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ORIGINAL ARTICLE

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Enhanced effect of high-dose leukocytapheresis using a large filter in rheumatoid arthritis

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Abstract To evaluate the efficacy of high-dose leukocytapheresis (LCAP) using a large filter in patients with refractory rheumatoid arthritis (RA), we conducted a multicenter, nonrandomized, open-label clinical study. Thirty patients with highly active RA were treated with high-dose LCAP performed 3–5 sessions at 1-week intervals using a CS-180S filter (CS-180S group); the treatment involves the removal of leukocytes from a higher blood volume per body weight (100 ml/kg). The clinical response was evaluated at 4 and 8 weeks after a series of LCAP using the 28-joint disease activity score (DAS28). Similar data of 53 patients treated with conventional LCAP (60 ml/kg) using a standard filter, CS-100, were compared as a control (CS-100 group). The CS-180S filter demonstrated a higher adsorption capacity for leukocytes, particularly lymphocytes. The CS-180S group exhibited significant improvements in each item of DAS28 after treatment although the CS-100 group did not demonstrate such improvements in the CRP level and the ESR. Compared to the CS-100 group, the patients of the CS-180S group exhibited a tendency toward improvement with respect to the CRP level and ESR (P = 0.057 and 0.041, respectively). According to the EULAR improvement criteria based on DAS28, 60% and 45% of the patients from CS-180S and CS-100 groups achieved moderate or more responses, respectively, at 4 weeks after treatment. These results suggest that compared to conventional LCAP, high-dose LCAP may enhance the suppression of RA disease activity.

Key words Extracorporeal circulation · Leukocytapheresis · Rheumatoid arthritis

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Introduction

Although the recent development of disease-modifying antirheumatic drugs (DMARDs), including biological agents, has created a wide range of therapeutic options for rheumatoid arthritis (RA), the alternative treatment modalities provided in the guidelines for the management of late-stage RA that fails to respond to therapy with multiple DMARDs are inadequate.[1] For example, infliximab provides great benefits to patients with early RA;[2] however, it is relatively less effective in patients of late-stage RA.[3] Further, the antitumor necrosis factor antibody therapy for RA may pose the risk of serious infections and malignancies.[4] There are many patients who insufficiently respond to multiple DMARDs or who cannot receive DMARDs because of complications and infections or due to old age. As for nondrug therapy, leukocytapheresis (LCAP) – a treatment that removes peripheral leukocytes by continuous flow cell centrifugation – was found to provide clinical benefits in patients with refractory RA.[5–11] Thereafter, simpler filtration LCAP was developed and reported to suppress disease activity in patients with active RA refractory to multiple DMARDs.[12–16] The filtration LCAP leads to considerable improvements in joint symptoms and global assessment of disease activity; however, acute-phase indicators such as C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) have exhibited limited improvement. In this study, in order to improve the therapeutic effect of LCAP by increasing the quantity of leukocytes that were removed, we conducted a multicenter, nonrandomized, open-label clinical study to investigate high-dose LCAP performed using a newly developed larger filter and

an increased dose of 100 ml/kg, i.e., the blood volume per body weight treated, as an alternative to conventional LCAP that used a standard filter and a dose of approximately 60 ml/kg.

Patients and methods

The patients eligible for the study had active RA that was refractory to drug therapy with inadequate response to multiple DMARDs, including biological agents, or could not receive a sufficient dose of DMARDs because of drug side effects or complications. In addition, eligible patients had ≥6 swollen joints among 46 joints, and a CRP level ≥3.0 mg/dl or an ESR ≥50 mm/h.

Thirty-two patients received high-dose LCAP performed using a large LCAP filter with a capacity of 270 ml (Cellsorba CS-180S; Asahi Kasei Medical, Tokyo, Japan), at a target dose of 100 ml/kg of blood volume per weight per treatment session once a week at 11 centers of LCAP Investigators in Kyushu College of Rheumatology (LIKCR). Of the 32 patients, clinical evaluation of 30 patients (Table 1, CS-180S group) was performed; these patients had completed three or more sessions of a target of five treatment sessions. Treatment was discontinued in one patient after a single session, and the clinical data after treatment were missing for another patient; these two were excluded from the study.

With the identical inclusion criteria as CS-180S group, 58 patients received conventional LCAP performed using a standard filter with a capacity of 170 ml (Cellsorba CS-100; Asahi Kasei Medical), at a standard dose of approximately 60 ml/kg once a week at the LIKCR centers. Of the 58 patients, 53 patients were retrospectively included as historical controls (Table 1, CS-100 group); these patients had completed 3 or more sessions of a target of five treatment sessions.

As the evaluation criterion for clinical efficacy, the 28-joint disease activity score (DAS28) was used.[17] The DAS28 score was calculated by an equation based on the following four parameters: the tender joint count of 28 joints (TJC28), swollen joint count of 28 joints (SJC28), the patients' assessment of global disease activity (Visual Analog Scale, VAS), and the ESR.[18] The formula is as follows.

$$\mathrm{DAS28} = 0.56 \times \sqrt{(\mathrm{TJC28})} + 0.28 \times \sqrt{(\mathrm{SJC28})} + 0.7 \ln(\mathrm{ESR}) + 0.014 \times (\mathrm{VAS})$$

The European League Against Rheumatism (EULAR) improvement criteria classifies efficacy based on the DAS28 scores before and after treatment into 3 levels – "good response," "moderate response," and "no response."[18] A "good response" is defined as a patient in whom the DAS28 improves by >1.2 and who has a DAS28 <3.2 at the time of evaluation. A "moderate response" is defined as a patient who has either an improvement of DAS28 of 0.6–1.2 and who has a DAS28 <3.2, or an improvement of DAS28 >0.6 and a current DAS28 of 3.2–5.1, or who has an improvement of DAS28 >1.2 and a current DAS28 >5.1. Patients who do not fulfill these criteria are considered to be "no response."

The adsorption efficiency of the LCAP filters was estimated by a method described previously.[13] Briefly, blood samples were sequentially taken from the inlet and outlet of the filter during LCAP, the leukocyte and platelet counts were measured, and the adsorption efficiency was calculated as follows.

$$\text{Adsorption efficiency (\%)} = (\mathrm{Count_{in}} - \mathrm{Count_{out}}) \times 100/\mathrm{Count_{in}}$$

Continuous data were indicated as mean ± standard deviation (SD). For changes within groups, the nonstandard distribution and rank data were analyzed using the Wilcoxon rank-sum test. Comparisons between groups were analyzed using the Mann–Whitney *U*-test. The test for contingency table data was analyzed using the Wilcoxon rank-sum test. $P < 0.05$ was considered statistically significant.

Table 1. Patient background and leukocytapheresis (LCAP) treatment

	CS-100 group (n = 53)	CS-180S group (n = 30)	P value
Study period	April 2004–June 2006	October 2005–August 2006	
Age, years	59.8 ± 12.9	58.4 ± 10.6	0.365†
Female, *n*	45 (85%)	19 (63%)	
Duration of disease, years	13.6 ± 10.2	13.7 ± 10.8	0.896†
DAS28	6.00 ± 0.98	6.37 ± 1.24	0.067†
High (>5.1)	40 (82%)	25 (83%)	
Moderate (>3.2 but ≤5.1)	8 (16%)	5 (17%)	
Low (≤3.2)	1 (2%)	0 (0%)	
Stage (I/II/III/IV)	4/12/8/29	3/6/7/14	0.610‡
Class (1/2/3/4)	1/31/19/1	1/20/9/0	0.392‡
LCAP treatment			
No. of sessions (3/4/5)	4/5/44	0/4/ 26	0.583‡
Treated blood volume per session, liter	3.02 ± 0.30	5.12 ± 0.99	<0.001†
Treated blood volume per weight, ml/kg	60.5 ± 11.1	103.0 ± 20.6	<0.001†

DAS28, 28-joint disease activity score
†Mann–Whitney *U*-test; ‡Wilcoxon rank sum test

Results

Adsorption efficiency of filters

We examined the adsorption performance of the LCAP filters based on the differential leukocyte counts in the blood samples taken from the inlet and outlet of the filter during treatments with CS-180S (n = 12) and CS-100 (n = 25). The data demonstrated a difference in the mean adsorption efficiency of the filters (Fig. 1). CS-180S and CS-100 both trapped almost all the granulocytes and monocytes passing through the filter. When 1500 ml or more of blood was treated, the adsorption efficiency of CS-180S for lymphocytes and platelets was found to be greater than that of CS-100. These data indicate that CS-180S has a higher adsorption capacity for leukocytes, particularly lymphocytes, than does CS-100.

Patient background and LCAP treatment

The patient backgrounds and the details regarding the LCAP treatment are summarized in Table 1. In both the groups, the mean duration of the disease was more than 13 years; further, 80% of the patients exhibited high disease activity (DAS28 >5.1), and 50% of the patients were classified under Steinbrocker Stage IV, indicating that the disease was highly active and advanced. The patient backgrounds were not significantly different between the groups. The CS-180S group was treated at a 1.7-fold higher blood volume per weight during a session of LCAP than the CS-100 group.

Clinical response

To evaluate the clinical efficacy of high-dose LCAP in RA, the DAS28 data were analyzed using baseline scores prior to conducting LCAP and scores at 4 and 8 weeks after the last LCAP session (Fig. 2). The data for the CS-100 group at 8 weeks after treatment were missing. The tender joint count (28 joints), swollen joint count (28 joints), and the

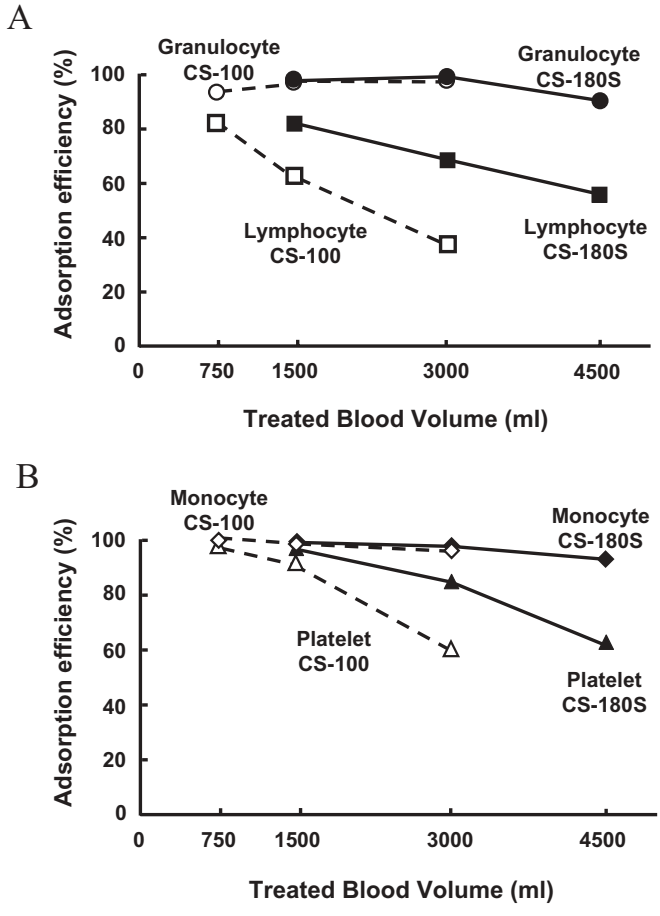

Fig. 1. Changes in leukocytes and platelet adsorption efficiencies of the leukocytapheresis filters, CS-100 (*white symbols, dashed line*) and CS-180S (*black symbols, solid line*). *Circle*, granulocyte; *rectangle*, lymphocyte; *diamond*, monocyte; *triangle*, platelet

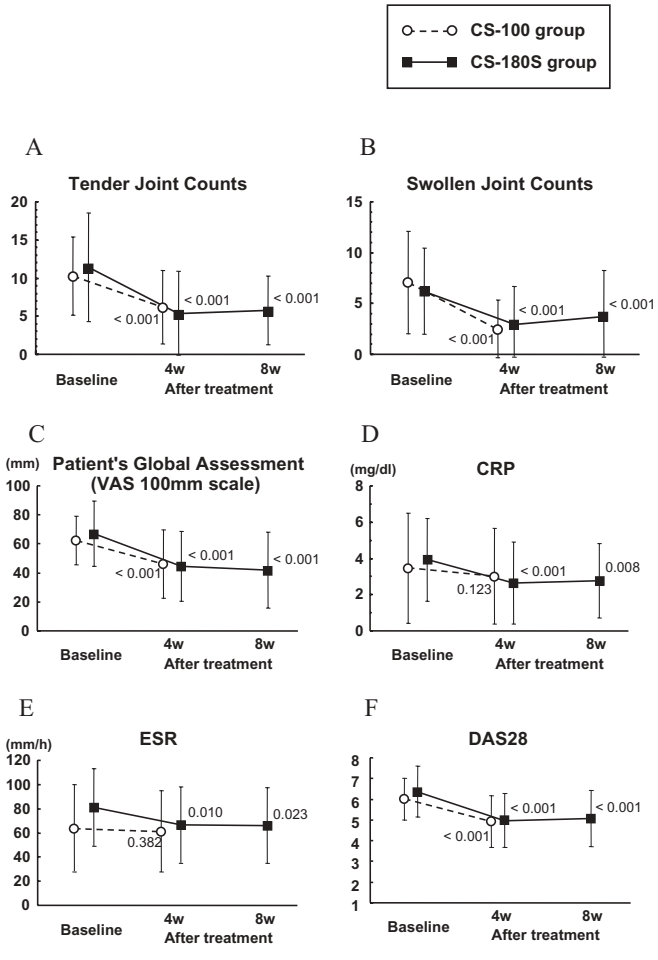

Fig. 2A–F. Changes in individual components of 28-joint disease activity score (*DAS28*) of CS-100 group (*white circle, dashed line*) and CS-180S group (*black rectangle, solid line*) at baseline and at 4 and 8 weeks (*w*) after treatment. The tender joint count (**A**), swollen joint count (**B**), patient's global assessment (100 mm VAS, **C**), CRP level (**D**), ESR (**E**), and DAS28 score (**F**). *P* values are compared with baseline by the Mann–Whitney *U*-test. *VAS*, Visual Analog Score; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate

patients' global assessment (100 mm scale, VAS) were significantly improved after LCAP in each group. The CRP levels and the ESR of the subjects in the CS-180S group were significantly improved at 4 and 8 weeks after LCAP; however, this improvement was not observed among those in the CS-100 group. Compared to the subjects in the CS-100 group, those in the CS-180S group demonstrated a tendency toward improvement with respect to the CRP levels and the ESR ($P = 0.057$ and 0.041, respectively). According to the EULAR improvement criteria based on DAS28, 2 (4%) and 22 (42%) patients of the CS-100 group and 2 (7%) and 16 (53%) patients of the CS-180S group exhibited good and moderate responses, respectively, at 4 weeks after LCAP. Further, at 8 weeks after treatment, 1 (3%) and 13 (43%) patients of the CS-180S group exhibited good and moderate improvements, respectively.

Safety

Side effect, of which relationship to LCAP cannot be denied, was evaluated in all 32 patients who underwent high-dose LCAP. Two patients experienced anemia (Hb = 9.0 and 8.9 g/dl, respectively) at the fourth session of LCAP and cancelled the fifth treatment. They recovered at 4 weeks after treatment (Hb = 10.4 and 10.7 g/dl, respectively), that is, the anemia was temporary.

Discussion

In this clinical study, we demonstrated that a large LCAP filter – CS-180S – increases the adsorption efficiency for leukocytes, particularly lymphocytes, and that high-dose LCAP significantly improves the clinical response in refractory RA patients not only with respect to joint symptoms and the patients' global assessment but also for CRP levels and ESR.

Analysis of adsorption efficiency indicated that CS-180S filter has a higher adsorption capacity for lymphocytes than does CS-100. There are differences between CS-180S and CS-100 filters not only in the capacities but also in the fiber diameters of the filters (mean, 1.7 μm and 2.5 μm, respectively; manufacturer's information). According to the research in leukocyte depletion filters for blood transfusion, small lymphocytes can be adsorbed more efficiently by filters with non-woven webs comprising ultrafine fibers of 1–2 μm diameter than that of >3 μm.[19] Our data are consistent with the adsorption properties of the ultrafine fibers.

There are some reports about the mechanisms of action of LCAP. Ueki et al. proposed that peripheral lymphoablation by LCAP may exert an immunomodulatory effect in patients with RA on the basis of their observation that the total lymphocyte number removed from responders – who received LCAP and achieved ACR20 criteria – was significantly more than that from nonresponders who did not achieve.[20] Hidaka et al. advocated that lymphoablation by LCAP may accelerate lymphocyte replenishment from their observation that the proportion of activated T lymphocytes (CD4+DR+, CD4+CD25+, and CD4+CD71+) decreased in the synovial fluid and increased in the peripheral blood from RA patients treated with LCAP significantly as compared with those of sham control group.[21] Their hypotheses are applicable to our data that high-dose LCAP using CS-180S filter introduces enhanced lymphoablation and improved clinical effectiveness.

Not only lymphocytes, but circulating monocytes are also suitable targets to be eliminated by LCAP because activated macrophages play a pivotal role in RA pathogenesis.[22] Hahn et al. demonstrated that circulating monocytes have been highly activated in RA patients and secreting prostaglandin E2, neopterin, interleukin 1β, and tumor necrosis factor-α; and that centrifugal LCAP can efficiently remove these cells and induce replenished monocytes which have lower activation status.[23] Since CS-180S filter can trap almost 100% of monocytes with a large adsorption capacity, monocyte ablation might play a part of improved therapeutic effect of high-dose LCAP.

CS-180S filter also has a higher adsorption capacity for platelets than CS-100. Although our patients did not show sign of thrombocytopenia during high-dose LCAP, precautions are needed. Recently, Wang et al. demonstrated that expressions of CD62P and CD63 on platelet have been elevated in active RA patients and that these cell surface markers were positively correlated with ESR.[24] Interestingly, these activated platelets have also increased in patients with active ulcerative colitis and that these cells were reduced after LCAP treatment.[25,26] Therefore, improvement of ESR in the present study might be partially mediated by reduction of increased platelet aggregation activities.

Recently, Onuma et al. reported an experience of pulse LCAP in 9 patients with methotrexate-resistant RA, in which the blood volume treated in one session was increased to 5000 ml with a conventional CS-100 filter; they observed that the CRP level altered significantly although this alteration was not observed for ESR.[27] In addition to the increase in the blood volume treated, the usage of a CS-180S filter that has an increased adsorption efficiency may enhance the suppression of RA disease activity, including ESR. Highly safe high-dose LCAP may be applicable to RA patients who are not eligible for the increase in the DMARD dose or for the application of biologics because of complications such as nephropathy, lung involvement, liver damage, and infection or due to old age.

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