A Pilot Study of Centrifugal Leukocyte Apheresis for Corticosteroid-Resistant Active Ulcerative Colitis

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Corticosteroids are effective in bringing about a clinical remission in patients with ulcerative colitis. However, in severely relapsed cases, corticosteroids are not always effective even when a high dosage is administered. In addition, the long-term use of corticosteroids often causes serious side effects. Therefore, an alternative treatment for active ulcerative colitis is necessary in order to avoid these clinical problems. In the present pilot study, the efficacy of leukocytapheresis using a centrifugal procedure was evaluated for corticosteroid-resistant, active ulcerative colitis. Fourteen patients with corticosteroid-resistant severely active ulcerative colitis were treated by leukocytapheresis. Thirteen patients (92.9%) achieved clinical remission within 4 weeks after the apheresis, and remained in remission for 8 months on average without any additional corticosteroid therapy. In the remaining patient, in whom remission was not induced, a total colectomy was performed immediately after the fourth course of leukocytapheresis. No significant side effects were noticed throughout the therapy. Both colonoscopic and histological examinations confirmed the beneficial effect of this procedure in terms of the reduction of severe inflammation of the affected colon. We found that the expression of two adhesion molecules, L-selectin and VLA4α, on the surface of peripheral leukocytes was decreased after this new therapy.

Key words: inflammatory bowel disease, leukocytapheresis, corticosteroid-resistance, cell adhesion molecules, VLA-4, L-selectin

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by clinical features of abdominal pain, severe diarrhea, hematochezia, fever and anemia. Although the etiology has not been fully elucidated, several altered autoimmune responses and genetic abnormalities are implicated (1−5). Generally, UC is associated with intervals of acute exacerbation, and the administration of corticosteroids is effective in bringing about a clinical remission. However, in some severely relapsed cases, corticosteroids are not always effective even when a high dosage of more than 1 mg/kg/day is administered orally or intravenously. In addition, the long-term use of corticosteroids often causes serious side effects such as hormonal disturbance, peptic ulcer, liver dysfunction and psychological problems. These problems sometimes cause the disruption of corticosteroid treatment and result in acute exacerbation. Therefore, an alternative treatment for active UC is necessary in order to avoid these clinical problems associated with corticosteroid therapy. Recently, the efficacy of leukocytapheresis using a leukocyte removal filter for inflammatory bowel diseases was reported (6).

In the present study, we conducted an open pilot study of centrifugal leukocyte apheresis in corticosteroid-resistant active UC, and evaluated the effectiveness to induce remission and the safety of this treatment. We also examined the expression of L-selectin and VLA4α on the peripheral leukocytes by flow cytometry before and after the apheresis to elucidate the therapeutic mechanisms.

Patients and Methods

Fourteen patients with active UC were treated by leukocyte...
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apheresis using a centrifugal separation apparatus (Multi Component System, Haemonetics Co., Braintree, MA). All of the patients were admitted to Asahikawa Medical College Hospital; informed consent was obtained from each patient prior to the therapy. A profile of the patients is given in Table 1. All the patients had severe active colitis and were resistant to the previous corticosteroid therapies. Nine patients had entire colitis and five patients had left-sided colitis. In Patients 1, 2 and 3, corticosteroid-resistant continuous inflammation persisted. In Patients 4–8, corticosteroid therapy was ineffective against severe inflammation while Patients 9 through 14 were in relapse during the course of steroid tapering. The cumulative amount of corticosteroid as prednisolone was 17,630 mg on average. All of the patients were corticosteroid-resistant patients in the previous six months.

One session of the treatment consisted of leukocytapheresis once a week for three weeks, except in Patients 1, 4, 5 and 6, in whom, because of extremely severe colitis, apheresis was carried out twice a week. In each session, leukocyte-rich fractions of the buffy coat layers were removed from 2,000 to 2,400 ml of patients' peripheral blood from cubital veins. We performed centrifugal leukocytapheresis by the intermittent method. In one cycle, from 400 to 500 ml of peripheral blood was treated, and five or six cycles were continued in one session. Less than 180 ml of ACD-A solution (including 3 w/v% citrate) was used for anti-coagulant. During the course of the therapy, all of the patients received total parental nutrition (TPN).

The severity of the disease was evaluated by clinical activity index (CAI), a systemic severity scoring system reported by Rachmilewitz (7). The CAI is composed of eight parameters: bowel habit, hematochezia, general condition, abdominal pain, body temperature, extra intestinal manifestations, sedimentation rate and hemoglobin. The effect of the therapy was also evaluated by CAI. In addition, we also checked serum C-reactive protein (CRP) and peripheral white blood cell count (WBC). Colonoscopy and histological examination of biopsied specimens were also performed both before and after the apheresis to confirm the effect on the inflamed colon. For the assessment of colonoscopic findings, the results were graded according to Mats' criteria (8). In this study, remission was defined as the clinical severity score being less than or equal to point 2 and the colonoscopic findings having a grade less than or equal to 2 without need for additional corticosteroid administration.

We also studied the expression of L-selectin (LECAM-1®, Becton Dickinson, San Jose) and VLA4a (SG/73®, Seikagaku Co., Tokyo) on peripheral mononuclear cells before and one day after leukocytapheresis in thirteen patients by using flow cytometry (EPICS-ELITE®, Coulter, Hialeah). Data of flow cytometry were calculated as the expression rate of each adhesion molecule, and mean values (SD) were obtained. Mann Whitney U test was used for statistical analysis.

Results

In each session of leukocyte apheresis, $3.4 \times 10^9 \pm 1.4 \times 10^9$ (mean +/- SD) leukocytes were removed; this was well tolerated by all the patients and no significant problems occurred. In thirteen of the fourteen patients (92.9%), the severity score dramatically decreased within 4 weeks of the apheresis and CRP became negative. In these patients, clinical symptoms such as abdominal pain, tenderness and hematochezia ceased completely and the severity score, CAI, became less than or equal to two. Colonoscopic examination revealed a definite reduction of inflammation, improvement in edematous change and cessation of bleeding in the colonic mucosa. In twelve patients, the colonoscopic score improved from grade 3–4 to grade 2. The changes of colonoscopic findings before and after leukocytapheresis in Patient 9 are shown in Fig. 1. Histological examinations of the biopsied specimens showed a significant

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Figure 1. Colonoscopic findings showed a remarkable improvement in Patient 9. Pre: Severe inflammation; ulceration, edema and bleeding were present in pre-leukocytapheresis. Post: In post-leukocytapheresis (4 weeks from started leukocytapheresis) these active inflammation disappeared, completely.

decrease of inflammatory cell infiltration. As shown in Fig. 2, thirteen of fourteen patients were considered to have entered into clinical remission by this therapy.

The differential counts of the removed leukocytes were: 41.4 +/- 15.6% (mean +/- SD) of neutrophils and 53.9 +/- 16.1% of lymphocytes. There was no significant change in the number of either peripheral white or red blood cells before and one day after the treatment. Although the peripheral platelet number decreased one day after apheresis, it returned to the original level within a few days. These patients remained in remission for 8 months on average (from 2 to 15 months) after the apheresis at an outpatient clinic; no additional medication with corticosteroids was needed during this period. In patients who were given oral corticosteroids during the apheresis, corticosteroids could be decreased or discontinued when apheresis was finished. The procedure took approximately 80 minutes, and cost ¥20,000-¥25,000 (approximately 170-200 U.S.$) for each session of leukocyte apheresis.

Flow cytometric analysis showed that the expression of L-selectin and VLA4a on lymphocytes was decreased at one day after leukocytapheresis (Fig. 3). The expression rate of VLA4a on lymphocytes before centrifugal leukocytapheresis (73.6 +/- 10.5%) was significantly (p<0.01) higher than one day after the apheresis (47.4 +/- 21.3%). The expression rates of L-selectin were 63.5 +/- 24.2% before, and 48.2 +/- 23.5% after. In the flow cytometric study, we concluded that circulating peripheral leukocytes changed due to the apheresis, from the viewpoint of the adhesion molecules, L-selectin and VLA4a.

Discussion

In 1985, Bicks et al (9) first reported in an abstract that remission induction was achieved in 8 patients with Crohn's disease (CD) by lymphocyte apheresis. They reported that a
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Figure 2. Changes of systemic severity score in clinical activity index (CAI), colonoscopic findings in Matts' criteria and histological findings in Matts' criteria. Pre- and post- (4 weeks from started) leukocytapheresis: each of them was evaluated.

Figure 3. Flow cytometric analysis of L-selectin and VLA4a expression on peripheral mononuclear cells before and one day after the centrifugal leukocytapheresis.

remission rate of 87% 2 years after seven procedures and that lymphocyte apheresis was beneficial for active CD. In 1993, Lerebours et al (10) reported a controversial result by conducting a randomized control trial of lymphocyte apheresis in CD. They concluded that lymphocyte apheresis worked only in terms of reducing the corticosteroid dependence to some degree but it could not prevent the occurrence of early relapse. Since they did the apheresis after induction of remission by steroid therapy, the aim of their study was totally different from that of Bicks et al (9). This is a point argued by Chadwick in his recent review (11). Therefore, the efficacy of lymphocyte apheresis in CD remains unclear. Furthermore, there is another argument that lymphocyte apheresis cannot be beneficial in CD, because the immuno-activated T lymphocytes are present only in the inflamed intestine and not in the peripheral blood.

Recently, Sawada et al (6) treated both UC and CD patients by leukocytapheresis using a leukocyte removal filter and showed that this procedure may be beneficial for both of the diseases. In their report, they mentioned that the clinical response was recognized in 6 of 8 patients with UC, and in 5 of 5 patients with CD, and that inflammatory bowel disease might be associated with the cell-mediated immune response. However, several lines of evidence indicate that the immunopathological background of UC is different from that of CD. For example, anti-neutrophil cytoplasmic antibodies (ANCAs) are reported to be associated with UC, whereas CD is reported to be less frequently associated with ANCAs (2). UC and CD seem to have entirely different HLA class II associations: DR2 in UC...
and DR1/DQ5 in CD (12). Therefore, it is not unreasonable to speculate that the clinical efficacy of apheresis is different between UC and CD. Further studies are needed to evaluate this point.

Corticosteroids are effective in bringing about a clinical remission in patients with UC. However, in severely relapsed cases, corticosteroids are not always effective and serious side effects may follow. In the present study, we targeted the apheresis on corticosteroid-resistant UC patients because a recent finding of leukocyte scintigraphy had revealed an abnormal accumulation of radionucleotides in the affected intestine of UC but not in CD (13), and we speculated that the extracorporeal removal of leukocytes might promote improvement of the disease stage. Rather than using a leukocyte removal filter, we used a newly developed centrifugal apparatus. This procedure has the advantage of gently removing whole buffy coat layers because no leukocyte adhesion to the filter occurs during the apheresis process. When we consider the clinical efficacy of this procedure, it is noteworthy that 92.9% of the UC patients in this study entered into remission. Since this is an open pilot study, a controlled study is in preparation to prove the definite efficacy of this therapy.

There remains a question of how leukocyte apheresis can reduce the severe inflammation of the colon of active UC. Activated neutrophils as well as lymphocytes are thought to play an important role in the pathogenesis of UC. In our centrifugal apheresis method, both lymphocytes and neutrophils were removed from the peripheral blood of the patients. One apheresis procedure removed massive circulating leukocytes; the absolute cell number of lymphocyte exceeds that of neutrophils. Such a rapid removal of neutrophils and lymphocytes from peripheral blood pools might have an effect on leukocyte production in the bone marrow. How can leukocytapheresis reduce the severe inflammation of the colon of active UC? It is supposed that rapid changes of adhesion molecules such as L-selectin and VLA4a in leukocytes are associated with the mechanism of this favorable effect. Our hypothesis is shown in Fig. 4. In apheresis, massive removal of peripheral leukocytes mobilizes another leukocyte pool. We pointed out two adhesion molecules: L-selectin and VLA4a, because these are target molecules on leukocytes and endothelial cell interaction (14, 15). Decrease of homing lymphocytes and neutrophil extravasation induces the disappearance of inflammation of the colon. Considered together, it might be suggested that the short-term rapid removal of leukocytes from the peripheral blood causes some kind of alteration of phenotype, particularly of the expression of the homing receptors of leukocytes to the colon. This hypothesis is now under study.

References


Massive removal of peripheral leukocytes

Mobilization of another leukocyte pool

L-selectin ↓
VLA4a ↓

Decrease of 'homing' lymphocytes and neutrophil extravasation

Disappearance of inflammation

Figure 4. A hypothesis on therapeutic mechanisms of centrifugal leukocytapheresis.