NIZ group did include more subjects with severe RE compared with the famotidine group (Sekiguchi *et al.*, 1983; Nakano *et al.*, 1984; Sekiguchi *et al.*, 1989). In addition, it has been reported that maintenance therapy from NIZ has a significantly higher non-recurrence rate than that of famotidine in patients with RE (Hamamoto *et al.*, 2005). RE is characterized by excessive esophageal acid exposure, which increases significantly according to the severity of RE (Iwakiri *et al.*, 2009). Since, as it has been reported, there is no difference in the healing rate of gastric ulcers among H2RAs (Barbara *et al.*, 1983; Naccaratto *et al.*, 1987; Inoue *et al.*, 1988; Judmaier *et al.*, 1988), it is considered that there is no significant difference in the level of acid suppression among H2RAs and it is therefore possible that NIZ has other positive effects on esophageal acid exposure besides acid suppression. Although it has been reported that NIZ enhances salivary secretion, bicarbonate output (Adachi *et al.*, 2002) and stimulates gastric emptying (Harasawa *et al.* 1993; Shiomi *et al.*, 2001), it is not clear whether or not NIZ has an effect on reflux itself.

RE is characterized by excessive esophageal acid exposure and because most acid reflux episodes, in healthy subjects and patients with RE, are caused by transient lower esophageal sphincter relaxations (TLESRs) (Iwakiri *et al.*, 2005; Hayashi *et al.*, 2008; Iwakiri *et al.*, 2009), if there is a decrease in the rate of TLESRs in patients with RE, it is possible that the number of acid reflux episodes also decreases and excessive esophageal acid exposure will normalize. Since there is no difference in the rate of TLESRs in healthy subjects and patients with RE, in this study we investigated the effect of NIZ on the rate of TLESRs, the rate of acid reflux during TLESRs and esophageal acid exposure in healthy subjects.

Methods

Subjects

Studies were carried out in 10 asymptomatic, healthy volunteers, who did not have hiatus hernia, defined as when the LES-crural diaphragm separation is more than 2 cm at inspiration, using high-resolution manometry. The subjects underwent esophagogastroduodenoscopy but there were no localized lesions in the esophagus, stomach, or duodenum and none had previously undergone gastrointestinal surgery. Informed consent was obtained from every subject and the study was approved by the Ethics Committee for Human Research at the Nippon Medical School.

Study design

The study was carried out in single-blind fashion, where two identical tablets were administered for both NIZ and the placebo, to ensure that the subjects could not identify the treatment arm. Subjects were randomly assigned to receive either NIZ or the placebo and the order of these treatments was also randomly determined. For 7 days, subjects were given NIZ (150 mg am before breakfast and 150 mg pm before dinner) or the placebo. Subsequently, after a washout period of at least 7 days, subjects crossed over to the other arm (placebo-treatment, treatment-placebo) (Fig. 1a). After taking NIZ or the placebo for 7 days, on day 8, concurrent esophageal manometry and pH monitoring was carried out.

On the day of the study, 60 min before breakfast (6:00 am), subjects took NIZ or the placebo and thereafter food was prohibited. Subjects again took NIZ or the placebo at 1:40 pm, after which, the

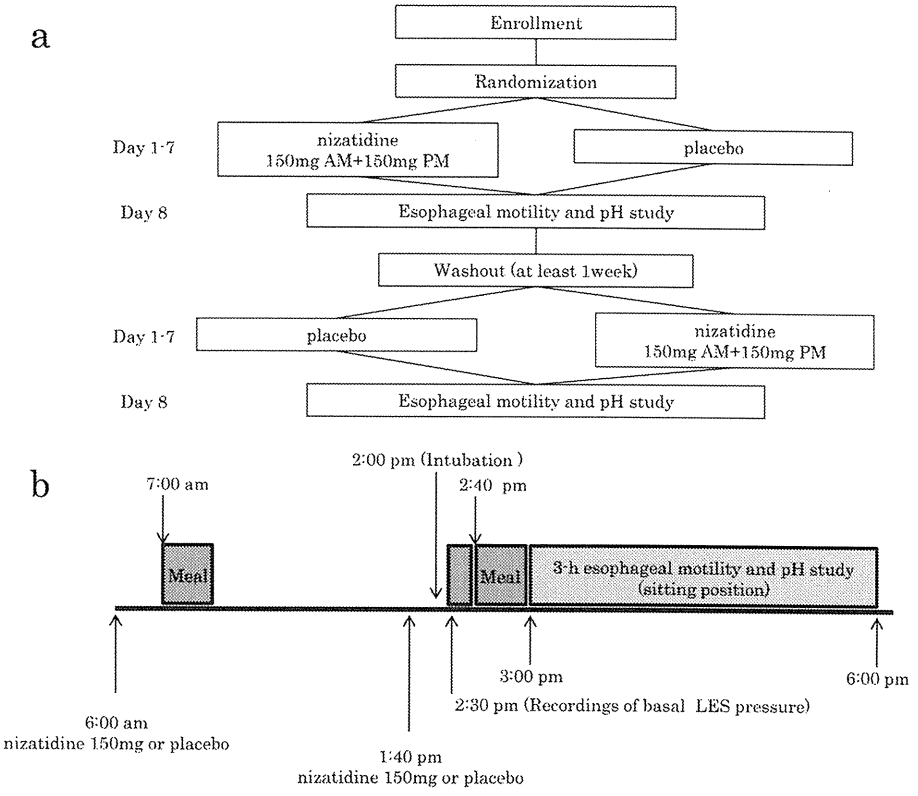

Fig. 1. Study design showing (A) randomized schedule and (B) dosing schedule, standardized meals and pharmacodynamics assessments. LES = lower esophageal sphincter.

manometric assembly and the pH electrode were passed via an anaesthetized nostril at 2:00 pm and positioned so that the LES was positioned 2 cm below the most proximal of the 1-cm interval side holes and the pH electrode positioned 2 cm above the proximal margin of the LES. The subjects were then allowed to adapt to the assembly for 30 min, after which recordings of LES pressure were taken for 10 min (2:30–2:40). At 2:40 pm, for a period of 20 min, the subjects were given a solid-liquid test meal, which consisted of a hamburger, ice-cream and 250 mL of mineral water (678 kcal, 34.1 % fat, 17.0% protein). After the meal, if necessary, the position of the assembly was adjusted and recordings were taken for 3 h (3:00–6:00 pm) while subjects remained seated (Fig. 1b).

Recording methods

Esophageal manometry was performed with a 21-channel manometric assembly (Dentsleeve Pty Ltd., Wayville, Australia). Ten side holes, spaced at 1-cm intervals, starting at 3 cm above the distal end of the assembly, monitored pressure from the proximal stomach, LES and distal esophagus. A further 7 side holes, spaced at 2-cm intervals, monitored pressure from the distal to the proximal esophagus, and 4 side holes, at 3, 6, 10, 13 cm above the most proximal of the 2-cm interval side holes, monitored pressure from the proximal esophagus to the pharynx. Each lumen was perfused with degassed, distilled water at 0.15 mL/min by a low compliance manometric infusion pump (Dentsleeve

Pty Ltd., Wayville, Australia). Esophageal pH was measured with an antimony electrode (Synectics Medical AB, Stockholm, Sweden) positioned at 2 cm above the proximal margin of the LES. Data were digitized with a computer and the digitized signals were displayed, stored, and analyzed, using Trace! Software (Dr. G.S Hebbard, The Royal Melbourne Hospital, Parkville, Australia).

Data analysis

Basal LES pressure was measured at end-expiration and referenced to end-expiratory intragastric pressure. One-minute visual means were taken for ten consecutive min and a mean basal LES pressure was calculated.

Acid reflux episodes were defined as an abrupt drop in esophageal pH below 4 for at least 4 seconds or, if basal esophageal pH was already below 4, as a further decrease in pH of at least 1 pH unit. For analysis of the occurrence of acid reflux during TLESRs, acid reflux was deemed to have occurred if there was an abrupt drop in pH of at least 1 pH unit (Holloway *et al.*, 1991).

For each reflux episode, the mechanism of reflux was determined by the patterns of LES pressure and esophageal body activity, their relationship to swallowing, and the occurrence of abdominal straining. These mechanisms were classified as TLESR, swallow-induced LES relaxation (including multiple swallows), LES pressure drifts, absent basal LES pressure, straining and non-interpretable. For the analysis of patterns of LES pressure associated with reflux, the profile of pressure was used across the full extent of the ten 1cm-spaced side-hole array, which straddled the gastroesophageal junction. TLESRs were defined as previously described (Holloway *et al.*, 1995). LES relaxations which lasted for more than 15 s and which were associated with a swallow, within 4 s before or 2 s after the onset of an LES relaxation, were also included as TLESRs (Kawahara *et al.*, 1997). LES relaxations lasting <15 s, whose onset was associated with a swallow within 4 seconds before its onset until maximal relaxation had been achieved, were judged to be swallow-induced. Persistently absent basal LES pressure was defined as LES pressure of less than 2 mmHg for more than 30 s. A drift in basal LES pressure was defined as a slow fall (<1 mmHg/s) in LES pressure to a level less than 2 mmHg above intragastric pressure, maintained for less than 30 s. Abdominal strain was defined as a sharp and simultaneous increase in gastric and esophageal pressure, greater than twice the normal inspiratory increase of intragastric pressure. Straining was judged to be the principal mechanism of reflux when the onset of an acid pH deflection occurred during a strain when there was detectable (>2.0 mmHg) LES pressure, and as a possible co-factor when LES pressure at the time of reflux was < 2.0 mmHg (Penagini *et al.*, 1996).

Statistical analysis

Values for the basal LES pressure, the rate of TLESRs, the rate of acid reflux during TLESRs and acid esophageal exposure were determined for each subject and from these, median values were calculated for the group as a whole. All data are presented as median (interquartile range) and were analyzed using Wilcoxon's test. A P value of <0.05 was considered significant.

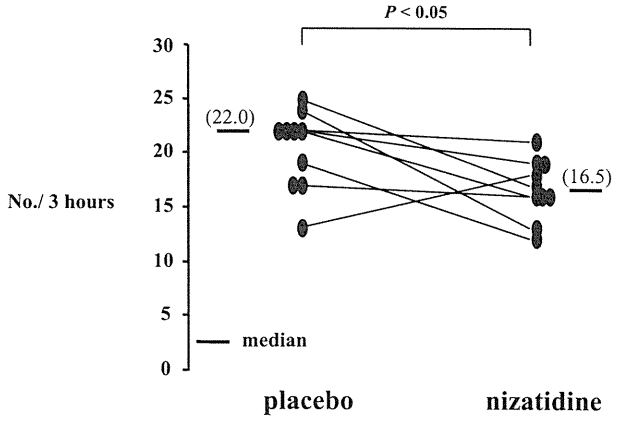

Fig. 2. The rate of transient lower esophageal sphincter relaxations in both the nizatidine and the placebo groups for the entire 3-hour postprandial period.

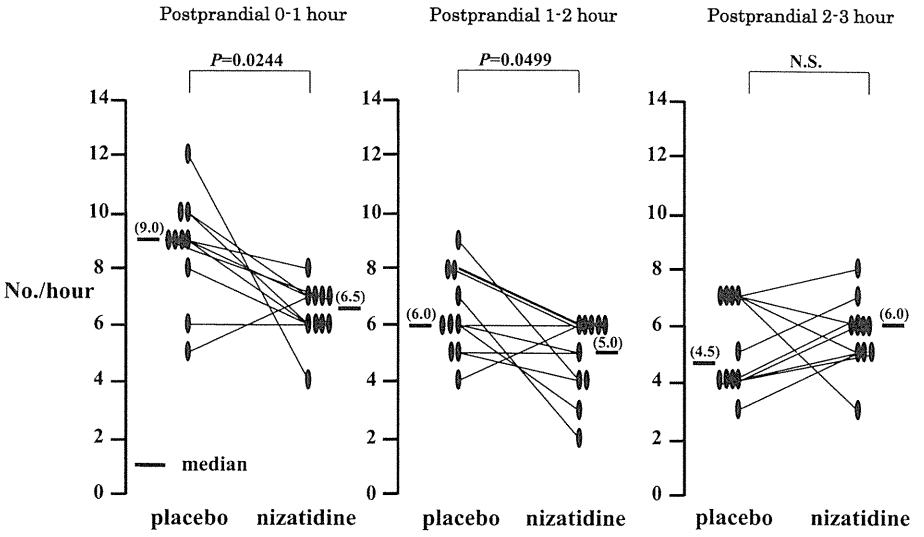

Fig. 3. The rate of transient lower esophageal sphincter relaxations in both the nizatidine and the placebo groups for each postprandial hour.

Results

Basal LES pressure

Basal LES pressure in the NIZ group (14.1 mmHg [11.9–18.0]) was significantly (P=0.0077) greater than that in the placebo group (8.5 mmHg [7.0–9.6]).

Mechanisms of acid reflux episodes in the NIZ group and the placebo group

All acid reflux episodes in the NIZ group and placebo group occurred during TLESRs.

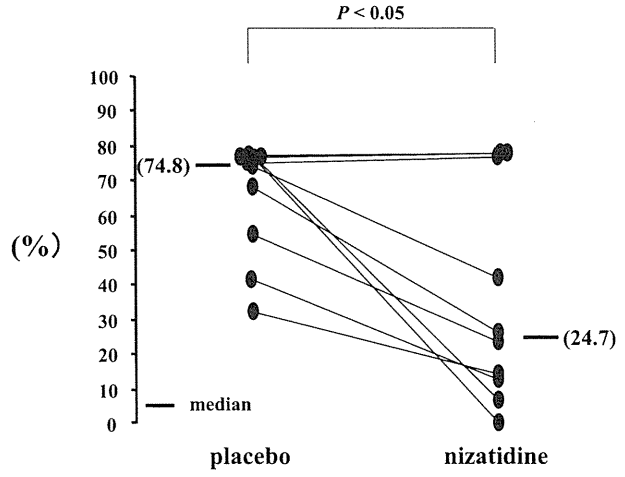

Fig. 4. The rate of acid reflux during transient lower esophageal sphincter relaxations in both the nizatidine and the placebo groups for the entire 3-hour postprandial period.

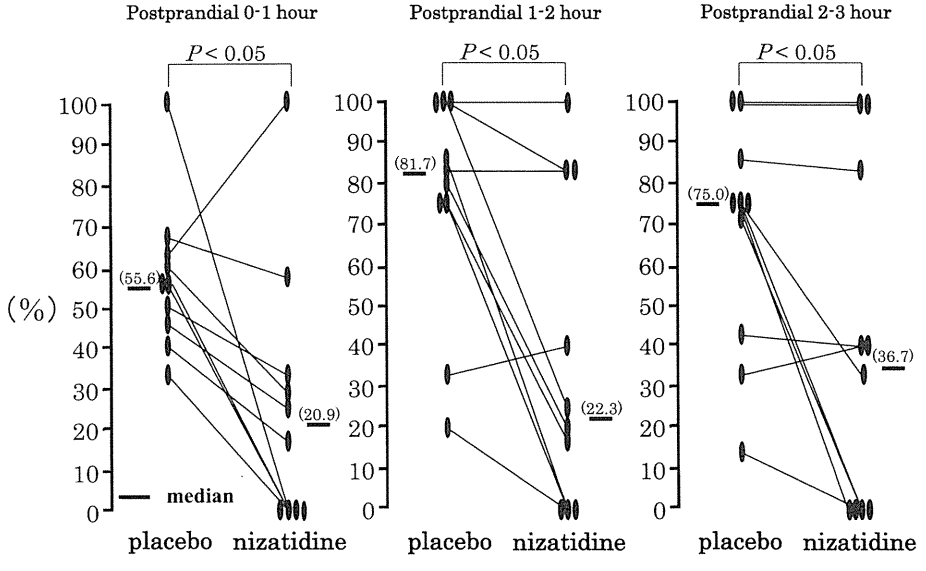

Fig. 5. The rate of acid reflux during transient lower esophageal sphincter relaxations in both the nizatidine and the placebo groups for each postprandial hour.

Rate of TLESRs

The rate of TLESRs in the NIZ group for the entire 3-hour period (0–3 h: 16.5/h, [16.0–19.0]) (Fig. 2), for the 0-1 hour (6.5/h, [6.0–7.0]) and for the 1–2 hour (5.0/h, [4.0–6.0]) postprandial hour (Fig. 3) were significantly less than that of the placebo group (0–3 h: 22.0/h, [17.0–22.0], $P<0.05$, 0–1 h: 9.0/h, [8.0–10.0], $P=0.0244$, 1–2 h: 6.0/h, [5.0–8.0], $P=0.0499$, respectively). However, for the 2–3 postprandial hour, there was no difference in the rate of TLESRs in the groups (NIZ group: 6.0/h, [5.0–6.0], placebo group: 4.5/h, [4.0–7.0]) (Fig. 3).

Table 1. Esophageal acid exposure in the placebo group and nizatidine group

	placebo group	nizatidine group
Postprandial 3 hours	2.8% (1.1–3.7)	0.2% (0.1–1.0)*
Postprandial 0–1 hour	1.5% (0.4–3.3)	0.3% (0–1.1)*
Postprandial 1–2 hour	4.0% (0.6–7.7)	0.2% (0–0.8)*
Postprandial 2–3 hour	0.7% (0.4–2.4)	0% (0–0.7)*

*, $P<0.05$ *vs.* placebo group. Median % (interquartile range).

Rate of acid reflux during TLESRs

The rate of acid reflux during TLESRs in the NIZ group for the entire 3-hour period (Fig. 4) and for each postprandial hour (Fig. 5) (0–3 h: 24.7% [12.5–76.4], 0–1 h: 20.9%, [0–33.3], 1–2 h: 22.5%, [0–83.3], 2–3 h: 36.7%, [0–83.3], respectively) was significantly less than that of the placebo group (0–3 h: 74.4%, [54.2–76.9], $P<0.05$, 0–1 h: 55.6%, [45.5–62.5], $P<0.05$, 1–2 h: 81.7%, [75.0–100], $P<0.05$, 2–3 h: 75.0%, [42.7–85.7], $P<0.05$, respectively).

Esophageal acid exposure

Esophageal acid exposure in the NIZ group for the entire 3-hour period and for each postprandial hour, was significantly ($P<0.05$) less than that of the placebo group (Table 1).

Discussion

In healthy subjects and patients with mild RE, most acid reflux episodes occur 0–3 hour after a meal and most are accompanied by TLESRs (Iwakiri *et al.*, 2005). In order to investigate the effect of NIZ on TLESRs and acid reflux, we administered NIZ 60 min before a meal so that the blood concentration of NIZ would be at its maximum, approximately 80 min after having administered. As a result, in the NIZ group, compared with the placebo group, NIZ significantly increased the basal LES pressure and significantly reduced the rate of the TLESRs at 0–1 and 1–2 postprandial hours, the rate of acid reflux episodes during TLESRs at each postprandial hour and the esophageal acid exposure at each postprandial hour.

Excessive esophageal acid exposure causes RE therefore in order to reduce this, it is important to decrease gastric acid secretion, improve delayed esophageal clearance and to reduce acid reflux itself. Considering that TLESRs are the major mechanism of reflux episodes in healthy subjects and patients with gastroesophageal reflux disease (GERD) (Iwakiri *et al.*, 2005; Hayashi *et al.*, 2008; Iwakiri *et al.*, 2009,), it is important to decrease the rate of TLESRs in the treatment of GERD.

At present, there are only a few pharmacological agents (morphine, cholecystokinin, atropine, baclofen) available for the treatment of acid reflux (Boulant *et al.*, 1997; Mittal *et al.*, 1997; Penagini *et al.*, 1997; Boeckxstaens *et al.*, 1998; Clavé *et al.*, 1998,), however it is very difficult to use most of these drugs because they need to be injected and /or they have side-effects. It is possible for only baclofen to be effective in the treatment of acid reflux and it has been reported that baclofen, a gamma-aminobutyric acid B agonist, has been successful in decreasing the rate of TLESRs, as

well as decreasing the number of acid reflux episodes in healthy subjects and patients with GERD (Lidums *et al.*, 2000; Zhang *et al.*, 2002). Baclofen has also been effective in reducing the symptoms associated with acid reflux and non-acid reflux in the postprandial period (Vela *et al.*, 2003). It can be used as a therapeutic option, however, its use is limited because of its side-effects and as well, the standard dose of baclofen used in Japan is half that used in Western countries. The result that NIZ decreases the rate of TLESRs, as found in this study, may be very valuable in the treatment of GERD.

Inhibition of TLESRs is theoretically feasible using either an antagonist or an agonist on the neurotransmitters and receptors, which are involved in the reflex arc underlying TLESRs (Hirsch *et al.*, 2002). Although the mechanisms of how NIZ affects TLESRs are unknown, in view of the fact that NIZ reduces the rate of TLESRs, it is possible that NIZ participates in the reflex arc underlying TLESRs.

It is possible also that the administration of NIZ increases basal LES pressure because it has been reported that NIZ has inhibitory activity on acetylcholine esterase and as expected, basal LES pressure significantly increases after the administration of NIZ. In healthy subjects and patients with mild RE, it has been reported that acid reflux episodes occurred almost exclusively during TLESRs, therefore an increase in LES pressure after the administration of NIZ in healthy subjects, does not directly affect the number of acid reflux episodes.

In summary, NIZ significantly reduces acid reflux episodes by inhibiting both the rate of TLESRs and acid reflux during TLESRs and suppressing esophageal acid exposure time in healthy subjects.

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A multicenter study of the efficacy and safety of leukocytapheresis therapy without concomitant systemic steroid treatment in patients with active ulcerative colitis

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ABSTRACT

We conducted a multicenter study to investigate the efficacy of leukocytapheresis (LCAP) without concomitant steroid therapy in active ulcerative colitis (UC) patients. Twenty patients were enrolled. LCAP was performed twice a week for 3 weeks. The results revealed a significant decrease of the Lichtiger's clinical activity index (CAI) from 11.7 ± 2.6 at baseline to 6.6 ± 4.1 after the therapy. The endoscopic index and serum C-reactive protein levels also decreased significantly after the therapy. Of the 20 patients, 15 (75%) were assessed as responders (CAI $\leqslant$ 4 or ΔCAI $\geqslant$ 3), and 7 (35%) achieved complete remission (CAI $\leqslant$ 4). No serious adverse reactions were encountered. The results suggest that LCAP is an effective and safe option for patients with active UC who had not received systemic steroid treatment.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease of unknown etiology and is characterized by repeated relapses and remissions [1]. In the treatment of UC, mainly 5-aminosalicylate (5-ASA), azathioprine and 6-mercaptopurine are used during the remission phase, and high-dose oral steroids or intravenous steroid therapy are used during the active phase. Intravenous cyclosporin therapy or infliximab treatment is given to some patients with severe disease [2,3]. However, all of these drug therapies are frequently associated with adverse reactions.

Cytapheresis is a procedure in which leukocytes are extracorporeally removed from circulating blood, and the procedure has been reported to be effective, with a

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relatively high safety profile, as a non-pharmacological therapeutic method for active UC [4,5]. Cytapheresis includes leukocytapheresis (LCAP) to remove leukocytes including granulocytes, monocytes and lymphocytes, and granulocytapheresis (GCAP) to eliminate granulocytes and monocytes. The efficacy of cytapheresis treatment of steroid-resistant UC has been evaluated in several studies [6–8]. Cytapheresis came to be used in combination with steroid therapy in active UC patients whose symptoms do not improve sufficiently with systemic steroid treatment alone. However, steroids, the primary drugs used for the induction of remission in UC, are associated with numerous serious adverse reactions, such as moon face, failure to thrive, peptic ulcer and osteoporosis [9–13]. Recently, cytapheresis without concomitant steroid therapy has been attempted in steroid-naïve UC patients [14–17]. These single-center studies have suggested that cytapheresis shows the promise of becoming a first-line therapy for UC.

In order to develop an effective alternative to steroid treatment for the induction of remission, we conducted the present multicenter study to investigate the efficacy and safety of LCAP in patients with active UC who had not received systemic steroid treatment.

2. Materials and methods

2.1. Patients

The study was conducted between May 2003 and March 2005 at 12 institutions. Prior to the study, the protocol was approved by the Ethics Committee of each hospital. We explained the purpose, procedures, efficacy and safety of LCAP to the patients. Informed consent was obtained from all of the patients. The inclusion criteria were as follows: patients with moderate to severe UC in active stage according to the UC severity criteria established by the Research Committee of Specified Diseases/Intractable Inflammatory Bowel Diseases of the Ministry of Health, Labour and Welfare [6], and were not on systemic (oral or intravenous) steroid treatment for at least 1 month prior to the study; patients who underwent endoscopy within 2 weeks prior to the study; and patients with left-sided colitis or pancolitis. The exclusion criteria were as follows: patients with proctitis; patients with serious cardiovascular diseases; patients with hypotension with systolic blood pressure of less than 80 mm Hg; and patients who started on immunosuppressive drugs within the previous month.

2.2. Treatment procedure

The LCAP was carried out using the leukocyte removal column, Cellsorba EX (Asahikasei Kuraray Medical Co. Ltd., Tokyo, Japan). The column is composed of polyester non-woven fabric that can trap leukocytes from whole blood. The filter mainly traps monocytes and granulocytes (>95%), partially traps lymphocytes and platelets (30–90%), and does not trap erythrocytes (<10%) [18].

Extracorporeal circulation was established as follows [6]: the patient's blood was pumped out from the cubital vein and introduced into the column. Nafamostat mesilate (50 mg) (Torii Pharmaceutical Co., Tokyo, Japan) for an anticoagulant was continuously injected into the inlet side of the column. The processed blood was subsequently returned to the patient via the cubital vein of the contralateral arm. About 2000–3000 ml of blood was processed within a period of approximately 1 h. Each treatment was performed in a closed circuit system. The treatment was conducted twice a week for 3 weeks. Drug administration schedules were not altered until the completion of the study.

2.3. Evaluation

For evaluation of the clinical efficacy of LCAP, Lichtiger's clinical activity index (CAI) [19], the endoscopic index (EI) established by Rachmilewitz [20], the serum level of C-reactive protein (CRP), and the Inflammatory Bowel Disease Questionnaire (IBDQ) [21,22] were used. Evaluations were performed before the LCAP therapy, and at 4 weeks after the start of the therapy. Remission was defined as a decrease of the CAI to 4 or less (CAI $\leqslant 4$), and improvement was achieved when the CAI decreased by 3 points or more from the value before the LCAP therapy (ΔCAI $\geqslant 3$) but remained above 4. The response rate of the CAI (CAI $\leqslant 4$ or ΔCAI $\geqslant 3$) was evaluated as the primary endpoint, and changes in the CAI, EI, serum CRP and IBDQ scores, and the incidence of adverse reactions during the therapy were assessed as the secondary endpoints.

2.4. Statistical analysis

Continuous data were expressed as mean ± standard deviation (SD). For changes within groups, non-normal distribution and rank data were analyzed by Wilcoxon's rank-sum test. A multivariate logistic analysis was carried out for evaluation of the factors predicting the response. $p < 0.05$ was considered to represent statistical significance. Statistical analyses were performed using the software StatView, Ver.5 for Windows (Hulinks, Tokyo, Japan).

3. Results

3.1. Patient background

Twenty patients with active UC who had not received systemic steroid treatment within the previous month (8 men and 12 women) were enrolled in the study. The background characteristics of the patients are shown in Table 1. The mean age was 29.9 (range, 16–51) years and the mean disease duration was 47.9 (range, 2–227) months. The mean CAI and EI of the patients were 11.7 ± 2.6 and 8.9 ± 2.5, respectively. The drug treatment received during the study included 5-ASA in 18 (90%) patients, salazosulfapyridine in 1 (5%) patient, steroid enema in 4 (20%) patients, and azathioprine in 1 (5%) patient. The patient treated with azathioprine had been on the drug since 6 months prior to the study. None of the patients received

Table 1
Baseline characteristics of the 20 patients enrolled in the study.

Number of patients	20
Sex (male/female)	8/12
Age, years	29.9 ± 11.6 (16–51)
Disease duration, months	47.9 ± 71.6 (2–227)
UC severity criteria (moderate/severe)	17/3
Extent of disease (pancolitis/left sided)	11/9
Clinical pattern (first attack/relapse-remitting/chronic continuous)	4/12/4
Lichtiger's CAI	11.7 ± 2.6
Rachmilewitz's EI	8.9 ± 2.5
CRP, mg/dl	1.4 ± 2.2

Values are mean ± SD. Values in parentheses represent the corresponding ranges. All other data represent the number of patients. CAI, clinical activity index; EI, endoscopic index; CRP, C-reactive protein.

systemic steroid treatment from a month prior to the study, to study completion. Eleven (55%) of the 20 patients had a previous history of steroid treatment.

3.2. Clinical efficacy

LCAP was performed twice a week for 3 weeks, that is, 6 sessions in total. Seven of the 20 (35%) patients showed remission (CAI of $\leqslant 4$ at Week 4 of therapy). In addition, 8 of the 20 (40%) patients showed improvement (ΔCAI $\geqslant 3$ at Week 4 of therapy). Five of the 20 (25%) patients were non-responders. Therefore, the response rate to LCAP (CAI $\leqslant 4$ or ΔCAI $\geqslant 3$), the primary endpoint, was 75% (15/20).

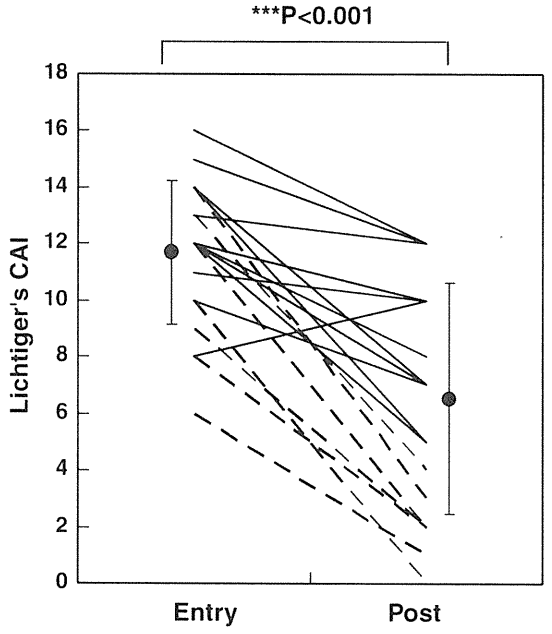

Fig. 1. CAI at entry and post-LCAP treatment in 20 patients with active UC. The dashed lines represent the clinical remission group (CAI $\leqslant$ 4). The mean CAI decreased from 11.7 ± 2.6 at baseline to 6.6 ± 4.1 after the LCAP treatment.

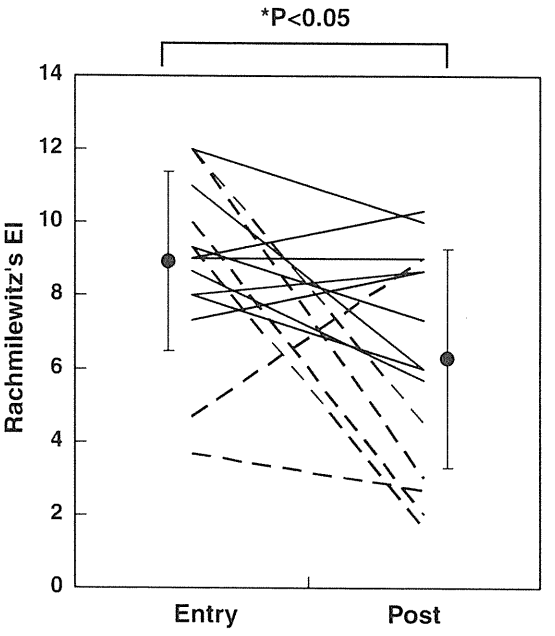

Fig. 2. EI at entry and post-LCAP treatment in 15 patients with active UC. The data of five patients with deep ulcers are not included. The dashed lines represent the clinical remission group (CAI $\leqslant$ 4). The mean EI decreased from 8.9 ± 2.5 at baseline to 6.3 ± 3.0 after the LCAP treatment.

Changes in the CAI and EI from the baseline to Week 4 after the initiation of LCAP therapy are shown in Figs. 1 and 2. The CAI decreased significantly from 11.7 ± 2.6 at baseline to 6.6 ± 4.1 after the LCAP treatment ($p < 0.001$). The mean EI decreased from 8.9 ± 2.5 at baseline to 6.3 ± 3.0 after the therapy ($p < 0.05$). The mean serum CRP level decreased from 1.4 ± 2.2 mg/dl at baseline to 0.45 ± 0.9 mg/dl after the therapy ($p < 0.05$). Nine of the 20 patients cooperated with the evaluation by the IBDQ and submitted their responses to the questionnaires before and after the LCAP therapy; an improvement of the IBDQ score from 4.0 ± 1.0 at baseline to 5.0 ± 0.5 after the therapy was noted ($p < 0.05$).

Univariate and multivariate analyses were performed to identify the background characteristics of the patients who were assessed as responders at Week 4 after the start of the LCAP therapy. The univariate analyses revealed no significant differences in the UC severity criteria (severe/moderate), age ($\leqslant$29 years/$\geqslant$30 years), disease duration (<2 years/$\geqslant$2 years), extent of disease (pancolitis/left-sided colitis), clinical pattern of the disease (first attack/relapse-remitting/chronic continuous type of disease) or baseline CAI ($\leqslant$11/$\geqslant$12) between the responders and non-responders. Additionally, we performed a logistic regression analysis, but due to the small number of the samples, we could not calculate an odds ratio or 95% confidence interval, or estimate precise contributions.

3.3. Safety

Adverse reactions associated with LCAP were noted in 4 (20%) of the 20 patients. The adverse reactions included

headache, nausea, malaise, anemia, general, discomfort, and fever. All were transient and mild in severity, and no severe adverse reaction associated with LCAP was observed.

4. Discussion

Adverse reactions caused by long-term continuous treatment with steroids are serious problems in UC patients [11–13]; thus, the development of a new and effective alternative to steroids has been desired. LCAP is a procedure in which leukocytes are extracorporeally removed from circulating blood. An open-label multicenter study showed that the efficacy of weekly LCAP with concomitant steroid therapy was higher (74%, 29/39) than that of high-dose steroid treatment (38%, 14/37) [6]. Additionally, weekly LCAP has been reported to be an effective substitute for steroids in steroid-naïve patients with UC in the early stage [14,15]. These single-center studies have suggested that LCAP shows the promise of becoming a first-line therapy for UC. We conducted the present multicenter study to investigate the efficacy and safety of LCAP without concomitant steroid treatment in patients with active UC.

There were 20 patients with active UC in the study. The patients had not received systemic steroid treatment for at least 1 month prior to the start of the study. The response rate to LCAP without concomitant steroid therapy in the 20 patients with active UC was 75% (15/20). This rate was similar to those reported in previous single-center clinical studies in steroid-naïve patients with UC (Nishioka et al. 88.9% (8/9) [14]; Umehara et al. 61.1% (11/18) [15]). Those results suggest that LCAP seems to have a therapeutic effect even in the absence of concomitant steroid therapy in patients with active UC.

In Japan, cytapheresis for steroid-resistant UC is covered by the National Health Insurance. The therapeutic schedule is generally once a week for a total of 5–10 sessions. However, with this schedule, it usually takes a while for the therapeutic effect to become apparent. In order to induce remissions rapidly, an intensive LCAP treatment for six patients with toxic megacolon associated with fulminant UC was performed [23]. LCAP was carried out 3 times a week for 2 weeks followed by 4 more times in the next 4 weeks. Meanwhile, since LCAP in our study was performed without concomitant steroid therapy, no concomitant effect with steroid was anticipated. For this reason, the intensive LCAP therapy was undertaken with twice-weekly sessions for 3 weeks, a total of 6 sessions to compensate for the absence of steroid effect. The assessment was made at Week 4 and significant decreases of the CAI and EI were found, from 11.7 ± 2.6 and 8.9 ± 2.5 at baseline to 6.6 ± 4.1 and 6.3 ± 3.0 at Week 4, respectively. In the randomized controlled trial of LCAP with concomitant steroid therapy conducted by Sawada et al., LCAP was performed once a week for 5 weeks, and the evaluation was made at Week 7 [11]. In their study, the CAI and EI significantly decreased from 13.9 ± 4.1 and 8.3 ± 2.2 at baseline to 7.4 ± 5.3 and 3.5 ± 3.6 at Week 7, respectively. The decrease in CAI, a clinical finding, was similar to that in our study. This suggested that even in the absence of concomitant steroid therapy, the intensive LCAP treatment quickly showed improvement similar to LCAP with concomitant steroid therapy; thus, the intensive treatment with twice-weekly LCAP sessions may shorten the time needed for remission induction. In contrast to the decrease of the CAI, the decrease in the EI, an endoscopic index, was relatively small in our study. This was considered to be attributable to endoscopic improvement which in general takes a longer time to become apparent than clinical improvement. Also the follow-up assessment conducted earlier, at Week 4, in our study compared to Week 7 in the study by Sawada et al. Nevertheless, in our study, a strong tendency for marked improvement of the endoscopic findings was also observed in the patients who showed remission (CAI of $\leqslant 4$ after therapy). This observation indicated that the therapeutic effects of LCAP alone without concomitant steroid administration brought not only improvement of the clinical features, but also endoscopic restoration of the intestinal mucosa and suppression of the inflammatory activity.

Furthermore, the serum levels of CRP, a marker of inflammation, also decreased. The score on the IBDQ which is a disease-specific QOL scale for IBD patients also improved; thus, LCAP could be a highly satisfactory therapy for patients.

In conclusion, LCAP without concomitant steroid therapy is a useful and safe therapy for patients with active UC. It is expected that LCAP therapy as an alternative to steroid treatment may allow avoidance of steroid treatment for relapses of UC, and thereby reduce the risk of the adverse reactions associated with long-term steroid treatment. In order to further investigate the efficacy of LCAP without steroid, it is necessary to conduct randomized controlled studies with control groups and seek appropriate patients for LCAP and predictive factors.

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Double-Blind, Placebo-Controlled Trial of Oral Tacrolimus (FK506) in the Management of Hospitalized Patients with Steroid-Refractory Ulcerative Colitis

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Background: We report a multicenter study of oral tacrolimus (FK506) therapy in steroid-refractory ulcerative colitis (UC).

Methods: In a placebo-controlled, double-blind study, 62 patients with steroid-refractory, moderate-to-severe UC were randomized into either a tacrolimus group or a placebo for 2 weeks. Patients were evaluated using the Disease Activity Index (DAI). As an entry criterion, patients had to have a total DAI score of 6 or more as well as a mucosal appearance subscore of 2 or 3. Clinical response was defined as improvement in all DAI subscores. Mucosal healing was defined as mucosal appearance subscore of 0 or 1. Clinical remission was defined as a total DAI score ≤ 2 with an individual subscore of 0 or 1.

Results: The mean total DAI score at study entry was 9.8 ± 1.61 in the tacrolimus group and 9.1 ± 1.05 in the placebo group. At week 2 the clinical response rate was 50.0% (16/32) in the tacrolimus group and 13.3% (4/30) in the placebo group ($P = 0.003$). The rate of mucosal healing observed was 43.8% (14/32) in the tacrolimus group and 13.3% (4/30) in the placebo group ($P = 0.012$) and the rate of clinical remission observed was 9.4% (3/32) in the tacrolimus group and 0.0% (0/30) in the placebo group ($P = 0.238$). The therapies in this study were well tolerated, with only minor side effects.

Conclusions: Oral tacrolimus therapy in patients with steroid-refractory UC shortened the acute phase and induced rapid mucosal healing. These results suggest that tacrolimus therapy is useful as an alternative therapy for steroid-refractory UC.

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Key Words: ulcerative colitis, immunosuppressive therapy, tacrolimus

Tacrolimus, a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*, a species of *Actinomyces*, was discovered in 1984 on Mt. Tsukuba in Japan. Fellermann et al[1] reported the results of a study of tacrolimus in patients with steroid-refractory, severe ulcerative colitis (UC). With patients initially treated by continuous intravenous infusion and subsequently transferred to oral administration, the study showed improved symptoms in five of six patients, with successful induction of remission and steroid tapering achieved in four patients. A report on oral and injectable formulations of tacrolimus stated, "most importantly, oral tacrolimus therapy appears to be effective and obviates the need for intravenous dosing."[2]

Baumgart et al[3] demonstrated the usefulness of low doses of oral tacrolimus (4–6 ng/mL) and Högenauer et al[4] reported, "Oral tacrolimus might be an effective alternative treatment to intravenous cyclosporine for treatment of steroid-refractory UC."

As no evaluation had yet been made of tacrolimus using a placebo as comparator, we conducted a dose-ranging study to evaluate oral administration over 2 weeks.[5] The study established a placebo group, a group with a target tacrolimus trough concentration of 10–15 ng/mL, and a group with a target tacrolimus trough concentration of 5–10 ng/mL. The results indicated a significant difference in efficacy between the 10–15 ng/mL group and the placebo group over the short 2-week period.

Here we report on a multicenter study which was a double-blind study of oral administration for 2 weeks,

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comparing a placebo group with a group having a target tacrolimus trough concentration of 10–15 ng/mL.

MATERIALS AND METHODS

Patient Selection

Patients with moderate-to-severe, active UC were eligible for inclusion in this study. UC was defined according to standard criteria for symptoms and standard radiographic and endoscopic criteria.[6] Before starting treatment, infectious diarrhea was ruled out by stool cultures and *Clostridium difficile* toxin testing. Endoscopies were performed during the week prior to the first dose of the study drug. The extent of colonic involvement was determined by total colonoscopy. All patients in the study had left-sided colitis and pancolitis and all were hospitalized.

Patients with known renal or severe hepatic dysfunction and pregnant women were excluded from the study. Pretreatment assessment included taking a history of the patient, physical examination, complete blood count, chemistry screening panel, and urinalysis.

Patients were classified as steroid-resistant or steroid-dependent. Patients with active UC were defined as steroid-resistant when the disease failed to respond to a systemic daily dose of 1 mg per kg of body weight, or 40 mg or more of prednisolone given over at least 7 days, or the equivalent of a daily dose of prednisolone of 30 mg or more over at least 2 weeks. Steroid-dependent patients were defined as patients with active UC in whom attempts to taper steroids had been unsuccessful. The steroid dosage remained the same from study initiation for 2 weeks, while only those patients in whom a dose of prednisolone of 60 mg/day or more was effective were permitted to decrease the dosage during this period. Efficacy was based on improvement in the frequency of stools and a decreased amount of blood in the stool.

Patients were evaluated using the Disease Activity Index (DAI).[7] The DAI score is a sum of subscores for the following four factors: stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment, each of which is graded on a scale from 0 to 3. The DAI score ranges from 0 to 12; the higher the score, the more severe the disease activity. As an entry criterion, the patient was required to have a total DAI score of 6 or more, as well as a mucosal appearance subscore of 2 or 3.

Patients who started taking azathioprine within 3 months prior to entering the study were excluded from the study, and patients were permitted to continue taking azathioprine at an unchanged dose over the period beginning 3 months prior to the start of the study, until completion of the study. Patients were permitted to continue taking 5-aminosalicylic acid during the study, as long as the drug dosage was not changed over the period beginning 2 weeks prior to the start of the study, until completion of the study. Receiving cytapheresis within 14 days prior to entry in the study was a reason for exclusion from the study. Patients receiving concomitant nutritional therapy continued to receive the same therapy during the study.

As UC therapy with cyclosporin, biological therapies, 6-mercaptopurine, or other immunosuppressants was not covered by health insurance in Japan, the concomitant use of these drugs was prohibited.

Protocol Review

The study protocol was reviewed and approved by each Institutional Review Board. Each patient read and signed a consent form before enrollment in the study.

Study Design

We conducted a multicenter study of oral tacrolimus treatment, consisting of a 2-week placebo-controlled, double-blind, randomized study in which patients with active UC were given either placebo or tacrolimus at an oral dose sufficient to achieve and maintain target blood concentrations of 10–15 ng/mL.

Open-label Extension

After week 2, patients received conventional treatment or tacrolimus open-label treatment. Data were collected during an open-label extension phase of the study. The effect of continuous treatment in the tacrolimus group was evaluated by comparing the condition of patients in the tacrolimus group at weeks 2 and 12.

Administration and Monitoring of Study Drug

The tacrolimus capsules used (Tacrolimus, Astellas Pharma, Japan) contained 0.5 mg or 1 mg of FK506. In consideration of safety, tacrolimus therapy was initiated at a small dose of 1–2.5 mg per time, twice daily. Dose adjustments were determined using proportional calculations of "blood trough concentration at steady state" and "target trough concentration" as shown in Table 1. To reach the target trough concentration quickly, the first dose adjustment occurred at an early stage. This increase required blood collection at 12 hours (C12h) and 24 hours (C24h) after the initial dose for determination of the trough concentration of tacrolimus in whole blood. Steady-state values were estimated to be 4 times the value at C12h, 2.5 times the value at C24h, or 3 times the mean value of C12h and C24h. The dose was adjusted by proportional calculation using a target concentration of 12.5 ng/mL. These equations were created based on the known pharmacokinetic profile of tacrolimus in healthy volunteers (data not shown).

For the next adjustment, measured values were checked against the target trough concentration. When the measured value was outside the range of 10–15 ng/mL, the dose was readjusted using blood trough concentration at steady state.

The randomization was performed by the Control Center (Bellsystem24, a third-party organization independent of study physicians and sponsor). To preserve blinding, blood trough

TABLE 1. Dose Adjustment of Tacrolimus

Dosage calculation method using trough concentration				
Blood trough concentration under the same food intake condition as at administration should be used (fed/fasted condition).				
For 2 weeks:				
The dose is increased to a target trough concentration of 10-15 ng/mL (target of 12.5 ng/mL).				
Initial adjustment (a, b, or c)				
Initial dose				
Weight (kg)	30≤<50	50≤<70	70≤<90	90≤ ≤100
Dose per time (mg), twice daily	1	1.5	2	2.5

The blood trough concentration at 12 hours (C12h) and/or 24 hours (C24h) after the initial dose.
a: Initial dose (mg) × target trough concentration (12.5 ng/mL) / (average of C12h & C24h × 3).
b: Initial dose (mg) × target trough concentration (12.5 ng/mL) / (C12h × 4).
c: Initial dose (mg) × target trough concentration (12.5 ng/mL) / (C24h × 2.5).
Next adjustment:
The blood trough concentration (C) was measured at steady-state, after 2 days or more following the prevopus adjustment, to check whether the value was within the range of 10-15 ng/mL.
When the measured value was outside the range of 10-15 ng/mL, the dose was readjusted.
Previous dose × target trough concentration (12.5 ng/mL) / C.

levels were measured by SRL (a third-party organization independent of study physicians and sponsor) and relayed to the Control Center (Bellsystem24). Dosages were calculated at the Control Center based on the trough levels. The clinical sites were informed of the adjusted dosage by 3 days after the blood sample was drawn. Patient doses in the placebo group were pseudo-adjusted to preserve study blinding. The Control Center used the equations shown in Table 1 to carry out dose adjustments.

Symptom Assessment and Study Endpoints

The primary endpoint was clinical response based on the DAI score.[7] Clinical response was defined as a reduction in DAI by at least 4 points and improvements in all categories (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment). A worse or unchanged score in any category was considered a treatment failure, even if all other scores improved. Secondary endpoints were mucosal healing and clinical remission.[8] Mucosal healing was defined as mucosal appearance subscore of 0 or 1. Clinical remission was defined as a total DAI score ≤ 2 with individual subscore (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment) of 0 or 1. When a patient's symptoms worsened at any time and the investigator decided the study drug could not be continued, the treatment was considered a failure.

Statistical Analysis

Fisher's exact test was used to compare the tacrolimus group with the placebo group for demography, efficacy, and safety. The Wilcoxon signed rank test was used to compare each timepoint with baseline for demography. All statistical tests were two-sided with a significance level of 0.05 unless otherwise specified.

Sample Size

Based on previous results,[5] the clinical response was assumed to be 50% in the tacrolimus group and 10% in the placebo group. We estimated that randomizing 31 patients to each group would be sufficient to show a difference in efficacy between placebo and tacrolimus based on the above assumptions and a two-sided alpha of 0.025 and power of 0.9 using a normal approximation.

RESULTS

Patient Population

This study was performed between August 2006 and February 2008. Sixty-two patients in total were recruited. The mean total DAI score of patients enrolled was 9.8 ± 1.61 in the tacrolimus group and 9.1 ± 1.05 in the placebo group.

Drug Exposure

The mean trough concentrations in the tacrolimus group were 1.4 ± 0.9 ng/mL at 12 hours, 2.2 ± 1.5 ng/mL at 24 hours, 9.6 ± 3.1 ng/mL at day 7, 10.3 ± 3.1 ng/mL at day 8, 11.6 ± 3.4 ng/mL at day 10, and 13.0 ± 4.4 ng/mL at day 14.

Efficacy

Figure 1 shows that a clinical response was observed in 50.0% (16/32) of patients in the tacrolimus group and 13.3% (4/30) of patients in the placebo group. Significantly more patients in the tacrolimus group showed improvements compared with the placebo group ($P = 0.003$).

The observed rate of mucosal healing was 43.8% (14/32) in the tacrolimus group and 13.3% (4/30) in the placebo group ($P = 0.012$) at week 2, and clinical

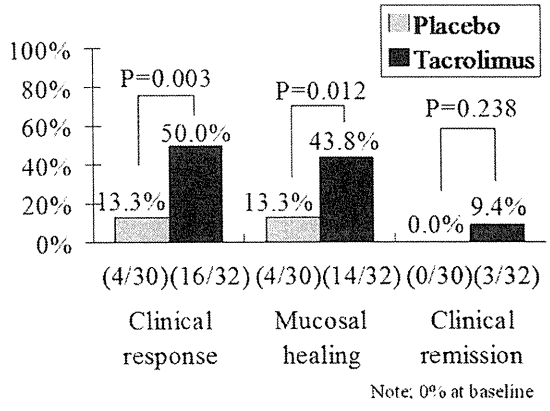

FIGURE 1. Efficacy result.

remission at week 2 was observed in 9.4% (3/32) of the tacrolimus group compared with 0.0% (0/30) in the placebo group ($P = 0.238$).

Twenty-seven of the 32 patients in the tacrolimus group achieved target trough levels. Among the 27 patients, the observed rate of clinical response, mucosal healing, and clinical remission were 59.3% (16/27), 51.9% (14/27), and 11.1% (3/27), respectively. Among the other five patients who did not achieve target trough levels, clinical response, mucosal healing, and clinical remission were not observed.

The rate of clinical remission was lower than that of mucosal healing. This was supposed to have been associated with the difference in criteria for the former and the latter. While mucosal healing was defined as achieving a mucosal appearance subscore of 0 or 1, clinical remission was more strictly defined as a subscore of 0 or 1 on each of the four factors (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment) and a total score of 2 or lower.

Safety

Adverse events and serious adverse events were evaluated in all patients who received at least one dose of the study drug (Table 2). No statistically significant difference in incidence of adverse events was seen between the tacrolimus group (81.3%) and placebo group (70%) ($P = 0.379$).

The most common adverse event seen in patients who received tacrolimus was numbness. All events were mild and did not interfere with the patients' normal functioning. There were no significant adverse events on body temperature, blood pressure, pulse rate, hematologic parameters, electrolytes, renal function, cholesterol levels, and blood glucose levels, and no opportunistic infections were observed. No clinically significant differences in vital signs or laboratory test values were found between the two groups.

The mean values of serum creatinine (mg/dL) in the tacrolimus group and in the placebo group were, respectively, 0.652 and 0.640 at baseline, and 0.633 and 0.672, respectively, at the end of the study. The mean values of BUN (mg/dL) in the tacrolimus group and in the placebo group were, respectively, 9.49 and 9.99 at baseline, and 11.59 and 9.29, respectively, at the end of the study.

Open-label Extension

After 2 weeks the treatment for 20 of the 62 patients in this study was changed to conventional treatment with drugs such as azathioprine. The remaining 42 patients continued to be treated with tacrolimus. Twenty-one of the 42 patients were in the tacrolimus group. The effect of continuous treatment in the tacrolimus group was evaluated by comparing the condition of 21 patients in the tacrolimus group at week 2 and week 12.

The results show an increase in mucosal healing from 66.7% (14/21) to 85.7% (18/21) and in clinical remission from 14.3% (3/21) to 28.6% (6/21) (Fig. 2a).

Seven of the 21 patients had failed azathioprine maintenance over the period beginning 3 months prior to the start of the study. Among the seven patients, the results also show an increase in mucosal healing from 71.4% (5/7) to 85.7% (6/7) and in clinical remission from 28.6% (2/7) to 57.1% (4/7). Among the other 14 patients the results also show an increase in mucosal healing from 64.3% (9/14) to 85.7% (12/14) and in clinical remission from 7.1% (1/14) to 14.3% (2/14).

Furthermore, the mean prednisolone dose was decreased (8.9 mg/day) from that at baseline (24.2 mg/day) (Fig. 2b). One patient was off steroids at week 12 and the total DAI score of this patient was 3. Although the prednisolone doses was not evaluated after week 12, the prednisolone doses in six patients who achieved clinical remission

TABLE 2. Safety Result

No. of Patients (%)	Tacrolimus (n=32)	Placebo (n=30)
Adverse events	26 (81.3)[a]	21 (70.0)
Related adverse events	19 (59.4)	10 (33.3)
Serious adverse events:	None	None
Related adverse events occurring in > 5% of patients in at least one of the treatment groups		
Nausea	4 (12.5)	3 (10.0)
Headache	4 (12.5)	3 (10.0)
Numbness	4 (12.5)	0 (0.0)
Finger tremor	3 (9.4)	1 (3.3)
Dysmenorrhea	3 (9.4)	1 (3.3)
Hot flushes	2 (6.3)	1 (3.3)
Abdominal pain upper	2 (6.3)	1 (3.3)
Back pain	2 (6.3)	1 (3.3)

[a]Fisher's exact test, $P = 0.379$ vs. placebo.

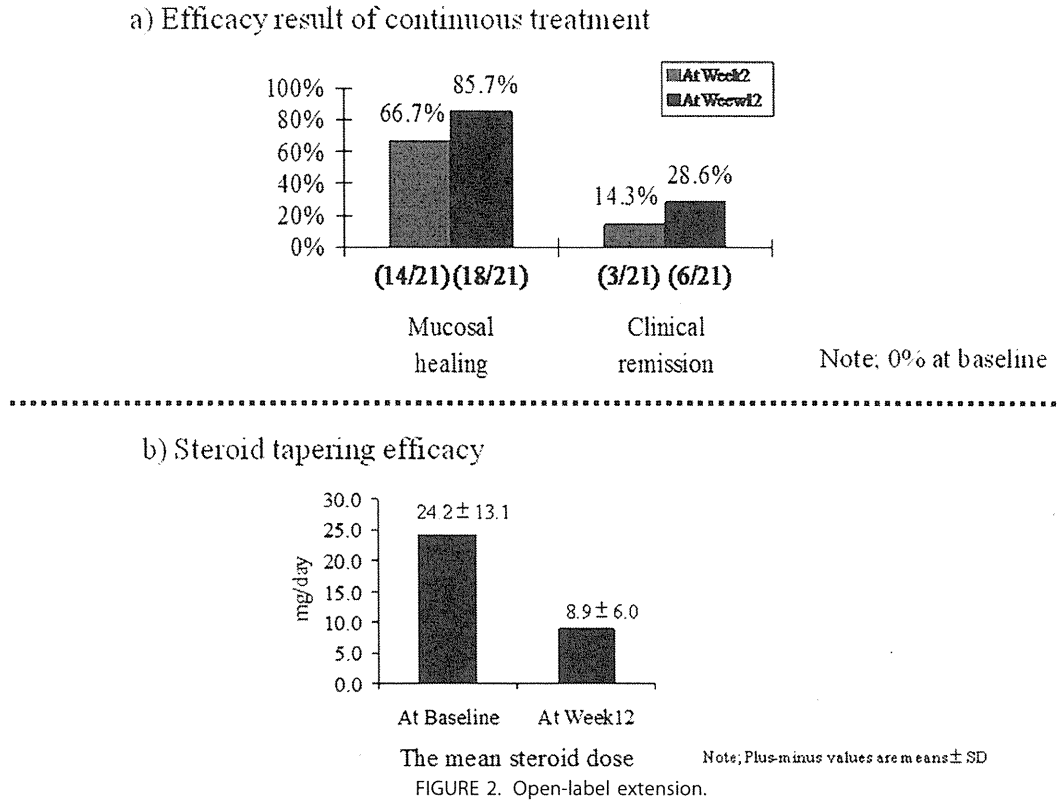

FIGURE 2. Open-label extension.

at week 12 were 10 mg/day, 10 mg/day, 5 mg/day, 5 mg/day, 2.5 mg/day, and 2.5 mg/day, respectively.

A smooth transition to the extension phase was achieved. The mean tacrolimus trough concentrations were 5.5 ± 1.5 ng/mL at week 4, 6.3 ± 1.7 ng/mL at week 8, and 6.7 ± 1.8 ng/mL at week 12.

This open-label extension phase of the study was well tolerated, with only minor side effects and no patients required colectomy.

Compliance

Patients were questioned by the investigator regarding compliance during the study. No cases of noncompliance could be identified.

DISCUSSION

Patients included in this study either had failed treatment with their most recent steroid treatment or were in immediate need of alternative treatment, including operative procedures. Because of these factors, a study design involving administration of placebo for 2 weeks or more was impossible both in terms of ethics and appropriate treatment. Although these results in the short duration of treatment should be treated with caution, it was demonstrated that oral tacrolimus therapy in patients with steroid-refractory, moderate-to-severe UC shortened the acute phase and induced rapid mucosal healing.

An open-label extension resulted in further improvements and a reduction in steroid dose. Remission induction rates, relapse rates, and surgery rates in patients treated with tacrolimus over the long term are now being investigated in a prospective study.

The efficacy of tacrolimus in severe steroid-refractory UC was also confirmed in another small open-label study, although these results were not published. While intravenous infusion of cyclosporine has been thought to be effective and recognized as an alternative therapy against refractory, severe UC,[9,10] administering oral tacrolimus therapy is more convenient than 24-hour continuous intravenous infusion of cyclosporine. Intravenous infusion imposes a great physical and psychological burden on the patient in hospital. Changing from intravenous injection to oral administration requires prolonged hospitalization to allow for the dose adjustment period; however, oral tacrolimus therapy can eliminate these disadvantages.

With regard to the long-term usefulness of tacrolimus, Baumgart et al[11] and Yamamoto et al[12] have reported the usefulness of long-term administration of tacrolimus for 12 weeks or more as remission maintenance therapy in open-label studies. More recently, Yamamoto et al[13] reported the efficacy of tacrolimus compared with

thiopurines for maintaining remission in patients with refractory UC. They concluded that maintenance therapy with tacrolimus for patients with UC could be considered an alternative to thiopurine therapy.

Naganuma et al[14] summarized how/when we should use tacrolimus in patients with refractory UC. Although our results suggest that tacrolimus therapy is useful as an alternative therapy against steroid-refractory UC, further investigation will be necessary to clarify the clinical usefulness of tacrolimus in comparison with biologics, such as infliximab, as a therapeutic strategy for refractory UC.

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