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【 ORIGINAL ARTICLE 】

Azathioprine Is Useful for Maintaining Long-term Remission Induced by Tacrolimus for the Treatment of Ulcerative Colitis: An Inverse Probability of a Treatment Weighing Analysis

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Abstract:

Objective The need for and efficacy of immunomodulators for maintaining remission after tacrolimus therapy have not been sufficiently defined. This study evaluated the efficacy of immunomodulators for maintaining remission in patients with ulcerative colitis after tacrolimus therapy.

Methods Patients with active ulcerative colitis who started oral tacrolimus between January 2009 and September 2017 and were responsive were retrospectively evaluated. Long-term outcomes were compared using Cox proportional hazard regression with inverse probability of treatment weighting.

Results Among the 63 patients in the study, 45 received immunomodulators. During the follow-up, 30 patients (47.6%) experienced a relapse. The relapse-free survival rate was significantly worse in the group that did not receive immunomodulators than in those that did (p=0.01, log-rank test); the 2-year relapse-free rates were 22.5% and 63.6% in the non-immunomodulator and immunomodulator groups, respectively. A multivariate analysis showed immunomodulator treatment to be an independent protective factor for clinical relapse (adjusted hazard ratio: 0.35, 95% confidence interval: 0.16-0.78, p=0.01). A Cox regression analysis using inverse probability of treatment weighting also showed that immunomodulator maintenance therapy was correlated with a longer relapse-free survival (hazard ratio: 0.31, 95% confidence interval: 0.15-0.64, p<0.01), A similar response was also observed in non-steroid-dependent patients (hazard ratio: 0.36, 95% confidence interval: 0.14-0.99, p=0.047). No serious adverse events occurred due to tacrolimus or immunomodulator, and immunomodulator use did not increase the incidence of adverse events caused by tacrolimus.

Conclusion Our data suggest that the use of immunomodulators to maintain remission after tacrolimus therapy is beneficial for patients with ulcerative colitis.

Key words: ulcerative colitis, tacrolimus, immunomodulator

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Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease affecting the colorectum; the etiology of the condition is unknown. Traditional therapies for UC include mesalamine, corticosteroids, and immunomodulators (IM; thiopurines). Patients with UC who fail to respond to these treatments are considered for second-line therapy with biologics or calcineurin inhibitors, such as cyclosporine A

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(CyA) or tacrolimus (1, 2). CyA has been shown to elicit beneficial short-term responses in patients with steroid-refractory UC in randomized controlled trials (RCTs) (3), and tacrolimus has been used increasingly frequently for the treatment of severe and steroid-refractory UC (4-6).

Since calcineurin inhibitors are rescue therapy options and are best discontinued within six months because of side effects, the European Crohn's and Colitis Organisation (ECCO) guidelines recommend the use of thiopurines as maintenance therapy for patients responding to calcineurin inhibitors (2). This approach is widely accepted. This use of thiopurines is justified given the high colectomy rate among patients with UC and the reported efficacy of thiopurines in reducing the need for colectomy after the induction of remission with CyA (19-20% in the thiopurine-treated group and 53-60% in the thiopurine-untreated group at 1 year following the introduction of CyA therapy) (7, 8). However, for tacrolimus therapy, apparently only two retrospective studies have assessed the efficacy of thiopurines after tacrolimus-induced remission (9, 10). Both studies examined heterogeneous populations consisting of both steroid-refractory and steroid-dependent patients with UC.

The present study aimed to evaluate the long-term prognosis of patients with UC in order to clarify the efficacy of IM as maintenance therapy after tacrolimus-induced remission. This study was conducted as a retrospective, comparative study using inverse probability of treatment weighting (IPTW) to reduce any impact of treatment selection bias and potential confounding factors. Furthermore, the study focused exclusively on non-steroid-dependent patients in order to assess the efficacy of IM without any confounding effects of steroid dependence.

Materials and Methods

Study design

The present study was a retrospective, observational cohort study conducted at a single center.

Patients

All patients with moderate-to-severe active UC who started taking oral tacrolimus between January 2009 and September 2017 were enrolled. Tacrolimus was administered orally, and the initial dose was 0.05 mg/kg twice per day. Blood tacrolimus levels were measured two or three times per week for the first two weeks. Doses were adjusted to achieve a high trough level of 10-15 ng/mL. After maintaining high trough levels for 2 weeks, the doses were decreased to achieve a low trough level of 5-10 ng/mL. The duration of tacrolimus administration is always limited to 12 weeks because of the absence of long-term data regarding the efficacy and safety of this regimen. Tacrolimus administration was terminated or continued according to clinical requirements at the discretion of the patients' physicians.

Given the study's aim to determine the efficacy of IM as maintenance therapy after tacrolimus-induced remission, patients who were nonresponsive to tacrolimus treatment by week 12 were excluded. Patients administered other therapies for maintaining remission (e.g., infliximab, adalimumab, golimumab, and cytapheresis), except for IM or mesalamine, were also excluded. Adverse events were recorded retrospectively using hospital records. The diagnosis of UC was based on clinical, endoscopic, and histopathological findings. Demographic, clinical, and laboratory data were obtained from the medical records. Cytomegalovirus (CMV) reactivations were validated by CMV antigenemia (C7-HRP). The CMV antigenemia was measured before the start of tacrolimus.

Patients were followed up from the time of tacrolimus administration until clinical relapse, loss of follow-up, or until the end of November 2017.

Study endpoints

The primary outcome measure was the clinical relapse of UC, defined as the exacerbation of gastrointestinal symptoms requiring secondary alternative therapies such as surgery, administration of corticosteroids, or anti-tumor necrosis factor (TNF)-α. The secondary outcome measure was the incidence of adverse events.

Definitions

The partial Mayo score (11) (p-Mayo) was used to assess the disease severity. Moderate-to-severe active disease was defined as p-Mayo≥4. Severe UC was defined as p-Mayo≥7. Clinical remission was defined as p-Mayo≤2. Clinical relapse was characterized as the exacerbation of gastrointestinal symptoms requiring secondary alternative therapies such as surgery, administration of corticosteroids, or anti-TNF-α. Clinical response was defined as a p-Mayo reduction of ≥3 points, accompanied by a decrease of at least 30% from the baseline and a decrease in the rectal bleeding subscore of ≥1 or an absolute rectal bleeding subscore of 0 or 1 (12). The IM group included patients who received thiopurines (azathioprine or 6-mercaptopurine) at the time tacrolimus treatment was terminated, while the non-IM group included patients who did not receive thiopurines. In the IM group, patients withdrawn from IM during the maintenance period, per their physician's decision, were censored at that time. In the non-IM group, patients who started IM after the termination of tacrolimus treatment were censored at the time of the administration of IM. The use of concomitant medications (mesalamine or corticosteroids) was recognized at the start of tacrolimus administration.

Steroid-refractory patients were defined as patients who had an active form of the disease despite receiving either intravenous prednisolone at more than 1 mg/kg/day over at least 1 week or oral prednisolone at more than 30 mg/day over at least 2 weeks. Steroid-dependent patients were defined as patients for whom the prednisolone dose could not be tapered below 10 mg/day without recurrent disease or who had a relapse within 3 months of stopping predniso-

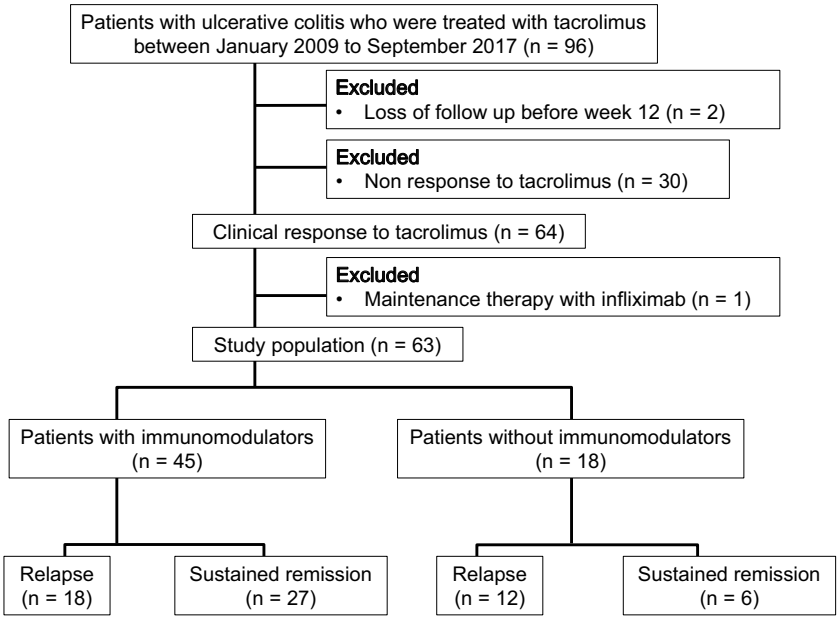


Figure 1. **Flow chart of the study population.**

lone. Adverse events were classified according to the Medical Dictionary for Regulatory Activities version 20.1.

Statistical analyses

Continuous variables are presented as the median and interquartile range (IQR). Differences in clinical characteristics were compared using either the chi-square or Fisher's exact tests for categorical variables and the Mann-Whitney U test for continuous variables. Prognostic factors for clinical relapse were evaluated to determine the cumulative relapse-free rate among the responders. Cumulative relapse-free rates were illustrated with Kaplan-Meier plots. Differences in survival curves were assessed with log-rank tests. A multivariate analysis was performed using a Cox regression model. Data were presented as hazard ratios (HRs) with 95% confidence intervals (CIs). Multivariate Cox regression analyses were performed to identify factors associated with clinical relapse; those factors speculated to be risk factors for clinical relapse were then evaluated in the multivariate analysis.

An IPTW analysis was applied to each observation in the Cox model in order to assess the relative effectiveness of IM. The IPTW analysis was derived using propensity scores on all observations before matching (13) in order to reduce selection bias by statistically adjusting for background factors, thereby enabling pseudo-randomization. Variables included in the IPTW analysis were age, sex, disease duration, disease location, concomitant mesalamine, concomitant corticosteroid, disease severity, serum albumin level, serum C-reactive protein (CRP) level, hemoglobin level, steroid-refractory state, steroid-dependent state, and presence of CMV antigenemia.

A p value less than 0.05 was considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). EZR is a modified version of R commander (version 1.6-3) that includes statistical functions frequently used in biostatistics.

Ethical considerations

This study was approved by the ethics committee of the relevant institution. The ethics committee granted an exemption from written informed consent to this study because the anonymity of clinical data that were retrospectively obtained after each patient agreed to treatment was maintained during the analysis. Nevertheless, all patients were notified of the content and information in this study and given the opportunity to refuse participation, although none of the patients did so. This procedure followed the Ethical Guidelines for Medical and Health Research Involving Human Subjects established by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare in Japan.

Results

Baseline characteristics and comparisons

Of the 96 patients with UC treated with oral tacrolimus, 2 were excluded as they were lost to follow-up before week 12, and 30 patients who were nonresponsive to tacrolimus therapy were also excluded. Sixty-four patients (68.1%) were considered responders, but 1 responder was excluded because he received infliximab as maintenance therapy. Thus, 63 patients were enrolled in the study.

Of these patients, 45 (71.4%) had received IM as maintenance therapy (Fig. 1). Among these 45 patients with IM maintenance therapy, 43 received azathioprine (2 received 25 mg/day, 34 received 50 mg/day, 2 received 75 mg/day, 3 re-

Table 1. Baseline Characteristics of Study Population.

	All patients	Non-IM group	IM group	p value
Number of patients	63	18	45	
Gender: male/female	35/28	9/9	26/19	0.589
Age at initiation of tacrolimus, years, median (IQR)	39.8 (27.9-50.9)	42.8 (24.4-56.3)	36.7 (28.8-49.3)	0.808
Age at onset, years, median (IQR)	32.2(22.4-42.2)	30.1 (20.6-45.3)	32.2 (23.1-39.8)	0.915
Disease duration, years, median (IQR)	2.6 (1.1-8.7)	2.6 (0.2-8.2)	2.7 (1.2-8.6)	0.626
Location: left-sided colitis/pancolitis	13/50	2/16	11/34	0.316
Response to corticosteroid				
Dependent, n (%)	22 (34.9%)	3 (16.7%)	19 (42.2%)	0.08
Refractory, n (%)	30 (47.6%)	8 (44.4%)	22 (48.9%)	0.787
Partial Mayo score, median (IQR)	7 (6-7)	7 (5.25-7)	7 (6-7)	0.458
Hemoglobin (g/dL), median (IQR)	11.6 (9.9-13.0)	10.3 (8.5-11.6)	12.0 (10.8-13.1)	0.032
Albumin (g/dL, median (IQR)	3.20 (2.70-3.60)	3.20 (2.52-3.70)	3.20 (2.70-3.60)	0.796
CRP (mg/dL), median (IQR)	2.60 (0.45-6.54)	3.30 (0.76-6.63)	2.25 (0.41-6.11)	0.654
Presence of CMV antigenemia	6 (9.5%)	2 (11.1%)	4 (8.9%)	1
Concomitant mesalamine at initiation of tacrolimus	59 (93.7%)	18 (100%)	41 (91.1%)	0.317
Concomitant corticosteroid at initiation of tacrolimus	44 (69.8%)	9 (50.0%)	35 (77.8%)	0.038
Concomitant IM at initiation of tacrolimus	13 (20.6%)	0 (0%)	13 (28.9%)	0.013
Duration of tacrolimus therapy (week), median (IQR)	13.9 (13.1-16.9)	14.9 (13.4-20.9)	13.6 (12.9-16.7)	0.357

IQR: interquartile range, CRP: C-reactive protein, CMV: cytomegalovirus, IM: immunomodulators

ceived 100 mg/day, and 2 received 150 mg/day) and 2 received 6-mercaptopurine (1 received 15 mg/day, and 1 received 30 mg/day) in the maintenance period. As the dose of IM was optimized according not to the exacerbation of gastrointestinal symptoms, but to the white blood cell count, the optimization of the dose of IM was not recognized as relapse. The remaining 18 patients did not receive IM maintenance therapy. The reasons for the absence of concomitant IM were a history of IM intolerance (n=1), non-steroid dependent state (n=3), achievement of clinical remission without any symptoms (n=7), achievement of clinical and mucosal healing (n=2), self-interruption (n=1), early adverse event of IM (n=2), comorbidity of malignant tumor (n=1), and patient's wish (n=1). In our hospital, patients select the use of IM as maintenance therapy after receiving a sufficient explanation from their physicians regarding the risks and benefits of the therapy, as few studies have evaluated the clinical utility of IM maintenance therapy after tacrolimus, and IM often causes severe adverse events. These 18 patients elected not to receive IM after a thorough consultation with their physicians.

The demographic characteristics of the patients are summarized in Table 1. In the 35 men and 28 women, UC was diagnosed at a median age of 32.2 years (IQR: 22.4-42.2), tacrolimus was started at a median age of 39.8 years (IQR: 27.9-50.9), and the median p-Mayo score at baseline was 7 (IQR: 6-7).

Influence of immunomodulators

Of the 63 patients enrolled, 45 received IM maintenance therapy at the time of the termination of tacrolimus treatment, all according to their physician's suggestion. Eighteen patients were treated without concomitant IM, and all of them received concomitant mesalamine. The proportions of concomitant corticosteroid usage and blood hemoglobin levels were higher in the IM group than in non-IM group, but no significant differences in any other baseline characteristics, including disease severity or response to corticosteroids, were noted (Table 1).

The median follow-up time after starting tacrolimus therapy was 14.2 months (IQR: 6.8-43.4). Of the 63 patients, 30 (47.6%) eventually relapsed during the follow-up period (Fig. 1). Of those, 40.0% (18/45) of the patients with IM eventually relapsed, compared with 66.7% (12/18) of the patients without concomitant IM (p=0.093, chi-square test).

Based on Kaplan-Meier survival estimator graphs, the probability of avoiding clinical relapse was 52.1% at 2 years and 41.9% at 5 years (Fig. 2). The Kaplan-Meier curve in Fig. 2 illustrates the relapse-free rate for both the IM and non-IM groups. The relapse-free survival rate was significantly worse in the non-IM group than in the IM group (p= 0.01, log-rank test); the 2-year rates were 22.5% and 63.6% in the non-IM and IM groups, respectively.

Concomitant IM was significantly conversely associated with clinical relapse in the unadjusted analysis (HR: 0.39, 95% CI: 0.19-0.83, p=0.01). No other clinical variables, including the age, sex, disease duration, age at onset, disease location, disease severity, response to corticosteroids, serum CRP, serum albumin, and presence of CMV antigenemia, showed a statistically significant association (Table 2).

Candidate factors associated with the long-term outcomes of tacrolimus therapy in the multivariate Cox regression model were the sex (14), age at the diagnosis (14), concomitant IM, steroid dependency, albumin (15), and disease location (16), These factors were previously reported to be associated with the clinical course of UC. Interactions among these 6 factors in the multivariate analysis revealed concomitant IM to be an independent protective factor for

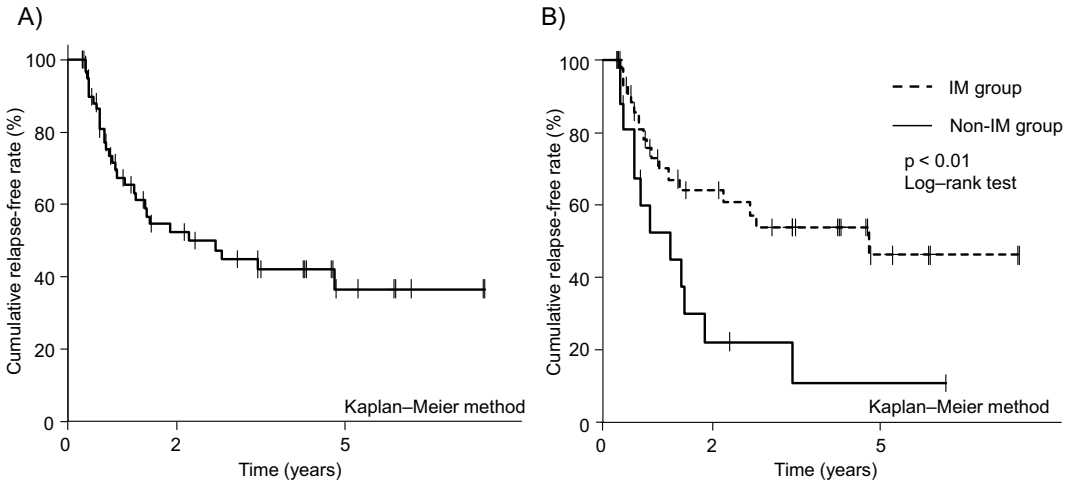


Figure 2. **The relapse-free survival after tacrolimus therapy. The overall relapse-free survival in 63 responders to tacrolimus (A) and the relapse-free survival based on concomitant immunomodulators (IM). The overall survival rate was significantly better in patients with IM than in those without IM (p<0.01, log-rank test) (B).**

Table 2. **Univariate and Multivariate Analysis of Clinical Relapse.**

	n	Case (%)	Unadjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value
Age (continuous, per 10 years old)	63		1.11 (0.87-1.42)	0.39		
Gender						
Male	35	17 (48.6)	1.00			
Female	28	13 (46.4)	0.88 (0.43-1.81)	0.73	0.86 (0.39-1.94)	0.72
Disease duration (continuous, per 10 years old)	63		1.02 (0.66-1.58)	0.94		
Age at onset (continuous, per 10 years old)	63		1.13 (0.87-1.46)	0.37	1.13 (0.86-1.49)	0.37
Disease location						
Left-sided colitis	13	5 (38.5)	1.00			
Pancolitis	50	25 (50.0)	1.06 (0.41-2.78)	0.90	0.81 (0.29-2.32)	0.70
Predonisolone dependent						
No	41	20 (48.8)	1.00			
Yes	22	10 (45.5)	0.81 (0.38-1.73)	0.58	0.72 (0.30-1.71)	0.46
Predonisolone refractory						
No	33	17 (51.5)	1.00			
Yes	30	13 (43.3)	0.93 (0.45-1.91)	0.84		
Immunmodulators						
No	18	12 (66.7)	1.00			
Yes	45	18 (40.0)	0.39 (0.19-0.83)	0.01	0.35 (0.16-0.78)	0.01
Severe ulcerative colitis at start of tacrolimus (partial Mayo score ≥7)						
No	27	15 (55.6)	1.00			
Yes	36	15 (41.7)	0.69 (0.34-1.41)	0.31		
CRP (continuous)	63		0.98 (0.90-1.06)	0.55		
Albumin (continuous)	63		1.36 (0.68-2.74)	0.38	1.55 (0.71-3.38)	0.27
Hemoglobin	63		0.99 (0.84-1.17)	0.94		
Presence of CMV antigenemia						
Negative	56	28 (50.0)	1.00			
Positive	6	2 (33.3)	0.80 (0.19-3.36)	0.76		

CI: confidence interval, HR: hazard ratio, CRP: C-reactive protein, CMV: cytomegalovirus

Table 3. Univariate and Multivariate Cox.

	HR (95% CI)	p value
Unadjusted	0.39 (0.19-0.83)	0.01
Adjusted	0.35 (0.16-0.78)	0.01
IPTW	0.31 (0.15-0.64)	<0.01

IPTW: inverse probability of treatment weighting, HR: hazard ratio

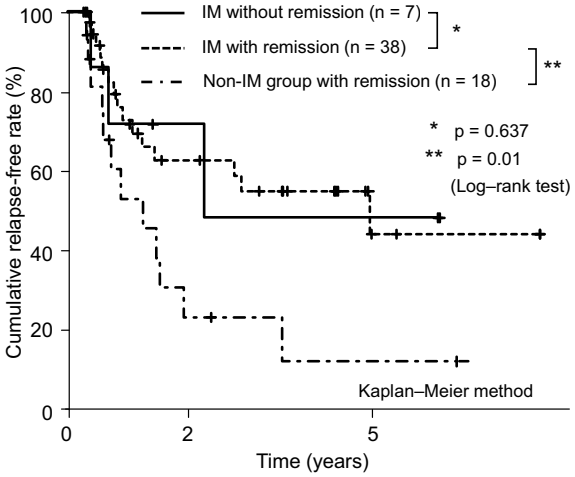
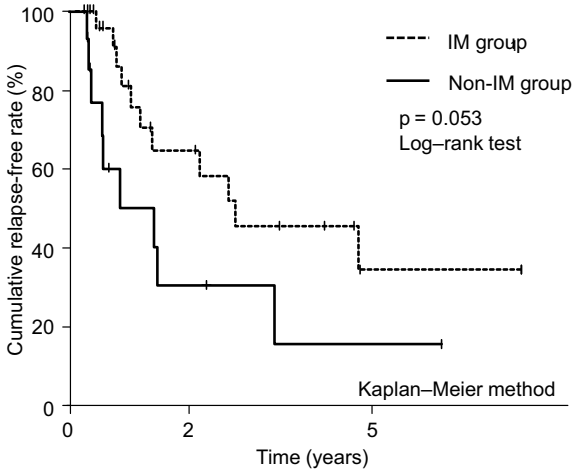


Figure 4. The relapse-free survival after tacrolimus therapy based on clinical remission after tacrolimus induction therapy. In the immunomodulators (IM) group, no significant differences were noted between patients with and without clinical remission. In contrast, among patients who obtained clinical remission, the relapse-free survival rate was significantly worse in the non-IM group than in the IM group (p=0.01, log-rank test).



Non- steroid dependent patients

Figure 3. The relapse-free survival after tacrolimus therapy among non-steroid-dependent patients. Even in the non-steroid-dependent group, patients with immunomodulators showed a tendency toward a higher relapse-free rate after tacrolimus therapy than those without immunomodulators (p=0.053, log-rank test).

relapse (adjusted HR: 0.35, 95% CI: 0.16-0.78, p=0.01), while the other 5 factors were not significantly associated with increased risks of clinical relapse (Table 2). After IPTW, IM maintenance therapy was also significantly associated with a longer relapse-free survival than non-IM therapy (HR: 0.31, 95% CI: 0.15-0.64, p<0.01) (Table 3).

Previous studies (9, 10) included both steroid-dependent and non-dependent patients, and the benefit of IM after inducing remission in non-steroid-dependent patients remained unclear. Since the current study population similarly consisted of both steroid-dependent and non-steroid-dependent patients, a subgroup analysis was performed to fill this knowledge gap. Even in the non-steroid-dependent group, patients with IM therapy showed a tendency toward a higher relapse-free rate after tacrolimus-induced remission than those without IM therapy (p=0.053, log-rank test) (Fig. 3). The 2-year relapse-free rates were 29.8% and 64.2% for the non-IM and IM groups, respectively. A Cox regression analysis using the IPTW method also identified concomitant IM as a protective factor for clinical relapse (HR: 0.36, 95% CI: 0.14-0.99, p=0.047).

Concerning the disease severity, the disease severity has been reported to be a risk of relapse (17). We therefore analyzed whether or not the disease activity influenced the prognosis. Among the 63 patients included in this study, 56 obtained clinical remission by tacrolimus induction therapy, and 7 did not. All seven of these patients without clinical remission received IM maintenance therapies. Fig. 4 shows the relapse-free survival rate based on clinical remission and IM maintenance therapy. In the IM group, no significant differences were noted between patients with and without clinical remission. However, among patients who obtained clinical remission, the relapse-free survival rate was significantly worse in the non-IM group than in the IM group (p=0.01, log-rank test).

Adverse events

Overall, 20 adverse events due to tacrolimus administration were observed in 16 of 63 total patients (25.4%), including 2 (11.1%) in the non-IM group and 14 (31.1%) in the IM group (Table 4). Concomitant IM treatment did not result in an increase in the incidence of these adverse events (p=0.121). Twelve adverse events due to IM were observed in 11 of 45 patients (24.4%) on IM maintenance therapy (Table 4). No serious adverse events occurred. All adverse events due to either tacrolimus or IM were reversible, and all patients recovered completely. While no patients had to discontinue tacrolimus, two had to discontinue IM due to adverse events (fatigue and elevated liver enzymes) in the early phases of treatment and were unable to receive IM as maintenance therapy. These 2 patients completely recovered following the discontinuation of IM.

Discussion

Tacrolimus treatment has been shown to be extremely ef-

Table 4. Adverse Events of Tacrolimus or Immunomodulators.

Adverse events, number (%)	Tacrolimus	Immunomodulators
Renal disorder	4 (6.3)	1 (2.2)
Tremor	3 (4.8)	0 (0)
Hypomaganesemia	3 (4.8)	0 (0)
Nausea	3 (4.8)	2 (4.4)
Headache	3 (4.8)	0 (0)
Fatigue	1 (1.6)	1 (2.2)
Hepatic disorder	1 (1.6)	0 (0)
Sweating	1 (1.6)	0 (0)
Hyperkalemia	1 (1.6)	0 (0)
Leucopenia	0 (0)	3 (6.7)
Alopecia	0 (0)	2 (4.4)
Dermatitis	0 (0)	2 (4.4)
Anemia	0 (0)	1 (2.2)

fective in patients with UC who otherwise would require colectomy. The 65.7% initial response rate in this study is consistent with the results of controlled trials (5). Data describing the long-term clinical course after tacrolimus discontinuation and the impact of IM on this clinical course are limited. The present study showed that the relapse-free survival was significantly longer in patients receiving IM than in those not receiving IM, and IM maintenance therapy was shown to be a protective factor for the relapse-free survival after adjusting for other confounding factors, as indicated using IPTW based on propensity scores. These results are consistent with those of previous studies showing that IM improved the long-term outcomes after tacrolimus-induced remission. Of note, those previous studies evaluated heterogeneous patient populations (9, 10). To our knowledge, our study is the first to assess the efficacy of IM as maintenance therapy after tacrolimus-induced remission for patients with UC, using IPTW to evaluate outcomes.

The 2- and 5-year relapse-free rates of 22.5% and 11.3% in the non-IM group were much worse than the previously reported relapse rates after initial steroid therapy (18). This result supports the recommendation in the ECCO guidelines that patients responding to calcineurin inhibitors should use IM as maintenance therapy, regardless of steroid dependency (2). However, owing to the lack of evidence regarding the long-term outcomes of IM maintenance therapy in non-steroid-dependent UC patients, the question remained as to whether or not non-steroid-dependent patients, who could theoretically maintain remission with only mesalamine treatment, would also benefit from IM maintenance therapy. Since the present study population included non-steroid-dependent patients, we were able to address this question.

Notably, the subgroup analysis shows that non-steroid-dependent patients treated with IM had significantly better long-term outcomes than those without IM. The 2- and 5-year relapse-free rates of 29.8% and 14.9%, respectively, in the non-IM group among the non-steroid-dependent population are still lower than those associated with a natural history of UC (18), indicating that IM may be beneficial after tacrolimus-induced remission, even for non-steroid-dependent patients. In the present study, patients experiencing their first UC occurrence or those who were refractory to steroid therapy were allocated to the non-steroid-dependent group by definition. However, some patients in this group may have been potentially steroid-dependent, since they had not been previously treated with steroids. Although some studies have analyzed the presence of predictive factors of steroid dependency before starting steroid treatment (19-21), it is difficult to determine actual steroid dependency before treatment.

Regarding disease severity, IM was useful for patients in whom clinical remission had been achieved with tacrolimus induction therapy (Fig. 4). Our analysis showed that even patients with clinical remission induced by tacrolimus induction therapy should receive IM as maintenance therapy to ensure a better long-term prognosis. However, in the IM group, no significant differences were noted between patients with and without clinical remission, although the very small sample size prevents us from concluding that the disease severity did not influence the long-term prognosis.

Some controversy exists as to which induction therapies should be chosen for UC flare-ups. Many studies have analyzed the efficacy and prognostic factors for UC patients treated with calcineurin inhibitors or infliximab (22-25), but almost all of these studies were unable to confirm the superiority of the short- or long-term efficacy of either therapeutic regimen (26-29). Patients receiving calcineurin inhibitors typically also received IM in such studies. The present study showed that the long-term outcomes after tacrolimus induction therapy without IM maintenance therapy were relatively poor, regardless of steroid dependency. Since long-term tacrolimus therapy is known to cause chronic renal dysfunction (30), maintenance therapies such as IM or anti-TNF-α antibodies are required. While tacrolimus treatment might be preferable in IM-naïve patients or patients receiving IM, other therapies (e.g., infliximab, adalimumab, golimumab, and cytapheresis) should be chosen in patients with IM failure or intolerance. In this study, the concomitant use of IM did not affect the frequency of adverse events due to tacrolimus administration, and no severe adverse events occurred due to tacrolimus or IM therapy. However, an increased risk of lymphoma has been reported to be associated with the use of IM (31, 32). Although no patients developed lymphoma in this study, we must consider the increased risk of lymphoma when prescribing IM. Patients tend to receive IM as a long-term therapy, as there is no unified view at the time of withdrawal of IM treatment. Particularly for younger patients, who have to receive maintenance therapies for long period, other maintenance therapies, such as anti-TNF-α, should probably be chosen after tacrolimus therapies. Indeed, Kotlyar et al. reported that men under 35 years of age are at a high risk for lymphoma (31). The risks of lymphoma and potential benefits of therapy should therefore be considered for all patients.

Several limitations associated with the present study warrant mention. First, the group sample sizes were small, patients' treatment history and therapeutic protocols were heterogeneous, and the study was retrospectively performed with relatively short observation periods at a single center. Not all variables could be controlled, leading to selection bias and other unmeasurable confounding factors. A prospective randomized study would be ideal for clarifying the efficacy of thiopurines as a maintenance treatment after tacrolimus therapy. However, such a study would be difficult to justify due to ethical concerns. Therefore, the accumulation of evidence from well-planned retrospective studies is essential. Despite the small number of patients and their heterogeneity, the study results support the conclusion that the use of IM as maintenance therapy is effective.

Furthermore, in the present study, we were unable to evaluate biomarkers such as fecal calprotectin or the endoscopic activity score. According to Ikeya et al., the endoscopic activity score predicts the long-term prognosis in patients with UC receiving tacrolimus therapy (33). Although mucosal healing is reported to be associated with the long-term prognosis after tacrolimus therapy, we were unable to assess its influence due to the retrospective design of the study; colonoscopy was not routinely performed after tacrolimus induction at a set time.

In conclusion, the present results show that the use of IM after tacrolimus therapy is a protective factor for the relapse-free survival in patients with UC, including non-steroid-dependent patients. Furthermore, no serious adverse events occurred due to either tacrolimus or IM therapy, and the use of IM did not increase the incidence of adverse events associated with tacrolimus administration. Retrospective data confirm the benefits and safety of IM for maintaining remission after tacrolimus therapy in patients with UC.

The authors state that they have no Conflict of Interest (COI).

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