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Granulocyteaphaeresis in steroid-dependent inflammatory bowel disease: a prospective, open, pilot study

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SUMMARY

Background: Uncontrolled studies suggest that granulocyteaphaeresis might be useful in the management of active ulcerative colitis.

Aim: To assess the efficacy of granulocyteaphaeresis treatment in active steroid-dependent inflammatory bowel disease.

Methods: We conducted a multicentre, prospective, open, pilot study in patients with steroid-dependent inflammatory bowel disease. All patients were started on 60 mg/day of prednisone; after 1 week, a five-session programme of granulocyteaphaeresis (once per week) was started. The steroid dose was tapered weekly if there was clinical improvement. Remission was defined as an inactive clinical activity index together with complete withdrawal of steroids at week 6. The patients were followed up for at least 6 months or until disease relapse.

Results: Twenty-six patients (14 ulcerative colitis, 12 Crohn's disease) were included. More than a half had been previously treated with immunomodulators. Remission was achieved in 62 and 70% of ulcerative colitis and Crohn's disease, respectively. During a median follow-up of 12.6 months, six of eight ulcerative colitis patients maintained their clinical remission; however, only one Crohn's disease patient remained in remission after the first 6 months of follow-up.

Conclusions: Granulocyteaphaeresis is a safe treatment option in inflammatory bowel disease. A five-session programme of granulocyteaphaeresis seems to be efficient in the treatment of steroid-dependent ulcerative colitis, but not in Crohn's disease.

INTRODUCTION

Systemic steroids are still the cornerstone of medical treatment in inflammatory bowel disease (IBD). However, 30–40% of IBD patients treated with systemic steroids develop steroid dependency.[1, 2] This scenario must be avoided because of the high rate of steroid side-effects, some of them even irreversible.[3, 4] Conventional immunomodulators (mainly azathioprine or methotrexate) or, more recently, infliximab, have demonstrated their efficacy in inducing disease remission and steroid withdrawal. Nevertheless, still a considerable proportion of these patients are refractory or intolerant to these drugs.[5–8]

Aphaeresis techniques have been used in the treatment of some refractory autoimmune disorders such as myasthenia gravis, psoriasis, rheumatoid arthritis, or systemic lupus erythematosis.[9–12] These techniques allow the removal of immune cells or inflammatory mediators from the bloodstream. Granulocyteaphaeresis

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(GCAP) is an aphaeresis procedure that has been used for the treatment of IBD [mainly ulcerative colitis (UC)] in the last few years in Japan. Some open series have been reported suggesting that GCAP might be useful in different clinical settings.[13–16] In addition, experimental and human studies, have recently demonstrated that GCAP removes granulocytes and monocytes by activation of the complement cascade mediated by its cellulose diacetate beads.[17, 18] GCAP could also induce functional changes in these cells, leading to a downregulation of inflammatory cytokines [interleukin (IL)-1, tumour necrosis factor (TNF)α, IL-6, or IL-8] and adhesion molecules (L-selectine), and to an increase in anti-inflammatory mediators (IL-1ra, soluble TNFα receptor).[13, 19, 20]

Although GCAP therapy has not been accurately evaluated in IBD, the autoimmune nature of the disease, the changes induced by GCAP on the immune cells and inflammatory mediators and the apparent safety profile of the device, makes it an interesting therapeutic approach in IBD. We report the results of the first prospective pilot study of the GCAP treatment in steroid-dependent IBD patients. The main objective of the study was to assess the efficacy in inducing and maintaining remission in patients with steroid-dependent Crohn's disease (CD) or UC. As a secondary objective, we assessed the safety profile of the device.

MATERIALS AND METHODS

This multicentre, prospective, open, pilot study was conducted at nine centres in Spain. The institutional review board at each participating site approved the study protocol. Written informed consent was obtained from all patients.

Patients

Inclusion criteria were: (i) prior diagnosis of UC or CD according to the Lennard–Jones criteria;[21] (ii) active disease, defined as a modified Truelove activity index >13 and ≤ 24 points for UC and Crohn's Disease Activity Index (CDAI) >150 points or a Van Hees Activity Index >120 points for CD and (iii) steroid-dependent disease defined as the impossibility to completely withdraw systemic steroids because of disease relapse or the occurrence of more than or equal to two flare-ups requiring systemic steroid therapy in a 6-month period.

Age under 18 years, pregnancy or lactation, white cell count $<4 \times 10^9$/L, platelet count $<100 \times 10^9$/L, haemoglobin concentration <8 g/dL, renal, cardiac, hepatic or pulmonary severe concomitant diseases and infliximab treatment during the last 3 months before the inclusion, were exclusion criteria. Concomitant treatment with immunomodulators (azathioprine, mercaptopurine, methotrexate and cyclosporine) in stable dosage was permitted.

Methods

At baseline, treatment with 60 mg/day of oral prednisone was started. Seven days later, a once-per-week GCAP treatment with Adacolumn (JIMRO; Takasaki, Japan) for 5 weeks, was started in an out-patient setting. GCAP is an extracorporeal vein-to-vein aphaeresis technique. This procedure allows functional changes in immune cells, by adsorbing and altering granulocytes and monocytes [13, 19, 20] from the patient's blood. Each column contains 35 000 beads of cellulose diacetate (2 mm diameter) soaked in isotonic saline within a 335-mL polycarbonate housing. Blood is drawn into the column from antecubital veins; in cases where this access was not available, other peripheral or even a central vein were catheterized. In normal conditions, a total of 1800 cm^3 of blood is processed in each session at a blood flow rate of 30 mL/min; therefore, each GCAP session takes 60 min. In each GCAP session, sodium heparin was continuously infused to the GCAP system at a rate of 25 UI/min to avoid coagulation problems of the extracorporeal system. All adverse events during the study protocol were registered.

If the clinical status of the patient improved, steroid dose was tapered by 10 mg per week, leading to complete steroid withdrawal at week 6. Disease activity was assessed weekly in all patients. In UC patients, the modified Truelove Activity Index by Rachmilewitz[22] was used and endoscopic activity was also assessed by the Rachmilewitz score at baseline and at week 6. In CD, the degree of disease activity was measured by CDAI or Van Hees Activity Index, depending on which one was usually applied in clinical practice in each participating centre.

Induction of remission was assessed at week 6. Clinical remission was defined as an inactive activity index (Modified Truelove Activity Index ≤ 13 points for UC and CDAI <150 points or Van Hees Activity Index <120 points for CD) together with a complete withdrawal of

steroids. In UC patients, endoscopic remission was defined as an Endoscopic Activity Index ≤ 4 points. All responders were followed up for at least 6 months or until relapse. Clinical and biological assessment were performed monthly during the first 12 months of follow-up; active clinical activity indexes and/or the need of specific medication for IBD other than 5-amino-salicylic acid (5-ASA) during this period were considered as loss of initial response.

Statistical analysis

Results are expressed as mean ± SD or frequencies. Quantitative variables were compared with the Student's *t*-test for paired data. All statistical analyses were performed using the BMDP package (BMDP; Statistical Software Inc., Los Angeles, CA, USA).

RESULTS

A total of 26 IBD patients (14 UC, 12 CD) were included in the study. Baseline characteristics are shown in Table 1. Sixteen of 26 patients had been previously treated with immunomodulators, including three patients treated with infliximab.

Table 1. Baseline characteristics of the patients

	UC ($n = 14$)	CD ($n = 12$)
Age (years)	39.9 ± 15	34 ± 9.9
Gender (M/F)	10/4	5/7
Time from diagnosis (months)	72.8 ± 34. 5 (12–129)	70.6 ± 42.4 (10–153)
UC extension (distal/extense)	6/8	–
CD location (L1/L2/L3/L4)	–	1/4/6/1
CD behaviour (B1/B2/B3)	–	8/–/4
Mean number of flare-ups requiring systemic steroids	4.2 ± 1.6 (2–7)	7.3 ± 4.2 (3–14)
Patients with previous immunomodulator therapy failure		
Azathioprine	9	5
Methotrexate	–	2
Infliximab	–	3

All data expressed as mean ± SD (range).
Crohn's disease location and behaviour based on Viena's classification criteria.[27]
L1, ileal; L2, colonic; L3, ileal and colonic; L4, upper gastrointestinal tract, B1, non-structuring non-penetrating; B2, structuring; B3, penetrating; UC, ulcerative colitis; CD, Crohn's disease.

Three patients (one UC, two CD) were excluded from the final analysis because of colonic cytomegalovirus (CMV) infection ($n = 1$) and violation of inclusion criteria ($n = 2$).

Induction of remission

In UC, eight out of 13 (62%) patients achieved remission at week 6 and two additional patients had an improvement in their clinical status without achieving remission criteria. Mean Modified Truelove Index decreased from 19.4 ± 1.84 at baseline to 12.5 ± 1.27 at week 6 in those patients with complete or partial response, whereas there were no significant changes in non-responders (Figure 1). Interestingly, all but one of these 10 responding patients also achieved endoscopic remission at week 6. Clinical and endoscopic improvement was also associated with a substantial decrease from baseline to week 6 in biological parameters such as C-reactive protein (CRP) (from 13.7 ± 14.5 to 3.9 ± 2.4 mg/L) or erythrocyte sedimentation rate (ESR) (from 47.3 ± 17.9 to 24.1 ± 12.8 mm/h) in these patients.

In CD, remission was achieved in seven out of 10 patients (70%). As in UC, CD responders evidenced a marked decrease in CRP (from 36.2 ± 36.9 to 12.2 ± 6.2 mg/L) and ESR (from 36 ± 27.3 to 17.1 ± 15.3 mm/h), from baseline to week 6. Four of these patients achieved remission within the first 3 weeks, whereas the remaining three patients did it at week 6 (Figure 2).

Duration of response

In patients with initial response, clinical and biological assessment was performed every month after the last

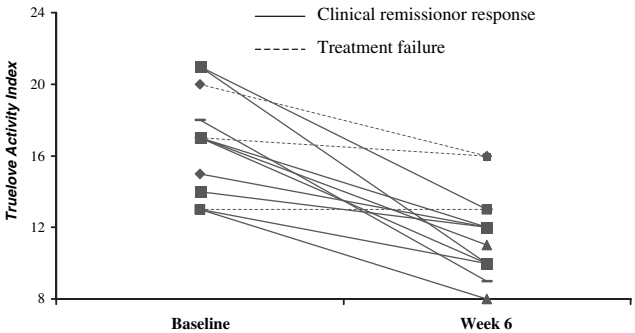

Figure 1. Evolution of Truelove Clinical Activity Index in ulcerative colitis patients after the granulocyteaphaeresis programme ($n = 13$).

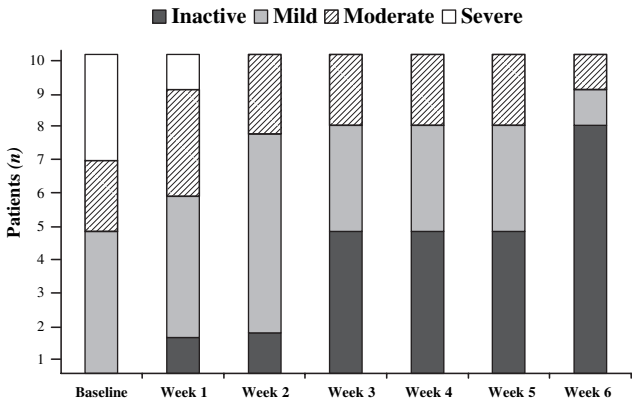

Figure 2. Evolution of disease activity in Crohn's disease patients during the treatment period.

GCAP session, for at least 6 months or until relapse. All patients were maintained on oral/topic 5-ASA and baseline immunomodulatory treatment was not modified. Loss of initial response was defined as a Truelove Activity Index >13 points in UC patients, and a Van Hees Activity Index >120 points or a CDAI >150, or the need of systemic steroids, surgery or new immunomodulatory agents.

Figure 3 shows the proportion of UC and CD patients who maintained the initial response during follow-up among those who achieved remission at week 6.

Six out of eight UC patients maintained clinical remission after a mean follow-up of 12.6 months (range 9–24). Only two UC patients relapsed 2 and 9 months after the last GCAP session, respectively; one of them required systemic steroids whereas the other presented peripheral arthropathy that required parenteral methotrexate administration. The two patients who presented partial response at week 6 achieved clinical remission 1 month after the last GCAP session; however, both of them relapsed at 4 and 6 months of follow-up, respectively.

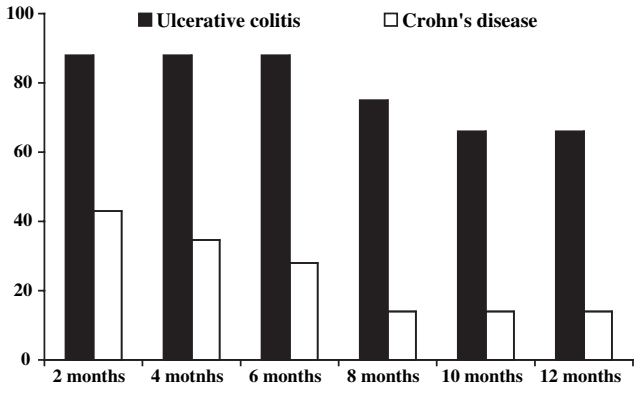

Figure 3. Proportion of patients (%) who achieved remission at week 6, in maintained remission during follow-up.

In CD three of seven patients who achieved remission at week 6 lost the initial response 1 month later; two additional patients relapsed 2 and 4 months after the last aphaeresis, respectively and another patient had to be excluded from the study because of the development of an allergic reaction to 5-ASA and the introduction of immunomodulators. In turn, only one patient remained in remission at the end of the follow-up (12 months).

Adverse events

Granulocyteaphaeresis was very well tolerated and only minor side-effects were registered in a few patients. During the study protocol, two patients reported mild headache. Two patients presented infectious problems (one pneumonia and one catheter's septicaemia); both of them required hospital admission and intravenous antibiotic treatment, but they were successfully discharged. Although they were not specifically evaluated, no major biochemical or haematological disturbances were reported.

DISCUSSION

Several authors have reported the efficacy of GCAP in inducing remission in active IBD (mostly in UC), in uncontrolled trials.[13–16] To our knowledge, this is the first reported prospective, open, pilot study evaluating the efficacy and safety of GCAP in steroid-dependent IBD. Recently, Naganuma *et al.* [14] reported their experience with the same GCAP session programme, in steroid-naïve, -refractory, and -dependent UC patients. In those patients with steroid-dependent disease ($n = 10$), a remission rate of 60% was achieved, with an additional 30% of patients having some clinical improvement. These results are very close to those obtained in our study, although in the Japanese study the initial dose and the tapering regimen of steroids was not the same in all the patients and complete withdrawal of steroids was not required to consider clinical remission. In our study, the outcome assessment at week 6 might be of low value because of, given the steroid-dependency, the clinical response could have been predominant because of the high-dose steroid therapy. In fact, the obtained response rates (62% in UC, 70% in CD) are closely to those reported in the literature for systemic steroids in acute IBD. However, it has to be pointed out that the study

protocol's course of steroid administration included a more rapid tapering dose regimen (6 weeks) than usual; in our country, an initial course of 60 mg of prednisone for an IBD flare-up, may take 8–9 weeks (in responders) to be completely discontinued. Moreover, >60% of our patients had been previously treated with immunomodulators, without success.

The present study also assessed endoscopic findings at baseline and at the end-of-study treatment protocol (week 6) in UC patients, demonstrating that clinical remission in UC was always correlated with endoscopic remission. Finally, biological parameters such as ESR and CRP also improved in responders, as Shimoyama *et al.*[13] had previously reported. Uncontrolled studies had suggested a therapeutic role for heparin in IBD, particularly in UC [23, 24]; during each GCAP session, sodium heparin was infused to the patients in order to avoid coagulation problems of the extracorporeal system. An additional therapeutic effect is unlikely because of the total amount of heparin administered per session (1500–3000 U) and its periodicity (once per week). In addition, a recent Spanish multicentre randomized trial demonstrated that a continuous infusion of unfractionated heparin was not effective in the treatment of active UC.[25]

Limited data about GCAP in CD are available. Matsui *et al.* [16] reported their initial experience in CD patients refractory to conventional therapy, obtaining clinical remission in five of seven patients with five to six GCAP sessions (one per week). In that study, responders were younger, had mainly colonic involvement and a short disease history. In our study, a similar proportion of patients (seven of 10) achieved clinical remission after five aphaeresis sessions. All but two patients had colonic involvement (colonic or ileocolonic disease) and no correlation of treatment efficacy with age or time from disease diagnosis could be observed.

Although the scheduled treatment programme achieved a similar remission rate at week 6 in UC and CD, the duration of the response during follow-up was not the same in both groups of patients. Most of those UC patients who achieved remission during the first 6 weeks, maintained their response and did not require systemic steroids during follow-up. These figures are similar to those reported by Naganuma *et al.*[14] who achieved long-term remission in 61% of 33 UC patients after a follow-up of 6–33 months.

We defined steroid-dependency as the impossibility to completely withdraw systemic steroids because of disease relapse or the requirement of two courses of systemic steroids in a short period of time, according to the definition used by most authors.[1, 2, 26] However, all but two UC patients were classified as steroid-dependent by the former criteria; thus, the therapeutic effect was not only explainable by the 6-week course of steroids, because most of these patients remained in remission and free of steroids during a mean follow-up of 12 months.

In CD patients, maintenance of remission was not the rule, showing that this treatment schedule was not adequate to induce long-term remission. There are no available data in the literature of long-term maintenance of remission after GCAP therapy in CD. The cause of this different long-term duration of remission between UC and CD is not known, but immunological mechanisms may be involved. In this sense, immune intestinal response is different in both diseases and, as the accurate mechanism of action of GCAP is still not perfectly known, it seems reasonable that the immunological changes induced by GCAP could have different qualitative implications in both UC and CD.

In uncontrolled and retrospective studies, GCAP has demonstrated to be a safe therapeutic technique, with a very low rate of side-effects.[13–16] GCAP sessions were generally well tolerated, they are not very time-consuming, and they allowed an out-patient management during the treatment period. However, in our study, there were three infectious adverse events that were most likely related to the immunomodulatory treatment (steroids, azathioprine and cyclosporine) than to GCAP itself. Probably, one of the potential limitations of this device is the accessibility to peripheral veins to perform the aphaeresis sessions, especially in steroid-dependent patients in whom vascular fragility is enhanced. In this study, some of our patients required the placement of a central venous catheter to be treated. Taking into account that they were mostly treated in an out-patient setting, the risk of using a central venous catheter during 4 weeks is not low and, in fact, one of our patients presented a catheter's septicaemia because of infection by *Pseudomonas aeruginosa* that required hospital admission and intravenous antibiotics.

In summary, GCAP seems to be effective and safe in the treatment of steroid-dependent UC. This technique induces remission and allows steroid discontinuation in a great proportion of UC patients, even in those refractory or intolerant to immunomodulators. Long-term remission is much more likely in UC than in CD; in turn, prolonged GCAP scheduled therapy must be

evaluated in CD patients. As the result of the scarce available data on GCAP, prospective, multicentric trials are needed to establish its role in IBD management, especially in some clinical settings such as steroid refractoriness or maintenance of remission.

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