vs. 11 of 51, 49.2% (CI 36.6–61.9) vs. 21.6% (CI 11.3–35.3), P=0.0034. Remission was seen in 10 of 65 ATM patients (15.4% (CI 7.6–26.5)) and in 6 of 51 placebo patients (11.8% (CI 4.4–23.9)) at 3 months (Figure 5c, P=0.79).

At 12 months after treatment completion, the clinical response rate and the mucosal healing rate were significantly higher in the ATM than in the placebo group (Figure 5a, 39 of 65 vs. 11 of 51, 60.0% (CI 47.1–72.0) vs. 21.6% (CI 11.3–35.3), P<0.0001, and Figure 5c, 36 of 65 vs. 12 of 51, 55.4% (CI 42.5–67.7) vs. 23.5% (CI 12.8–37.5), P<0.0001). The remission rate in the ATM group at 12 months was significantly higher than that in the placebo group (Figure 5c, 19 of 65, 29.2% (CI 18.6–41.8) vs. 6 of 51, 11.8% (CI 4.4–23.9), P=0.025).

In those with inactive UC who had a Mayo score of <6, there were no significant differences in response and remission rates at 3 and 12 months between the ATM and placebo groups. In those with active and inactive UC, the distributions of age, sex, disease duration, disease severity, extent of disease, concomitant medication, rates of corticosteroid-refractory and -dependent disease, and Mayo scores were well balanced between the ATM and placebo groups.

**Antibodies against F. varium**

In each group, 98 patients had serum samples available for assessment of antibodies to *F. varium*. The clinical characteristics of 10 patients whose sera were not available were similar to those of the other 196. Before treatment, baseline titers of immunoglobulins to *F. varium* were similar in the two groups (mean±s.e., ATM: 9.1±0.7, placebo: 9.4±0.6, P=0.541). Mean serum titers of immunoglobulins to *F. varium* in the ATM group were decreased at 3 (Δ mean: −0.02 U/ml) and 12 months (Δ mean: −0.11 U/ml) and were significantly lower than those in the placebo group at 12 months (P=0.0071; Δ mean: +0.12 at 3 months, +0.17 at 12 months).

**Responses in F. varium antibody-positive patients**

In this study, 142 of the 196 patients (72.4%) were positive for *F. varium* antibodies (≥5 U/ml; 76 of 98 placebo and 66 of 98 ATM patients). Among 66 ATM patients positive for *F. varium* antibodies, a clinical response was achieved in 30 patients (45.5%) at 3 months and in 32 patients (48.5%) at 12 months after treatment completion. In responders, serum titers of immunoglobulins to *F. varium* were decreased at 3 months (mean±s.e.; pretreatment, 12.2±1.6 U/ml; 3 months, 11.7±1.5 U/ml; Δ mean −0.55 U/ml, P=0.080) and significantly decreased at 12 months (mean±s.e.; before treatment, 11.8±1.5 U/ml; 12 months, 10.9±1.5 U/ml; Δ mean −1.20 U/ml, P=0.036). No serum titer decreases were observed in nonresponders at 3 months (pretreatment, 12.7±0.9; 3 months, 12.7±0.9; Δ mean +0.03, P>0.2) or at 12 months after treatment completion (pretreatment, 13.2±0.9; 12 months, 12.7±0.9; Δ mean −0.50, P=0.15).

Among the patients positive for *F. varium* antibodies (≥5 U/ml), the clinical response rate was significantly higher in the ATM (30 of 66, 45.5%) than in the placebo group (16 of 76, 21.1%) at 3 months (P=0.0023) and at 12 months (32 of 66, 48.5%) vs. the placebo group (14 of 76, 18.4%, respectively; P<0.0001), after treatment completion. Among patients negative for *F. varium* antibodies (<5 U/ml), the response rates in the ATM group were 37.5% (12 of 32) at 3 months and 46.9% (15 of 32) at 12 months but only 27.2% (6 of 22) at 3 months and 31.8% (7 of 22) at 12 months in the placebo group (P>0.2).

**Compliance and adverse events**

Compliance was similar in the placebo (mean 99.6%, range 0–100%) and ATM (mean 94.8%, range 30–100%) groups. No serious drug-related toxicities were observed during the trial. Significantly more ATM (52.4%) than placebo (14.9%) group patients experienced adverse events (P<0.0001). The proportions of ATM patients with nausea, fever, and watery diarrhea were 17.1, 12.4, and 7.6%, respectively. These symptoms all resolved promptly after treatment completion. Six ATM patients had reversible urticaria and fever, which resolved rapidly after antibiotic discontinuation. Stool cultures revealed no pathogens in any of our patients at any time during this study.
DISCUSSION

Bacteria have increasingly been recognized as having important roles in mucosal inflammation (28), and there is a growing body of evidence that colonic luminal bacteria contribute to clinical activity in UC (29,30), suggesting that, in addition to traditional immunosuppression, appropriate antimicrobial therapy may be important in UC management. Originally, antibacterial treatment for UC focused on sulfasalazine. However, definitive antibiotic activity has never been shown for this agent.

Truelove and Jewell (11) treated severe UC with prednisolone and tetracycline for 5 days, and 36 of 49 patients (73%) achieved complete remission. Their patients received both drugs and there were no controls. Dickinson et al. (13) reported that UC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n=101)</th>
<th>ATM (n=105)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean duration of follow-up, mean ± s.d. (weeks)</td>
<td>26.9 ± 20.6</td>
<td>32.6 ± 20.2</td>
<td>0.0416</td>
</tr>
<tr>
<td>Mayo score</td>
<td>Mean</td>
<td>Δ Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Before treatment</td>
<td>5.79</td>
<td>6.38</td>
<td></td>
</tr>
<tr>
<td>At 3 months</td>
<td>5.24</td>
<td>−0.52</td>
<td>4.28</td>
</tr>
<tr>
<td>At 12 months</td>
<td>5.18</td>
<td>−0.58</td>
<td>4.02</td>
</tr>
<tr>
<td>Stool frequency</td>
<td>Before treatment</td>
<td>1.29</td>
<td>1.47</td>
</tr>
<tr>
<td>At 3 months</td>
<td>1.22</td>
<td>−0.07</td>
<td>0.96</td>
</tr>
<tr>
<td>At 12 months</td>
<td>1.23</td>
<td>−0.05</td>
<td>0.89</td>
</tr>
<tr>
<td>Rectal bleeding</td>
<td>Before treatment</td>
<td>0.98</td>
<td>1.06</td>
</tr>
<tr>
<td>At 3 months</td>
<td>0.82</td>
<td>−0.16</td>
<td>0.70</td>
</tr>
<tr>
<td>At 12 months</td>
<td>0.82</td>
<td>−0.16</td>
<td>0.66</td>
</tr>
<tr>
<td>Physician’s global assessment</td>
<td>Before treatment</td>
<td>1.74</td>
<td>1.94</td>
</tr>
<tr>
<td>At 3 months</td>
<td>1.60</td>
<td>−0.14</td>
<td>1.28</td>
</tr>
<tr>
<td>At 12 months</td>
<td>1.55</td>
<td>−0.19</td>
<td>1.20</td>
</tr>
<tr>
<td>Sigmoidoscopy subscore</td>
<td>Before treatment</td>
<td>1.78</td>
<td>1.91</td>
</tr>
<tr>
<td>At 3 months</td>
<td>1.59</td>
<td>−0.16</td>
<td>1.33</td>
</tr>
<tr>
<td>At 12 months</td>
<td>1.56</td>
<td>−0.19</td>
<td>1.29</td>
</tr>
<tr>
<td>Clinical response, no. (%)</td>
<td>At 3 months</td>
<td>23 (22.8)</td>
<td>47 (44.8)</td>
</tr>
<tr>
<td>At 12 months</td>
<td>22 (21.8)</td>
<td>52 (49.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clinical remission, no. (%)</td>
<td>At 3 months</td>
<td>16 (15.8)</td>
<td>20 (19.1)</td>
</tr>
<tr>
<td>At 12 months</td>
<td>15 (14.9)</td>
<td>28 (27.6)</td>
<td>0.044</td>
</tr>
<tr>
<td>Clinical remission and corticosteroid discontinuation in corticosteroid-dependent patients</td>
<td>No./total no. (%)</td>
<td>At 6 months</td>
<td>6/51 (11.8)</td>
</tr>
<tr>
<td>At 9 months</td>
<td>7/51 (13.7)</td>
<td>17/49 (34.7)</td>
<td>0.019</td>
</tr>
<tr>
<td>At 12 months</td>
<td>7/51 (13.7)</td>
<td>17/49 (34.7)</td>
<td>0.019</td>
</tr>
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</table>

ATM, amoxicillin, tetracycline, and metronidazole.

*P value for difference in Δ mean between ATM and placebo groups by Mann–Whitney U-test.
temporarily stabilized in 16 of 18 (89%) patients who were given oral vancomycin, in contrast to only 8 of 15 (53%) placebo controls, although there was no significant difference in long-term outcomes. Intravenous metronidazole has been used for severe UC as an adjunct to intensive intravenous regimens (14). Although a double-blind, placebo-controlled trial found...
no benefit in the metronidazole group, no studies have focused on the possible efficacy of oral metronidazole. Burke et al. (15) studied 84 patients with acute relapse or a first attack of UC who were randomized to receive either oral tobramycin or placebo for 7 days as an adjunct to steroid therapy. At the 18- to 21-day end point, 31 of 42 patients (74%) in the tobramycin and 18 of 42 (43%) in the placebo group showed clinical remission ($P = 0.008$). However, on the basis of the same study, Lobo and Burke et al. (16) reported that although this difference between the two groups remained significant at 6 months, it had disappeared by 1 year. Furthermore, Mantzaris et al. (17) reported that a combination of intravenous tobramycin and intravenous metronidazole, administered with steroid therapy, provided no benefit for acute, severe UC. Mantzaris et al. (18) also reported that a short course of ciprofloxacin did not increase the proportion of patients with active UC achieving remission.

In contrast, in a double-blind, placebo-controlled study, Turunen et al. (19) showed that adding 6 months of ciprofloxacin to conventional therapy with steroids, mesalazine, or sulfasalazine was significantly superior to treatment with placebo during the first 6 months of administration. However, there was no significant difference between the ciprofloxacin and placebo groups in endoscopic, histological, or clinical findings at 12 months. Oral rifaximin was reportedly effective for severe UC attacks refractory to steroid treatment in a small, double-blind, placebo-controlled trial (20). However, the response rate was not significantly higher in the rifaximin compared with the placebo group, and long-term follow-up results have not yet been reported. Therefore, previous antimicrobial therapy for UC has tended to be associated with short-term benefits and none has translated into long-term improvement. In contrast, in this multicenter, randomized, double-blind, placebo-controlled study, the addition of a short course of therapy with three antibiotics to traditional therapy proved effective in patients with any stage of active UC, from mild to severe, despite conventional treatment. We showed ATM administration for only 2 weeks to be effective in UC patients with active disease, not only in the short term, but also for at least 12 months after treatment completion. The numbers needed to treat to obtain one clinical response at 3 and 12 months were estimated to be 4.5 (95% CI 2.9–10.6) and 3.6 (CI 2.5–6.6), respectively, and that to obtain one clinical remission at 12 months was 8.5 (CI 4.4–114.0). These small numbers needed to treat support the use of ATM as an adjunct to conventional therapies. Interestingly, in this study, clinical response and remission rates in the ATM vs. the placebo group were higher at 12 than at 3 months. Mayo scores, the subscores for stool frequency, physician’s global assessment, and the endoscopic score, except for rectal bleeding, all showed more improvement at 12 months in the ATM than in the placebo group. When patients were divided into active and inactive groups with a Mayo score of 6–12 vs. <6, ATM treatment was more effective in the active group, as reflected by the clinical response and mucosal healing rates at 3 and 12 months, and the remission rate at 12 months after treatment completion. It is reasonable that there were no significant effects in the inactive group. It is noteworthy that approximately half of the patients in these two groups were steroid dependent, and that ATM was not only effective in these patients but actually facilitated tapering and withdrawal of corticosteroids.

Because previous reports showed a low eradication rate for Helicobacter pylori using a single drug (31,32), we chose three antibiotics to which F. varium was highly susceptible. The decreases in F. varium antibody titers and mucosal F. varium densities at both short- and long-term follow-up in the ATM group, in both the present and our previous study (23,24), suggest that this therapy can suppress F. varium in the mucosa. Interestingly, F. varium antibody titers of nonresponders in the ATM group were not decreased, suggesting that additional studies are needed to identify a regimen that will routinely yield high eradication rates (e.g., longer treatment duration or the use of other antibiotics to which this microorganism is susceptible). The cumulative remission rate on long-term follow-up was significantly higher in the ATM than in the placebo group, suggesting a possible contributory relationship between F. varium, or an as yet unidentified but susceptible organism, and UC, possibly similar to that between H. pylori and gastroduodenal ulcers. However, as these three antibiotics are lethal to many bacterial species besides F. varium, we cannot rule out the possibility that a nonspecific sterilization effect may explain these UC remissions.

Although the proportion of patients with adverse events was significantly higher in the ATM than in the placebo group, the frequency and types of adverse events, such as nausea, watery diarrhea, and fever, were similar to those reported in up to 50% of patients undergoing eradication therapy for H. pylori (31,32). The safety findings in these studies were similar to the data obtained in clinical studies of antibiotic treatments in patients with UC or those receiving H. pylori eradication treatment. It is not possible to avoid adverse events with antibacterial treatment, but the events in this trial were mild to moderate and the completion and compliance with treatment rates were similar in the ATM and control groups.

A limitation of this study is that a rather large proportion of patients were unavailable for follow-up at 12 months. Many refractory UC cases were included in both groups (59.4% in the placebo and 59.1% in the ATM). Thus, these patients, especially those in the placebo group, tended to relapse and were unavailable for long-term follow-up. In analyzing those lost to follow-up, there were fewer dropouts in the ATM than in the placebo group and follow-up duration was much longer in the former. This confirmed the advantage experienced by the ATM group.

In conclusion, a 2-week antibiotic combination therapy was effective and safe in patients with active UC in this double-blind placebo-controlled multicenter trial. We propose ATM to be considered alongside conventional therapy in patients with relapsing UC as an alternative to undertaking surgery.

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CONFLICT OF INTEREST
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Study Highlights

WHAT IS CURRENT KNOWLEDGE
- Luminal bacteria in mucosal inflammation contribute to spontaneous colitis in models such as cytokine (interleukin (IL)-2 or IL-10)-knockout and other related gene-knockout mice.
- Spontaneous colitis in animal models is completely inhibited by a combination of broad-spectrum antibiotics.
- Microbial agents have long been implicated in the initiation and/or exacerbation of ulcerative colitis (UC) in humans, suggesting a possible rationale for antibiotic treatment of UC.
- Antibacterial therapy trials for UC have produced conflicting results.

WHAT IS NEW HERE
- A 2-week antibiotic combination therapy consisting of amoxicillin 500 mg t.i.d., tetracycline 500 mg t.i.d., and metronidazole 250 mg t.i.d. produced improvement, remission, and steroid withdrawal in active UC more effectively than a placebo.
- *Fusobacterium varium* antibody titers decreased in responders but not in nonresponders, and more in the antibiotic than in the placebo group.
- We propose ATM (amoxicillin, tetracycline, and metronidazole) to be considered alongside conventional therapy in patients with relapsing UC as an alternative to undertaking surgery.

REFERENCES