Cytapheresis in Patients with Severe Ulcerative Colitis after Failure of Intravenous Corticosteroid: A Long-Term Retrospective Cohort Study

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Background/Aims: Cytapheresis (CAP) is a novel strategy for ulcerative colitis (UC). However, there is insufficient data on the long-term outcome of UC patients who achieve remission by CAP. This study involved patients with severe UC who refracted to intravenous (iv) corticosteroid. **Methods:** Forty-seven UC patients who had received CAP therapy for the first time within 1 year after UC diagnosis were followed for 36 months. One of the inclusion criteria was a clinical activity index (CAI) of ≥ 7 points at the end of a 2-week iv course of corticosteroid therapy. CAP therapy consisted of ten sessions over 10 weeks. **Results:** CAP induced clinical remission ($CAI \leq 4$) in 70.2% patients (33/47). The number of submissions for colectomy was higher for severe UC at entry ($CAI \geq 12$, $n=25$) than for moderately severe UC at entry ($7 \leq CAI < 12$, $p=15$; $p<0.02$). The cumulative rates of avoiding surgery and relapse were 54.5% and 24.2%, respectively, at 36 months in patients who responded to CAP therapy. This was similar to that of iv cyclosporine reported recently. **Conclusions:** This study suggest that CAP is an effective therapy in patients who are refractory to conventional medications including iv corticosteroid. Increased remission rates should be expected in refractory patients with moderately severe UC. (*Gut and Liver* 2009;3:41-47)

Key Words: Inflammatory bowel disease; Corticosteroid; Ulcerative colitis; Leukapheresis

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the two major phenotypes of inflammatory bowel disease (IBD). The etiology of UC is still incompletely understood, but a functional immune disturbance is thought to be the major immunopathologic factor in this relapsing-remitting disease.¹ For several decades, intravenous (iv) administration of glucocorticosteroids has been accepted as an intensive therapy for patients with a severe flare of UC.² Further, because about 40% of patients do not respond to iv corticosteroids,² continuous iv cyclosporin A (CsA) has been administered to treat patients with severe UC unresponsive to iv corticosteroids.³ However, adverse events induced by aggressive suppression of the human-immune function are a potential risk to be reckoned with.^{4,5}

A new non-drug strategy introduced in recent years is cytapheresis (CAP) for the treatment of patients with active UC, including patients with severe refractory UC.⁶⁻¹² The primary action of CAP is to regulate the dysregulated immune response by removing inflammatory cytokine producing circulating leukocytes, especially granulocytes, which are destined for infiltration into the mucosa.¹² Currently, filtration leukocytapheresis (LCA),⁷ and adsorptive granulocyte/monocyte apheresis (GMA)⁸⁻¹² are the two major CAP therapies for patients with an acute UC flare.^{9,10} Impressive clinical efficacy has been reported by some of these studies (albeit mostly in uncontrolled studies).⁶⁻¹²

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GMA and LCA were approved by the Japan health insurance scheme for patients with active UC in 2000 and 2001, respectively. Over an 80,000 individuals have registered in the national UC database, and in 2006, approximately 30,000 CAP sessions were performed in Japan alone. However, there is lack of data on the long-term prognosis of patients who were successfully treated with CAP. In this study, we report on the efficacy of CAP in patients who failed to respond to iv corticosteroid. To say that these patients were candidates for iv CsA in Europe or in the USA. Further, by using this category of patients, we believe some of the anxiety related to the lack of a control group is relieved. Additionally, we present data on a 3-year follow-up of these severe patients who responded to CAP.

MATERIALS AND METHODS

1. Patients

A retrospective follow-up of 47 patients with UC who had been treated with either LCA or GMA within 1 year after their first UC diagnosis in our institute during 2000-2006 was done in this study. Of 47 patients, 21 were male, the rest were female. Mean age was 30.7 ± 14.1 yr, range 15-69 yr. Likewise, the mean duration of UC was 177.5 ± 198.2 days, range 1-741 days. After CAP therapy, these patients were followed for 36 months. Table 1 shows major demographic background of the 47 patients at entry. All of the 47 patients had shown active inflammation, which was defined as higher than 7 points in the clinical activity index (CAI) published by Lichtiger *et al.*³ The mean CAI score was 13.0 ± 3.7 points, range 7-19 points in spite of receiving 0.8-1.5 mg/kg/day (53.0 ± 11.0 mg/day) of iv prednisolone (PSL) for at least two weeks prior to CAP. The patients were randomly assigned to either LCA (n=19) or GMA (n=28), and there

were no statistical differences between the LCA and the GMA groups at entry. During both induction of remission and follow-up periods, patients who had been treated with immunosuppressant, like azathioprine (AZA), 6-mercaptopurine (6-MP), or an anti-TNF were excluded from this study (did not receive CAP).

2. Induction of remission by CAP

LCA was performed using a leukocyte removal filter column, Cellsorba EX (Asahi Kasei Kuraray Medical, Tokyo, Japan),^{6,7} and GMA was done by using an immuno-adsorptive column, the Adacolumn (JIMRO, Takasaki, Japan),^{8,11} set on simple one-way hemofiltration circuits. Cellsorba removes approximately 100% of neutrophils and monocytes, 64% of lymphocytes and 35% of platelets from peripheral blood that passes through the column,^{6,9} while the Adacolumn selectively adsorbs from blood in the column about 65% of granulocytes, 55% of monocytes/macrophages and a smaller fraction of platelets; lymphocytes are spared.¹² The duration of both LCA and GMA sessions was 60 minutes. Blood flow rates for LCA and GMA were 40 mL/min and 30 mL/min, respectively as used in previous studies.⁶⁻¹⁰ Optimal amount of Nafamostat mesilate (Futhan; Torii Pharma, Tokyo, Japan) was mixed with saline and was added to the column inflow line as an anticoagulant before infusion into Cellsorba or Adacolumn. Five, once a week CAP sessions were defined as one series, and a maximum of 10 sessions formed two series for each UC flare up. Initially, on average, 6.5 ± 3.3 LCA and 7.8 ± 3.3 GMA sessions were performed for each of the enrolled patients. Concomitant iv PSL was continued at the same dosage; then was tapered in line with improvements of clinical symptoms. Sigmoidoscopic evaluations were done within a week before starting CAP in 44 patients, 3 patients had to start

Table 1. Demographic Background of the 47 Patients Included in This Study

	Total (n=47)	GMA (n=28)	LCA (n=19)	p-value
Age, yr (range)	30.7 (15-69)	32.1 (15-69)	28.6 (16-47)	0.948
Gender (Male/Female)	21/26	10/18	11/8	0.151
Extent of UC (Total/Left sided colitis)	43/4	25/3	18/1	0.638
Disease duration (days)	177.5 (1-741)	160.8 (5-669)	202.1 (1-741)	0.374
CAI (range)	13.0 (7-19)	13.3 (7-19)	12.4 (7-19)	0.513
CRP, mg/dL (range)	3.4 (0.00-17.7)	3.3 (0.00-10.9)	3.6 (0.00-17.7)	0.380
Concomitant medication	Steroid only: 5 Steroids+5ASA: 42	Steroid only: 3 Steroids+5ASA: 25	Steroid only: 2 Steroids+5ASA: 17	1.000
Dosage of iv PSL (mg/day)	53.0 (30-80)	54.6 (30-80)	50.5 (30-60)	0.260

Data are mean and range values.

GMA, granulocyte/monocyte apheresis; LCA, leukocytapheresis; UC, ulcerative colitis; CAI, clinical activity index; CRP, C-reactive protein; 5ASA, 5-aminosalicylic acid; iv, intravenous; PSL, prednisolone.

their CAP therapy without endoscopy due to severe UC. The endoscopic findings were interpreted by using a pre-established Mats' criteria of endoscopic index (EI).¹³ Mucosal damage severer than grade 2-3 and ≥ 4 were defined as moderate and severe, respectively.

3. Maintenance therapy

All patients who responded to CAP therapy were discharged on 1.50-2.25 g/day of oral 5-aminosalicylic acid (5-ASA, Pentasa®; Ferring Pharmaceuticals, Lausanne, Switzerland) and low-dose (5-10 mg/day) oral PSL. On the basis of clinical response to CAP, the dosage of PSL was reduced to 5-10 mg in two weeks. Maintenance treatment with Pentasa was continued at the same dosage, but without AZA, 6-MP or other immunosuppressant (these were not approved for UC patients in Japan during our observation period). Relapse was defined when a patient had experienced an acute flare severe enough to start high dose PSL and/or another set of CAP during the following up period.

4. Statistics

Quantitative variables were compared by using the Mann-Whitney U test (2 sided). Two measures of long-term outcomes were assessed by the Kaplan-Meier survival analysis, and categorical data were analyzed by the Fisher exact test (2-sided). A $p < 0.05$ was considered statistically significant.

Table 2. Demographic Comparison between Responder and Nonresponder Groups

	Responder (n=33)	Nonresponder (n=14)	p-value
Gender (M/F)	15/18	6/8	1.000
Age (years)	29.4 (15-59)	33.8 (16-69)	0.545
Duration of UC (days)	163.4 (1-741)	210.7 (16-647)	0.530
Type of colitis Total/Left sided	30/3	13/1	1.000
First onset	19	7	0.752
CAI	11.9 (7-17)	15.6 (7-19)	0.001
Pre-endoscopic finding Severe/Moderate	24/9	9/2	0.701
CRP (mg/dL)	2.5 (0-9.9)	5.7 (0.3-17.7)	0.012
Dose of iv PSL (mg/day)	50.3 (30-60)	59.2 (40-80)	0.011

Data are mean and range values.

UC, ulcerative colitis; CAI, clinical activity index; CRP, C-reactive protein; iv, intravenous; PSL, prednisolone.

5. Ethical considerations

The study protocol was reviewed and approved by the local Ethics Committee and all subjects provided both oral and documented informed consents.

RESULTS

1. Clinical efficacy and safety of CAP

CAP therapy was well tolerated, and all patients completed their 2 series, 10 once a week CAP therapy. Thirty-three of the 47 enrolled patients (70.2%) showed clinical response ($\text{CAI} \leq 4$) and avoided colectomy during their initial hospitalizations. In demographic comparison between the responder and the nonresponder (surgery) groups at entry, significant differences were seen in the CAI score ($p=0.01$), C-reactive protein (CRP) value ($p < 0.02$), and the initial dosage of iv PSL prior to CAP ($p < 0.02$) (Table 2). We could not detect significant difference in the pre-endoscopic EI scores between these 2 groups, but the 3 patients who started CAP without receiving sigmoidoscopic assessments had to undergo colectomy because of insufficient improvement (unremitting severe UC). Further, only 2 of 47 patients (4.3%) experienced side effects, but the severity of side effects was not serious enough to stop CAP therapy. One patient had nausea at the end of the first GMA session and one patient had mild dyspnea during the second LCA session. These side effects were transient and resolved with appropriate adjustments; nausea disappeared by temporarily reducing the flow rate from 30 to 20 ml/min. This never appeared again during the subsequent sessions. Likewise, dyspnea receded by switching the anticoagulant from nafamostat mesilate to sodium heparin.

2. Long-term outcomes following CAP therapy

The mean follow-up time of the 33 responder patients was 558.5 ± 372.4 days, range 16-1,401 days. Among these 33 patients, 15 (45.5%) underwent colectomy during the observation period, average $383.6 \pm 2,501$ days; range 16-947 days. Life table analysis of avoiding colectomy following successful CAP at 1, 2 and 3 years follow-up were 78.4%, 50.1% and 41.8%, respectively (Fig. 1).

Fig. 2 shows the time to next relapse of the 33 patients who initially responded to CAP. The probability of avoiding relapse following successful CAP at 1, 2 and 3 year follow-up were 39.9%, 12.5% and 12.5%, respectively. Life table predicts a 60% relapse possibility within 1 year. Twenty of 33 responders (60.6%) could completely stop their concomitant steroid during the follow-up period. Their mean period of maintenance without steroid was

316.8±306.1 days (range: 28-1,174). The remaining 13 patients (13/33; 39.4%) could not stop PSL therapy, and showed a significantly higher risk of surgery by the end of the follow-up period ($p=0.02$; Table 3). Patients were to receive rescue therapy by increasing the dosage of PSL and/or starting another set of CAP therapy when they had a severe relapse of UC during maintenance period. We found that the group of patients who were re-treated with CAP had a significantly higher possibility of being without colon at the end of the observation period ($p<0.03$; Table 3). The mean interval between the 1st and the 2nd set of CAP was 258±211 days, range 21-702

days.

No adverse side effect was observed during the follow-up period. Among the drop-out patients, one patient had mild nausea during a CAP re-treatment in spite of finishing the first CAP therapy without any complication. No newly diagnosed malignancies or dysplasia were reported in any of the 47 patients included, although no specific investigations were performed in this regard. No post-operative complications were reported.

Fig. 1. A Kaplan-Meier-like plot showing the percentage of patients who avoided colectomy during the 36-month follow-up in this study. Data are based on 33 of 47 patients who initially achieved clinical remission by CAP therapy.

Fig. 2. Percentage of patients who remained relapse free during the 36-months of follow-up after initial CAP-induced clinical remission. Data are based on 33 of 47 patients who initially achieved clinical remission by CAP therapy.

Table 3. Demographic Comparison between Patients with and without a Colon at 36 Months after the Initial CAP for First Remission (n=33 Patients)

	With colon (n=18)	Without colon (n=15)	p-value
Follow up period (days)	737.6 (237-1,401)	383.6 (16-947)	0.005
Gender (M/F)	7/11	8/7	0.494
Age (years, range)	29.6 (15-52)	29.1 (16-59)	0.957
Duration of UC (days, range)	138.3 (5-741)	190.1 (1-669)	0.325
Type of colitis			
Total/Left sided	17/1	13/2	0.579
First onset	13	6	0.085
CAI (range)	11.4 (6-16)	12.4 (3-17)	0.190
Pre-endoscopic finding			
Severe/Moderate	13/5	11/4	1.000
CRP (mg/dL)	2.3 (0-9.9)	2.4 (0-8.0)	0.929
Free from PSL	16 (88.9%)	7 (46.7%)	0.020
Re-treated with CAP	8 (44.4%)	13 (86.7%)	0.027

Data are mean and range values.

CAP, cytapheresis; UC, ulcerative colitis; CAI, clinical activity index; CRP, C-reactive protein; PSL, prednisolone.

DISCUSSION

Approximately 15% of patients with UC will have a severe relapse of colitis necessitating admission to hospital to receive iv corticosteroid in their lifetime,¹⁴ and 30-40% of these patients fail to respond to iv corticosteroid.^{14,15} In the present study, we determined the immediate response and the long-term outcome of CAP therapy in a cohort of patients with severe episodes of UC who were treated between 2000 and 2006 in our institute. Only patients who did not show improvements following at least 2 weeks of iv corticosteroid were selected for CAP therapy, which was added to their ongoing iv PSL and then PSL was tapered in responder patients. We thought that the selection of these corticosteroid refractory patients should alleviate some of the concerns on the lack of a control group. Therefore, the results of this study should be taken as a strong indication that CAP might serve as a novel adjunct therapy in patients who do not respond to conventional medications including iv corticosteroids. The short-term survival rate, free from colectomy (70.2%; 33 of 47 patients) in the group of patients with severe disease is similar to previous reports.^{7,8,16} Currently, in the Western countries, the patients included in this study could be candidates for iv CsA therapy, which has been widely accepted as a strong therapeutic agent.³ Accordingly, a relatively high initial response rate of 60-80% was reported for iv CsA in the population of UC patients with a similar steroid refractory background as in our study.^{3,17-22} In Japan, the weekly CAP therapy has been developed and used as an original therapeutic option for such cases, substituted for iv CsA.⁷⁻¹⁰ Our initial response rate of 70.2% in the weekly CAP therapy seems to be well-matched by the result from iv CsA therapy.

The therapeutic mechanism of CAP for active UC has never been fully explained, but selective removal of activated peripheral leukocytes achieved during CAP has been shown to reduce the mucosal levels of neutrophils²³ and this is expected to alleviate intestinal inflammation and promote disease remission. This assertion is supported by a study that showed a strong association between intestinal inflammation and mucosal levels of neutrophils in patients with IBD.²⁴ Further, in patients with active IBD, peripheral blood granulocytes and monocytes/macrophages are elevated, show activation behavior and increased survival time.^{11,25-29} As these leukocytes are major sources of inflammatory cytokines,^{30,31} they are suspected to contribute to the exacerbation and perpetuation of IBD.^{32,33}

In this study, we looked at the demographic background of the enrolled patients and found that those who

had responded to CAP had significantly lower levels of serum CRP, CAI and the dose of iv PSL at entry. These observations might indicate that patients with moderately active UC are more likely to respond to CAP than those with severely active UC.

In Japan, oral AZA was not approved for maintenance therapy until the end of 2007. Our analysis showed that 39.9% and 12.5% of patients could maintain their remission without AZA for 12 and 36 months, respectively after weekly CAP was discontinued. These data are comparable to the outcome for iv CsA reported by Actis *et al.*³⁴ In their study, relapse-free rates in the AZA-untreated patients at 12 and 36 months after iv CsA were 36% and 10%, respectively.³⁴ Similarly, the long-term colectomy-free rate in the AZA-untreated patients after 12 and 36 months were 47% and 15%, respectively³⁴ compared with 78.4% and 41.8%, respectively for CAP in this study, bearing in mind that the latter has an unrivalled safety profile.¹⁰ However, several investigators have reported improved remission time in patients who were given oral AZA as maintenance therapy after iv CsA.^{17,18,22} Their data show a 30% relapse-free rate at 36 months, and up to 70% of patients could remain with colon at 36 months after their initial remission by iv CsA. On the basis of these hitherto observations, we believe that the remission time following a course of CAP can be improved by oral AZA. Complications and side-effects in the present cohort were a mere 4.3% during the initial CAP therapy and 3.0% in the long-term follow-up of 36 months. In contrast, 40-60% of patients in CsA therapy showed serious adverse side effects, including *Pneumocystis carinii*, *Aspergillus* sp. and seizures.^{17,18,34} Similarly, Domènech *et al.*²² reported that an 18.5% of patients had to reduce their dosage of AZA during follow-up due to severe side effects (neutropenia, lymphopenia and hepatotoxicity). Hence, weekly CAP and without AZA has very favourable safety. However, it might be appropriate to comment here that the clinical efficacy hitherto reported for CAP has been very variable, from an impressive 85%³⁵ to an insignificant level.³⁶ Given this variability, we share the views that CAP as a non-pharmacologic treatment strategy, its clinical efficacy is likely to be impacted by factors related to its operations and the conditions during the procedure.³⁷

In summary, this is the first study that looked at the immediate response and long-term outcome of patients treated by CAP for severe UC that was refractory to iv corticosteroid. Initial remission response was 70.2%, and these patients were selected for subsequent maintenance therapy and follow-up without immunosuppressants like AZA. After 1 year 60.1% had relapsed and after 3 years 87.5% had relapsed. Further, after 3 years, 58.2% had

had colectomy. A future comparative study is warranted with immunosuppressants like AZA given during follow-up. In line with this thinking, we have started a new study in which AZA is given after the weekly CAP therapy.

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