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Leukocytapheresis for Ulcerative Colitis: A Comparative Study of Anticoagulant (Nafamostat Mesilate vs. Dalteparin Sodium) for Reducing Clinical Complications

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Abstract: Leukocytapheresis (LCAP) is a therapeutic strategy for extra corporeal immunomodulation that has been used to treat several immunological disorders, including ulcerative colitis (UC), with encouraging results, inducing remission in steroid-resistant patients. However, we have experienced some complications during or after LCAP therapy. Common adverse effects include fever, chills, nausea, vomiting, and hypotension. One of the reasons for these adverse effects might be the use of nafamostat mesilate (NM) as an anticoagulant. In the present study, 75 patients with UC were divided into two groups, an NM group and a dalteparin sodium (DS) group. The clinical efficacy of these treatments, improvement after treatment, changes in leukocyte differential count, and adverse effects after LCAP therapy were then compared. The clinical efficacy, improvement after treatment, and changes in leukocyte classification were not significantly different between the two groups, while some adverse effects were observed in the NM group but not in the DS group. In conclusion, LCAP therapy is a useful therapy for patients with moderate to severe UC who fail to respond to glucocorticoid therapy, however, a safe anticoagulant should be used to avoid its related adverse effects.
Key Words: Complications, Dalteparin sodium, Leukocytapheresis, Nafamostat mesilate, Ulcerative colitis.

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by abdominal pain, severe diarrhea, hematochezia, fever and anemia (1,2). While, the etiology of UC has not been fully elucidated, several altered autoimmune responses and genetic abnormalities have been implicated (3). Recently, the efficacy of leukocytapheresis (LCAP), which involves the use of a filter to remove leukocytes from the blood, for the treatment of inflammatory bowel diseases (IBD) was reported (4–10). Because patients with UC have a high risk of intestinal bleeding, a serine protease inhibitor which has a short-acting regional activity called nafamostat mesilate (NM; Torii Pharmaceutical Co., Ltd, Tokyo, Japan), is commonly used for an anticoagulant in LCAP (11). Nafamostat mesilate (NM) is the only anticoagulant recommended for use with leukocyte removal filters in Japan. However, Nagase et al. reported some complications associated with LCAP, including anaphylactoid reaction, nausea, vomiting and abdominal pain, and they showed that some of those adverse effects might be related to NM (12,13). Furthermore, other reports of therapeutic apheresis therapies have indicated some complications associated with NM (14–16). Likewise, we have experienced some patients who developed some adverse reactions in LCAP, and those reactions disappeared after NM was replaced with dalteparin sodium (DS; KISSEI Pharmaceutical, Matsumoto, Japan), without changing any other factors. Thus, we comparatively examined the use of two anticoagulants, NM and DS, to evaluate the safety of LCAP with DS for the treatment of patients with moderate to severe UC who have a high risk of bleeding.

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MATERIALS AND METHODS

Patients

The major inclusion criteria were a diagnosis of active-stage and moderately severe, severe, or fulminating UC according to the diagnostic criteria for UC

TABLE 1. *Anticoagulants and patient characteristics*

Anticoagulant	Male	Female	Age (mean ± SE)
NM	23	12	36.2 ± 5.8
DS	18	22	31.2 ± 5.8

DS, dalteparin sodium; NM, nafamostat mesilate.

severity established by the Research Committee of Inflammatory Bowel Disease (IBD) of the Ministry of Health and Welfare in Japan in 1985. Seventy-five patients with UC were treated using an LCAP filter (Asahi Kasei Medical, Tokyo, Japan). Informed consent was obtained prior to the start of therapy, and all the treatments were carried out at Miyazaki University Hospital between October 2001 and January 2005. The patients were divided into two groups. The group that received NM (NM group) consisted of 23 men and 12 women with a mean age of 36 years (range: 14–64). The group that received DS (DS group) consisted of 18 men and 22 women with a mean age of 31 years (range: 16–68) (Table 1). In the NM group, 16 patients had left-side colitis and 19 patients had entire colitis; in the DS group, 18 patients had left-side colitis and 22 patients had entire colitis. Disease severity was evaluated based on clinical, hematological, barium enema, and endoscopic findings and the Seo activity index (17). In the NM group, 15 patients had severe colitis and 20 patients had moderate colitis; in the DS group, 16 patients had severe colitis and 24 patients had moderate colitis. The cumulative amount of prednisolone used in each patient was 8–10 g before the treatment period (Table 2).

Treatment protocols

All patients were admitted to the hospital for treatment. LCAP was carried out using a Plasauto 1000 apheresis unit (Asahi Kasei Medical, Tokyo, Japan) equipped with a Cellsorba leukocyte removal filter (Asahi Kasei Medical). In each patient, a mean total blood volume of 3000 mL was processed over a period of approximately 1 h (extracorporeal flow rate was approximately 50 mL/min). The access and return lines were connected via the antecubital veins. This therapy was carried out once per week for 5 weeks. The patients were assigned to either the NM or the DS anticoagulant groups as an observational study. NM was infused continuously at 50 mg/h, and DS was infused at 5000 U/h. The clinical efficacy of the treatment and the ratio of improvement were assessed after five LCAP sessions. Changes in leukocyte differential count, and adverse effects were assessed after the first LCAP session. A post-treatment leukocyte analysis was carried out 1 h after the LCAP therapy. If a patient experienced adverse effects after receiving NM, the anticoagulant was switched to DS.

Statistical analysis

The data were compared using a one-way analysis of variance and Student's *t*-tests. A P-value of <0.05 was considered significant.

RESULTS

Clinical efficacy and the ratio of improvement are shown in Table 3. The clinical responses of the NM

TABLE 2. *Clinical features of patients with ulcerative colitis*

Anticoagulant	Location	Severity	Cumulative amount of prednisolone (g)
NM	Left sided: 16	Severe: 15	8.6 ± 3.5
	Entire: 19	Moderate: 20	11.7 ± 4.8
DS	Left sided: 18	Severe: 16	7.4 ± 4.2
	Entire: 22	Moderate: 24	9.3 ± 3.8

DS, dalteparin sodium; NM, nafamostat mesilate.

TABLE 3. *Clinical response and frequency of adverse effects after leukocytapheresis (LCAP) therapy*

					Adverse effects		
Anticoagulant	Efficacy	Improvement			Allergic reaction	Digestive reaction	Cardio-pulmonary reaction
NM	YES	27	77.1%	*	4 (11.4%)	2 (5.7%)	1 (2.8%)
	NO	8					
DS	YES	31	77.5%		0	0	0
	NO	9					

DS, dalteparin sodium; NM, nafamostat mesilate.
*not significant.

and DS groups were not significantly different: 77.1% of the patients in the NM group and 77.5% of the patients in the DS group entered remission after LCAP treatment. As shown in Table 3, there were four instances of allergic reactions (including eruption and urticaria), two digestive reactions (involving nausea, vomiting and abdominal pain) and one cardio-pulmonary reaction (palpitation, dyspnea and chest discomfort) observed in the NM group, with no adverse effects observed in the DS group during the course of LCAP treatment. Next, we investigated a reduction ratio of leukocytes by LCAP. As shown in Fig. 1, after the first LCAP session, 26.2% of the leukocytes were reduced in the NM group (before, 12 131 ± 672/μL vs. after, 8862 ± 881/μL) and 28.7% of the leukocytes were reduced in the DS group (before, 11 432 ± 1121/μL vs. after, 8151 ± 821/μL). No significant difference was observed between the NM and DS groups. Furthermore, we also estimated the changes in the leukocyte classification after LCAP. The differential counting of leukocytes after the first session showed that the numbers of reduced neutrophils, lymphocytes and monocytes before and after LCAP therapy were not significantly different between the NM and DS groups (NM group-neutrophils: before, 8746 ± 732/μL vs. after, 5971 ± 416/μL; lymphocytes: before, 2087 ± 406/μL vs. after, 1851 ± 781/μL; monocytes: before, 1055 ± 306/μL vs. after, 840 ± 496/μL; DS group-neutrophils: before, 7808 ± 742/μL vs. after, 5286 ± 485/μL; lymphocytes: before, 2446 ± 654/μL vs. after, 2082 ± 713/μL; monocytes: before, 892 ± 314/μL vs. after, 732 ± 212/μL). Similar results were observed after the second LCAP session (data not shown).

DISCUSSION

The etiology of ulcerative colitis is unknown, but multifactorial genetic, environmental, immunological, and microbiological factors are likely to be

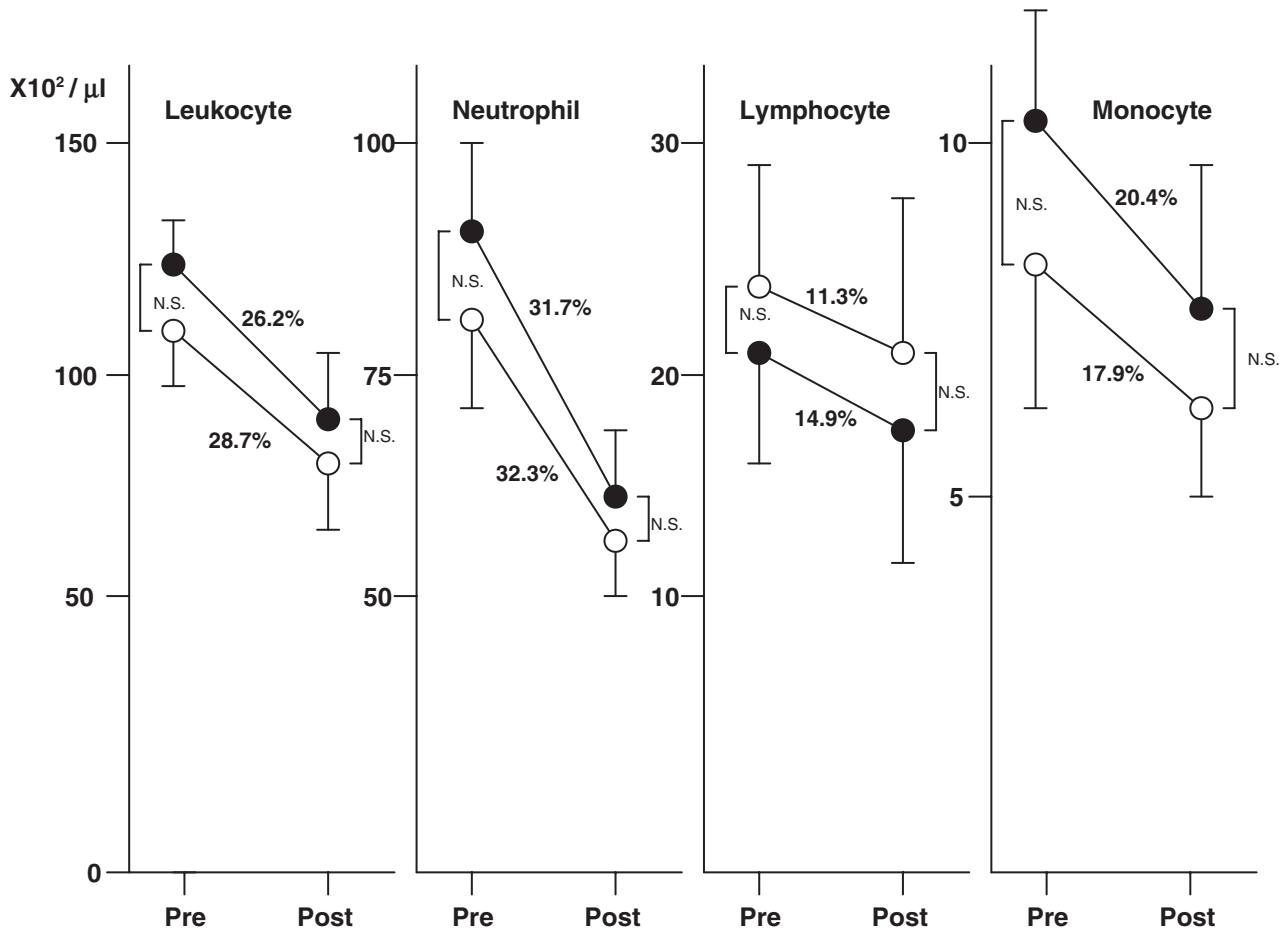

FIG. 1. Leukocyte classification before and after the first leukocytapheresis (LCAP) session. Samples from 35 patients in the NM group and 40 patients in the DS group obtained before (pre) and after (post) the first LCAP session were analyzed. The post-treatment leukocyte analysis was carried out 1 h after the LCAP therapy. The closed circles indicate the nafamostat mesilate (NM) group, and the open circles indicate the dalteparin sodium (DS) group. The bars indicate the means ± SD. N.S., not significant.

involved. Inflammatory mediators released from leukocytes, such as cytokines and eicosanoids, might be released during active ulcerative colitis, causing inflammation of the colonic mucosa (3). 5-ASA, salazosulfapyridine, corticosteroids, and immunosuppressive drugs have been used to treat ulcerative colitis, and the usefulness of these drugs is generally accepted (18–20). However, when patients experience a severe attack of ulcerative colitis, corticosteroid therapy might be ineffective, and urgent surgical resection might be required.

Recently, LCAP has been reported to be a useful treatment for diseases like IBD and rheumatoid arthritis. LCAP, using the leukocyte removal filter Cellsorba (Asahi Kasei Medical, Tokyo, Japan), is an online leukocyte removal system. Leukocytes are removed from venous blood passing through this filter via extra corporeal circulation technologies (7).

The present study shows that DS can be considered a safe anticoagulant with no adverse effects, while mild adverse effects were noted in seven patients (20%) in the NM group. In the NM group, four allergic reactions, two digestive reactions and one cardio-pulmonary reaction were observed. Most of these reactions were mild. However, we changed the anticoagulant from NM to DS in patients who experienced adverse reactions to NM, because these patients were already experiencing gastrointestinal symptoms caused by their condition (abdominal pain, severe diarrhea, hematochezia, fever and anemia, etc.). These seven patients who experienced adverse reactions could continue LCAP therapy series afterwards by using DS effectively and safely. None of the 40 patients in whom DS was used experienced an adverse reaction. Moreover, the clinical responses, including states of intestinal hemorrhage, of the NM and DS groups were not significantly different. We postulate that DS could be continuously used as a safer anticoagulant in UC patients receiving LCAP.

Akizawa *et al.* described NM as a useful anticoagulant for hemodialysis (HD) in patients with high risk bleeding, while adverse effects (headache, systemic pruritus, nausea and vomiting) caused by NM were observed in a few (5.6%) HD patients (11). In the present study, some of the adverse effects which occurred after the first session of LCAP therapy in the NM group might have been allergic reactions to the NM, because all of the patients who experienced adverse reactions in NM group did not relapse their symptoms after replacement of the anticoagulant by DS without any further change in treatments. Additionally, for the same reason, several known causes of adverse effects during therapeutic apheresis procedure (21–23) such as hypersensitivity reactions to ethylene oxide (ETO) gas used for sterilization of blood tubes, bradykinin activation during filtration (especially in patients given ACE-inhibitors), or allergic reactions against for the structure of leukocyte removal filter can be excluded. However, we have not yet confirmed our hypothesis because lymphocyte transformation tests (16), skin reaction tests (15) and measurement of anti-NM IgE (13) were not carried out in the NM group. Further examination will be necessary to determine other causes of adverse effects of LCAP related to NM. Also, because an anaphylactoid reaction induced by DS was also reported (24), albeit a solitary case report, we need careful follow-up regarding LCAP with DS.

In conclusion, no significant difference in the effectiveness between our NM and DS groups was observed, and there was no patient who developed an aggravation of intestinal hemorrhage induced by DS. LCAP with DS as an anticoagulant is considered a safe and effective therapy for the patient with moderate to severe UC.

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