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Long-term outcomes of antibiotic combination therapy for ulcerative colitis

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Abstract

Aims: An antibiotic combination of amoxicillin, tetracycline and metronidazole (ATM) is effective for ulcerative colitis (UC), but this regimen is discontinued in some cases due to adverse events. This study aimed to assess a revised combination, namely, amoxicillin, fosfomycin and metronidazole (AFM), in UC patients with the goal of reducing side effects while maintaining therapeutic efficacy.

Methods: A prospective open-label trial was undertaken in 104 adult UC patients. A combination of oral amoxicillin (1500 mg), fosfomycin (3000 mg) and metronidazole (750 mg) was administered to patients daily for 2–4 weeks in addition to their conventional medication. Clinical assessment was performed using the Lichtiger index before treatment and at 0, 3, 6, 9 and 12 months and 2 and 3 years. Endoscopic evaluation was performed using the Mayo score before treatment and at 3 and 12 months.

Results: The compliance rate was 99.2%. Response and remission rates were 80.8% and 63.5% at completion, 73.1% and 64.4% at 3 months, and 39.4% for both at 12 months, respectively. Of the 41 patients who were in remission at 12 months, 63.4% maintained that status until the 2-year follow-up. Similarly, 69.2% of those in remission at 2 years remained relapse free at the 3-year follow-up. Side effects were observed in 44.2% of the participants. Fever occurred in one patient (1.0%), which was lower than the rate observed with ATM therapy.

Conclusion: These results indicate that AFM therapy induces remission and is appropriate for long-term maintenance of UC while producing fewer and milder adverse events than ATM therapy.

Clinical trials: This study was registered in the University Hospital Medical Information Network (No. R000046546).

Keywords: antibiotic combination therapy, long-term outcome, ulcerative colitis

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Introduction

Gut microbiota dysbiosis has recently become a major topic of discussion in the effort to better understand and control ulcerative colitis (UC).[1–3] It is thought that in addition to psychological, genetic and environmental factors and immunopathological mechanisms, the gut microbiota is related to inflammatory bowel disease (IBD).[4,5] In general, the ideal balance of the gut microbiota is lost in the intestine of UC patients, and harmful bacteria are enriched. This dysbiosis results in an increase in inflammatory cytokine levels and mucosal permeability, leading to further dysfunction and damage of the intestinal wall.[6] Aiming to suppress this intestinal inflammation, the therapeutic of choice for UC has evolved in recent years. Along with conventional treatment options, including 5-aminosalicylate, corticosteroids, sulphasalazine, immunosuppressive drugs and anti-tumour necrosis factor (TNF) therapy, antibiotics have been gradually recognized for their efficacy in UC suppressing the excessive proliferation of harmful bacteria.[7–9]

Prior studies show variable outcomes on the effectiveness of administering a single or a combination of antibiotic(s) to UC patients. For instance, a randomised controlled trial (RCT) in acute UC reported that oral tobramycin for 7 days as an adjunct to steroid therapy achieved higher rates of symptomatic remission than placebo.[10] In another randomised study, 10 days of oral rifaximin administration was observed to be effective for steroid refractory patients with moderate to severe symptoms.[11] A double-blind study reported that the need for colectomy in adult UC patients with disease exacerbation decreased after the use of vancomycin.[12] A long-term, 6-month administration of ciprofloxacin added to conventional treatment of mesalazine and predonisone also demonstrated benefits on induction and maintainance of remission in UC.[13] Their results at 6 months showed a lower treatment-failure rate in the ciprofloxacin-treated group than in the placebo group. A systematic review suggested that antibiotics were significantly effective in inducing remission in chronic pouchitis.[14] Conversely, mild to severe acute UC patients who received either oral or intravenous ciprofloxacin for 2 weeks as an adjunct to corticoid therapy did not show significant improvement in remission compared with the placebo group.[15,16] Similarly, no significant difference was observed in an RCT between severe patients who were treated for 5 days with 500 mg intravenous metronidazole every 8 h and those treated with placebo.[17]

Regarding the use of multiple antibiotics, we demonstrated that the antibiotic cocktail ATM (amoxicillin, tetracycline and metronidazole), which targets *Fusobacterium varium* in the intestinal mucosa of UC patients, significantly improved symptoms and inflammatory markers in adult UC patients.[18,19] For younger individuals aged between 8 and 20 years, a combination of amoxicillin, metronidazole, doxycycline and vancomycin was also effective.[20] However, in another randomised trial with severe acute UC patients, no difference in treatment outcome was observed between the treatment group given intravenous ceftriaxone and metronidazole on top of the standard care and the placebo group.[21] Collectively, meta-analyses have concluded that the effectiveness of antibiotics for active UC is statistically significant, with an odds ratio of 2.17 for UC improvement.[22,23] Accordingly, certain choices of antibiotics might contribute to suppressing increased UC disease activity, yet due to the heterogeneity of previous studies, it remains unclear which agents are effective for UC patients.

In this study, we explored the long-term effectiveness of the combination of amoxicillin, fosfomycin and metronidazole (AFM). This is an improved regimen based on ATM therapy with demonstrated long-term effectiveness up to 1 year in a prior RCT of adult UC patients, with a side effect rate of 52.4%.[18] In particular, fever was the major side effect that led patients to discontinue treatment. To minimize adverse events, which are considered to occur mainly because of tetracycline, we designed this regimen to replace tetracycline with fosfomycin while maintaining *F. varium* sensitivity. Additionally, our ATM study results revealed the future necessity of and interest in observing long-term outcomes of antibiotics therapy, which has rarely been investigated. Therefore, with this AFM regimen, to further investigate the long-term outcomes of post-antibiotic therapy, we additionally aimed to have a longer follow-up period than employed in prior related studies.

Methods

Patients

A prospective, open-label study was conducted at the Jikei University Kashiwa Hospital in Japan from October 2008 to March 2017. All patients provided written informed consent. The eligibility criteria were an age of at least 18 years and active UC. Exclusion criteria included penicillin allergy, pregnancy, comorbid serious diseases, medication history of antibiotics within 2 weeks and ongoing use of biologic therapy. Patients who were positive for pathogens in stool culture or for *Clostridium difficile* toxin at baseline were also excluded.

Study design

All patients were administered a combination of oral amoxicillin (1500 mg/day), fosfomycin (3000 mg/day) and metronidazole (750 mg/day) for 2 weeks in addition to their regular medication in the hospital or at home. No changes in concomitant medications were made during the AFM treatment period. The duration of AFM therapy was extended for up to 4 weeks when the participant had severe disease activity based on Truelove and Witts criteria and when remarkably

deep ulcers were observed *via* endoscopy at baseline. The Lichtiger index score (ranging from 0 to 21 based on frequency of stool, nocturnal diarrhoea, proportion of bloody stool, incontinence of faeces, severity of stomach ache, performance status, abdominal tenderness and use of antidiarrheal drugs; a more serious status indicates a higher score) was recorded at baseline, 2 weeks, 3 months, 6 months, 9 months, and 12 months. An endoscopic Mayo score (score ranging from 0 to 3, indicating normal or inactive, mild, moderate and severe mucosal appearance, respectively[24]) was evaluated at baseline, 3 months and 12 months.

Patients were classified as steroid-refractory if their condition had not improved after at least 2 weeks of intravenous or oral administration of more than 30 mg/day of prednisolone, and patients were labelled as steroid-dependent if they had experienced a relapse while tapering prednisolone to at least 10 mg/day and were unable to discontinue steroids without relapse.

The primary endpoint was the change in the Lichtiger index at 12 months after AFM therapy. The secondary endpoint was the Lichtiger index at completion and 3 months. Based on prior studies that were comparable to the present study, we defined clinical response as a decrease in the Lichtiger index by more than three points from baseline and clinical remission as a Lichtiger index score of less than or equal to 3.[25,26] Relapse was defined as a Lichtiger index higher than baseline and requiring intensification of treatment, including a dosage increase and a switch to a new medication. In the absence of relapse, medication was not changed during the study period. Mucosal healing was defined as an endoscopic Mayo score of 0 or 1. Patients who had been under oral steroid therapy at baseline were followed to determine whether the use of steroids could be successfully withdrawn within 12 months after completion. Adverse events were recorded either in the hospital during the course or at an outpatient clinic at the completion of AFM therapy. Liver dysfunction was recorded when patients showed elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) above the upper limit of normal in their blood test at any follow-up point.

Additionally, patients who were in remission at 12 months were followed up to 3 years for further evaluation of the long-term response and remission rate.

Statistical analysis

We used Fisher's exact test to analyse clinical scores, response rates, remission rates and comparisons of side effects between ATM and AFM. We applied Friedman's test for analysis of the change in endoscopic Mayo score. All calculations were performed using STAT VIEW software, version J 5.1 (SAS Institute, Inc., Cary, NC, USA). A p value < 0.05 was considered statistically significant.

Ethical considerations

This study was reviewed and approved by the ethics committee of the Jikei Institutional Review Board, the Jikei University School of Medicine (Tokyo, Japan) and the clinical study committee of the Jikei University Kashiwa Hospital [No. 20-088 (5378)]. This study was also registered in the University Hospital Medical Information Network (No. R000046546). All procedures were performed in accordance with the Helsinki Declaration.

Results

Patient characteristics

A total of 104 patients with active UC symptoms were enrolled in this study (Table 1). Of them, 93.2% displayed severe to moderate symptoms of UC, 82.7% had repeated exacerbation and remission, 10.6% were in a chronic state and 6.7% were diagnosed with UC for the first time. In total, 46.1% were either steroid dependent or resistant. The median Lichtiger index was 9 at baseline.

All 104 participants were administered AFM therapy. Disease severity was classified based on the Truelove and Witts severity index. Probiotics included *Bifidobacterium longum, Bifidobacterium infantis, Clostridium butyricum, Streptococcus faecalis, Bacillus subtilis* or *Bacillus mesentericus*. Of the participants, 88 received the 2-week regimen, nine took the antibiotics for 3 weeks and seven took the antibiotics for 4 weeks. The total compliance rate was 99.2% (50–100%). Three patients (2.9%) could not complete the whole course due

Table 1. Patient characteristics.

Variables	Numbers
Median age – years (range)	36 (18–79)
Male/female – number of patients (%)	66/38 (63.5/36.5)
Median disease duration – years (range)	7.4 (0.1–28)
Extent of disease – number of patients (%)	
Extensive colitis	64 (61.5)
Left-sided colitis	29 (27.9)
Proctitis	11 (10.6)
Clinical severity of disease – number of patients (%)	
Severe	33 (31.7)
Moderate	64 (61.5)
Mild	7 (6.7)
Clinical course of disease – number of patients (%)	
Exacerbation and remission	86 (82.7)
Chronic	11 (10.6)
New onset	7 (6.7)
Concomitant medication – number of patients (%)	
Corticosteroid	27 (26.0)
Sulphasalazine	27 (26.0)
5-Aminosalicylic acid	71 (68.3)
Azathioprine	10 (9.6)
Probiotics	83 (79.8)
Steroid use – number of patients (%)	
None	56 (53.8)
Steroid dependent	38 (36.5)
Steroid resistant	10 (9.6)
Total steroid (predonisolone) amount – mg, mean (SE)	15,745.4 (17,323.5)

to adverse events caused by the antibiotics, such as nausea, stomatitis, fever and drug-related rash.

Clinical outcomes

The average Lichtiger index showed a significant decrease after AFM therapy [Figure 1(a)]. The score declined from 9.39 ± 4.37 at baseline to 3.71 ± 3.76 ($p < 0.0001$) at the completion of AFM, sustaining a lower state at 2.48 ± 2.92 ($p < 0.0001$), 1.90 ± 2.44 ($p < 0.0001$), 1.50 ± 2.64 ($p < 0.0001$) and 1.63 ± 3.09 ($p < 0.0001$) at 3 months, 6 months, 9 months and 12 months, respectively.

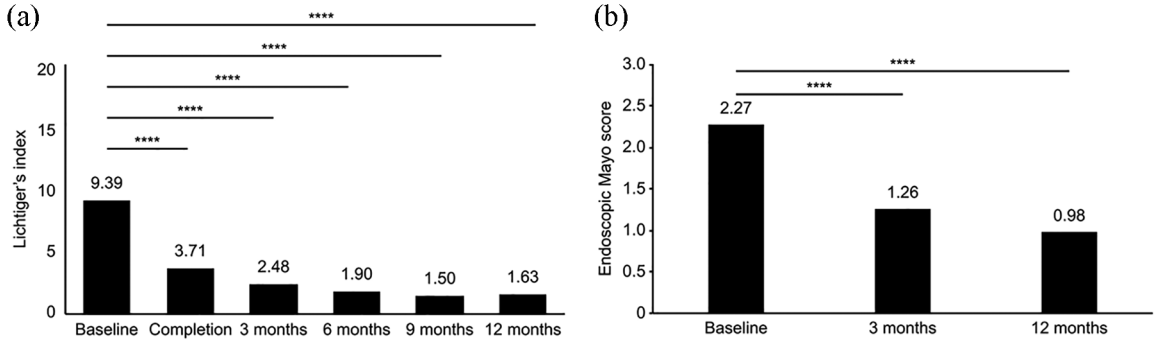

Figure 1. Changes in (a) the Lichtiger index and (b) the endoscopic Mayo score from baseline to 12 months. (a) The Lichtiger index decreased significantly after AFM therapy and was maintained at a lower value at up to 12 months of follow-up (****$p < 0.0001$). (b) The endoscopic Mayo score decreased significantly from 2.27 at baseline to 1.26 at 3 months post treatment (****$p < 0.0001$). Those who were followed by endoscopy at 12 months maintained an even lower score of 0.98 (****$p < 0.0001$).
AFM, amoxicillin, fosfomycin and metronidazole.

Significant improvement was confirmed by endoscopy [Figure 1(b)]. The average endoscopic Mayo score before AFM was 2.27, and it decreased to 1.26 at the 3-month follow-up ($p < 0.0001$). Among 46 participants who underwent endoscopy at the 12-month follow-up, an average low score of 0.98 was recorded ($p < 0.0001$).

Clinical response and remission

The overall flow of patients up to 1 year is displayed in Figure 2(a). When the regimen was completed, AFM induced a clinical response in 80.8% of the participants and remission in 63.5%; however, 13.5% did not experience a radical change, and 5.8% had markedly increased scores (Figure 3). At 3 months, 73.1% were in clinical response and 65.4% remained in remission. A total of 1.9% of the patients maintained a score similar to that at baseline, 20.2% relapsed, and five patients did not show up for the follow-up outpatient clinic visit. At the 12-month follow-up, 39.4% still showed a response and remained in remission, whereas 24.0% relapsed during this follow-up period. Oral steroids were successfully withdrawn in 21 of 27 patients (77.8%) who had been under treatment at baseline. Twelve participants dropped out and could not be followed. For endoscopic evaluation, 74 patients underwent endoscopy at 3 months. 56.8% of the patients scored 0 or 1 on endoscopic Mayo score. Similarly, 46 patients took endoscopic investigation at the 12-month follow-up, with 71.7% of the patients scoring 0 or 1.

Based on the response to corticosteroid treatment, the immediate response to AFM was weak among steroid-dependent patients, and the long-term outcome was better in non-steroid users (Figure 4). After completing AFM, 65.8% of the steroid-dependent patients experienced a clinical response. This rate was statistically lower than that of the other two groups of non-steroid (87.5%) and steroid-resistant (100%) patients ($p = 0.0086$). At 12 months, significantly elevated remission and response rates were maintained in non-steroid users ($p = 0.0193$). Both rates were exactly the same because all the patients showing a clinical response were in remission at that time.

Focusing on the extent of the disease, the effectiveness of AFM in reaching a clinical response or remission was not significantly different among those with extensive colitis, left-sided colitis or proctitis at completion, at 3 months or at 12 months (Table 2).

We also analysed disease severity based on Truelove and Witts criteria. As there were only a small number of participants involved whose symptoms were mild, we calculated this parameter exclusively for moderate and severe cases. The response rate in patients with severe disease at the completion of AFM was significantly higher than that in patients with moderate disease ($p = 0.0109$) (Table 3). There was no other significant difference among the groups in either response or remission rate at any time point.

Long-term progress of 2 years or more

Of 41 patients who were in remission at 12 months, 63.4% maintained their condition until the 2-year

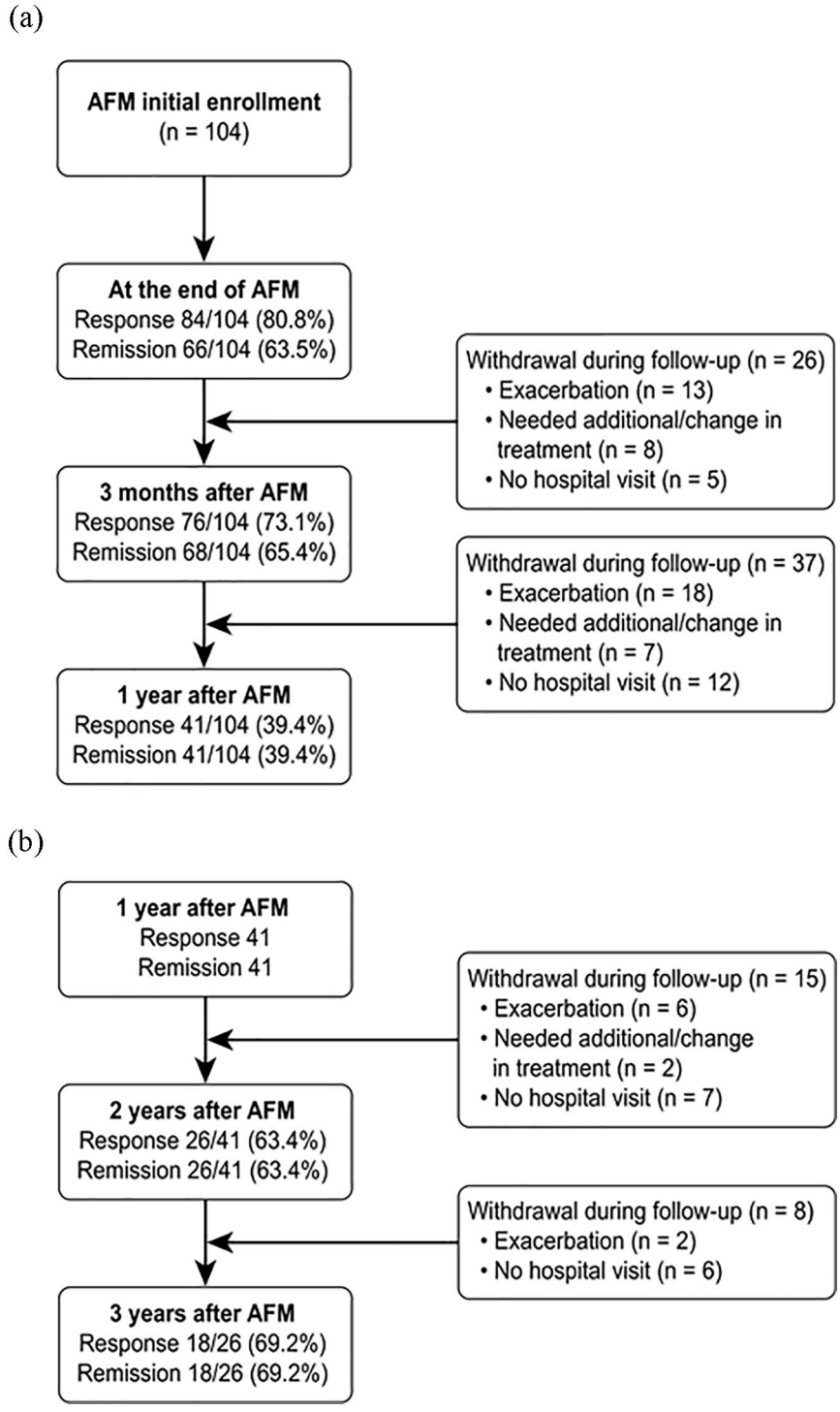

Figure 2. The patient flow (a) from enrolment to the 12-month follow-up and (b) after the 12-month follow-up visit to the 3-year follow-up visit. The response rate includes patients in remission. (a) After completing the AFM regimen, 26 patients dropped out due to relapse, increase/change in medication or missed follow-up visit. Thirty-seven participants withdrew between 3 months and 12 months. Forty-one patients stayed in remission 12 months after AFM therapy. (b) In total, 63.4% of participants who were in remission at 12 months maintained that status for another year, while 15 patients withdrew during that period. Of those who were in remission at 2 years, 69.2% remained in remission, two patients relapsed, and six were lost to follow-up at the 3-year follow-up visit.
AFM, amoxicillin, fosfomycin and metronidazole.

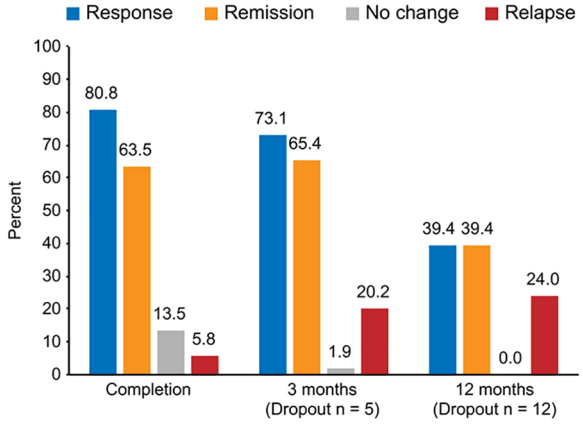

Figure 3. Overall response rate from baseline to 12 months. The numbers of participants who missed follow-up visit are shown in brackets under each axis title. The clinical response was 80.8% at completion and 73.1% at the 3-month follow-up, with the majority of patients in remission, 63.5% and 65.4% of the total participants, respectively. Clinical remission was also maintained in almost half of those patients at 12 months. A total of 13.5% did not show substantial changes, nor did 1.9% at 3 months. Relapse was seen in 5.8% at completion, 20.2% at 3 months and 24.0% at 12 months of follow-up.

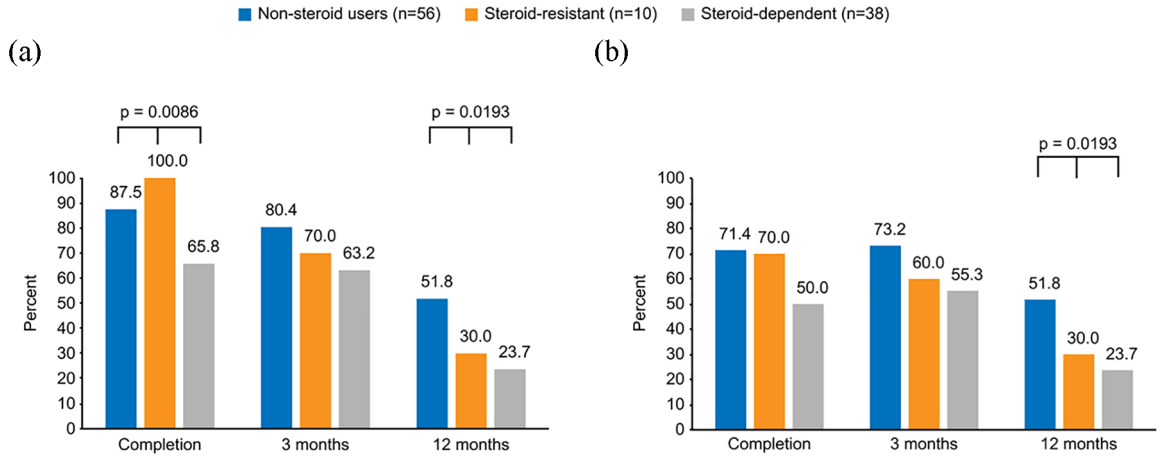

Figure 4. (a) Response rate and (b) remission rate segregated by steroid response; among non-steroid-using, steroid-resistant and steroid-dependent patients, the response rate at completion was lower in steroid-dependent patients than in non-steroid users and steroid-resistant patients ($p = 0.0086$). Both the response and remission rates at 12 months were highest in non-steroid-using patients among the three patient groups ($p = 0.0193$). Neither rate showed a significant difference among the groups at the 3-month follow-up.

follow-up. Furthermore, 69.2% of those in remission at 2 years did not experience relapse and were followed for 3 years [Figure 2(b)].

Adverse events

Side effects were observed in 46 patients (44.2%) (Table 4). The most common symptom was diarrhoea (17.3%), followed by hypogeusia (8.7%), nausea (7.7%) and liver dysfunction (5.8%). There were no serious adverse events throughout the study period. Three participants discontinued the therapy because of drug-related rash, nausea or oral ulcers. All other symptoms were manageable while completing the whole course and were resolved immediately after the completion or discontinuation of the regimen.

Discussion

Establishing an effective regimen to suppress the disease activity of UC and to induce long-term remission is one of the major goals of UC treatment. Given the chronicity of the disease, it is also

Table 2. Numbers and proportions of patients showing a clinical response and in remission according to disease extent; there was no significant difference in the effectiveness of AFM depending on the extent of disease at completion, 3 months or 12 months. Patients with mild symptoms were excluded due to small numbers. At completion, AFM achieved a higher response rate in patients with severe disease than in those with moderate disease. There was no other significant difference in response or remission rate between moderate and severe cases.

Disease extent	Completion		3 months		12 months	
	Response	Remission	Response	Remission	Response	Remission
Extensive colitis –*n* = 64 (%)	53 (82.8)	41 (64.1)	50 (78.1)	44 (68.8)	25 (39.1)	25 (39.1)
Left-sided colitis –*n* = 29 (%)	23 (79.3)	17 (58.6)	20 (69.0)	20 (69.0)	11 (37.9)	11 (37.9)
Proctitis – *n* = 11 (%)	8 (72.7)	8 (72.7)	6 (54.5)	4 (36.4)	5 (45.5)	5 (45.5)
p value	*p* = 0.7154	*p* = 0.0711	*p* = 0.9057	*p* = 0.1014	*p* = 0.2233	*p* = 0.9057

AFM, amoxicillin, fosfomycin and metronidazole.

Table 3. Numbers and proportions of patients showing a response/in remission stratified by disease severity. Patients with mild symptoms were excluded due to the small sample size. At completion, AFM yielded a higher response rate in patients with severe disease than in those with moderate disease. There was no other significant difference in response or remission rate between patients with moderate and severe disease.

Disease severity	Completion		3 months		12 months	
	Response	Remission	Response	Remission	Response	Remission
Moderate –*n* = 64 (%)	46 (71.8)	41 (64.1)	48 (75.0)	42 (65.6)	23 (35.9)	23 (35.9)
Severe –*n* = 33 (%)	31 (93.9)	18 (54.5)	22 (66.7)	20 (60.6)	12 (36.4)	12 (36.4)
p value	*p* = 0.0109	*p* = 0.3630	*p* = 0.3856	*p* = 0.6258	*p* = 0.9670	*p* = 0.9670

AFM, amoxicillin, fosfomycin and metronidazole.

important to explore therapeutic options with reduced adverse events. This AFM therapy showed a certain potential to effectively suppress exacerbations in patients whose disease activity could not be fully controlled with conventional medication.

The combination of the AFM antibiotics in the present study was clinically effective in patients with active UC. Regarding short-term effectiveness, 80.8% and 73.1% of the participants in this study successfully achieved a clinical response at completion and at 3-month follow-up, respectively. The same regimen was administered in the study by Ishikawa *et al.*[26] to adult UC patients as a pretreatment for faecal microbiota transplantation (FMT). They observed a clinical response in 68.4% of the AFM monotherapy patient group at the 4-week follow-up. Another set of ATM treatment in an RCT demonstrated a 44.8% clinical response rate at the 3-month follow-up.[18] As the response rate of the present result was not inferior to that for ATM, the revised AFM regimen would reasonably be considered a treatment option for inducing clinical remission.

Several factors, such as the spectrum of antibiotics and duration of the intervention, appear to influence the effectiveness of antibiotics therapy. For instance, Mishra *et al.*[21] administered a combination of ceftriaxone and metronidazole to acute severe patients, and the outcomes did not show a significant difference between the antibiotics and placebo groups.[21] Their primary outcome was on day 3, and their focus was exclusively on severe patients. Similarly, Chapman *et al.*[17] treated severe patients with either metronidazole alone or placebo for 5 days, and the results indicated no significant difference in clinical improvement. A

Table 4. In total, 46 patients experienced some degree of side effects. There was one case of fever and three cases of discontinuation due to adverse events, but no life-threatening episodes were observed during the study period.

Side effect	*n* = 46
Diarrhoea	18
Hypogeusia	9
Nausea	8
Liver dysfunction	6
Loss of appetite	3
Drug-related rash	2
Headache	2
Oral ulcer	2
Coated tongue	1
Eyestrain	1
Fatigue	1
Fever	1
Numbness	1
Redness of distal portion of extremities	1

similar period of 14 days of antibiotics administration was utilized in a study by Mantzaris *et al.*,[15,16] who used ciprofloxacin to treat mild to severe patients, but again, no significant difference was observed between the treatment and placebo groups. Interestingly, Turunen *et al.*[13] administered ciprofloxacin for 6 months to UC patients and showed a lower treatment-failure rate in the antibiotics group than in the placebo group. Based on these studies, the spectrum and combination of antibiotics and the duration of the therapy appear to influence the treatment effectiveness. In addition, the influence of the gut microbiota and probiotics intake will be discussed later in this section.

The long-term effect of AFM therapy should also be noted. Although little has been demonstrated about the duration of clinical remission after antibiotics therapy, this is of great importance in treatment of UC. In the present study, 39.4% of the participants maintained clinical remission for 12 months after the completion of therapy. A total of 63.4% of those remained in remission for 2 years, and 69.2% of the patients in remission at 2 years stayed in remission until the 3-year follow-up. When considering all participants, 26 of 104 patients (25.0%) appeared for the 2-year follow-up in remission, as did 18 of 104 (17.3%) at 3 years. In a part of another study to investigate long-term FMT with or without AFM therapy, the respective response rates in the AFM monotherapy group at 1 and 2 years were 16.2% and 12.8%.[27] We suspect that the lower response rate than that in the present study was due to the smaller group size and differences in patient recruitment criteria, as participants in that study were limited to those with severe symptoms with either a Lichtiger index ≥ 5 or an endoscopic Mayo score ≥ 1. Regarding the relapse rate, a longitudinal prospective study reported that 36% of patients receiving maintenance medications relapsed during the follow-up period of 1 year and that the mean duration to relapse was 14.4 months.[28] Another prospective study observed that 57.7% of inactive UC cases relapsed during their 1-year follow-up period, whereas the relapse rate at 12 months was 24.0% in the present study.[29] Indeed, there are several risk factors for relapse, including young age, extensive colitis, and mucosal inflammation in clinical remission.[28,30,31] As the present regimen exerted considerably good short-term effectiveness in steroid-resistant and severe patients and induced a long-term clinical response and mucosal healing regardless of the extent of disease, AFM is a possible choice, especially for intractable cases with those risk factors.

AFM therapy caused fewer severe side effects than ATM therapy. In this study, adverse events occurred in 44.2% of patients, all of which were manageable after the treatment and not life threatening. Ishikawa *et al.*[26] noted that less than 20% of patients experienced adverse events during the same regimen. In addition, Ohkusa *et al.*[18] reported that the side effect rate of ATM therapy was 52.4% in an antibiotic group and 19.4% in a placebo group. Compared with the ATM regimen, the present AFM regimen resulted in significantly fewer cases of fever ($p = 0.0013$) and more diarrhoea ($p = 0.0377$). Considering that diarrhoea is seen as both a symptom of UC and a side effect of antibiotics, we might have included some patients with UC diarrhoea as a side effect. Thus, the actual number of adverse events of diarrhoea in the AFM group might be smaller. Accordingly, we consider that the high compliance rate in the AFM group was due to its low occurrence of severe side

effects, including fever, which was the main reason for discontinuation of ATM therapy. Therefore, AFM therapy effectively suppresses disease activity while reducing the major adverse events that occur with ATM therapy.

From the long-term results of the present study, AFM may not be inferior to other treatments regarding effectiveness, and AFM shows superior safety. Indeed, none of our previous studies of ATM and AFM regimens revealed life-threatening episodes or long-term side effects, whereas serious side effects have been increasingly reported with other treatments, including biological products.[18,26,32] For example, regarding anti-TNF agents, recent studies in UC patients show response rates of 35–69% at week 8 and 30–45% at week 52, with severe adverse events, including deaths, malignancies, lymphoma, neurologic diseases and serious infections.[33–39] Considering that an onset of anxiety and mood disorders in IBD patients at 2 years before the start of anti-TNF therapy is suggested to increase the risk of discontinuing the therapy, careful management is especially needed with those agents.[40] Apart from anti-TNF agents, other biologic therapies, such as ustekinumab and vedolizumab, have also demonstrated effectiveness, although some crucial adverse events, including deaths, malignancies and infections, should be carefully observed and managed.[41,42] In addition, methotrexate, which has been used frequently in recent years, is suspected to have side effects of sperm DNA fragmentation and teratogenicity.[43] Overall, these agents are recognized as effective treatments that improve health-related quality of life; however, considering that we achieved similar outcomes, no serious adverse events and lower costs, AFM therapy may be a practical option due to its effectiveness, safety and cost.[44]

We consider that AFM therapy suppresses UC exacerbation by modifying dysbiosis of the gut microbiota. The composition of the gut microbiota among UC patients shows some alterations compared with that among healthy humans, though it is not yet thoroughly understood.[45] As previous studies indicate, there is reduced diversity of bacteria and increased abundance of *Fusobacterium* in the gut of UC patients.[46,47] Additionally, there is a decrease in the abundances of Bacteroidetes and *Lactobacillus* species, though effects on the Firmicutes group remain controversial.[48,49] Initially, ATM therapy was designed with a focus on *F. varium*, which is present in the intestinal mucosa of UC patients.[32,50,51] Regarding *Fusobacterium*, a study of FMT in UC patients showed that those who did not achieve remission had an increased level of *Fusobacterium* in their gut microbiota.[52] Although we did not conduct microbiota analysis in this study, it can be hypothesized that the combination of antibiotics in this study had a sufficiently broad spectrum to rebalance the gut microbiota to a more preferable composition, suppressing symptoms in UC patients. This hypothesis is additionally supported by the results of the present study showing that 79.8% of participants had taken probiotics from the baseline until either relapse or the 3-year follow-up. Probiotics supposedly contribute to a better gut microbiota balance and might have enhanced the outcome of this study.

Similarly, antibiotic agents have been used as pretreatment for FMT in UC patients in several previous studies, showing better outcomes when a combination of antibiotics was employed instead of a single agent.[26,53,54] Furthermore, preferable recovery of Bacteroidetes species was observed in the AFM pretreatment group, which was not observed in either the AFM or the FMT monotherapy group.[55] A combination of antibiotics may effectively initialise microbiotal balance, enabling transplanted biomes to engraft to the intestine successfully. Further investigation including analyses of the gut microbiota profile will provide a better understanding of the characteristics of responders and non-responders to this AFM therapy.

Our study has limitations. First, this is an open-label study at a single site. Randomized studies at multiple hospitals with a larger number of participants will be required for future studies. An arrangement of placebo or another comparator will also be necessary to more precisely assess the effectiveness of AFM therapy. Second, we did not measure calprotectin in this study, which may have limited our ability to thoroughly observe the disease condition. Patient smoking status could also have been collected for detailed analyses, considering its influence on UC condition. In addition, the mechanism of how antibiotics modify UC disease activity needs to be explored further to specify effective agents and establish an evidence-based regimen. Nonetheless, this study will serve as a milestone for future attempts to increase treatment options for UC exacerbation.

Author contributions

Toshifumi Ohkusa contributed to the design of the study and collected the data. Toshifumi Ohkusa and Yuriko Nishikawa analysed the data. Yuriko Nishikawa drafted the manuscript. All authors have approved the final version of this manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Ethical approval

This study was reviewed and approved by the ethics committee of the Jikei Institutional Review Board, the Jikei University School of Medicine (Tokyo, Japan), and the clinical study committee of the Jikei University Kashiwa Hospital [No. 20-088 (5378)]. All procedures were performed in accordance with the Declaration of Helsinki.

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Informed consent

All patients in this study signed written informed consent and agreed to participate.

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