Original Article

Adsorptive Granulocyte/Monocyte Apheresis for Maintenance of Remission in Patients with Ulcerative Colitis: A Prospective Randomized, Double Blind, Sham Controlled Trial

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ABSTRACT

**Background:** Weekly granulocyte/monocyte adsorption (GMA) to deplete elevated and activated leucocytes should serve as a non-pharmacological intervention to induce remission in patients with ulcerative colitis (UC). This trial aimed to assess the efficacy of monthly GMA as maintenance therapy to suppress UC relapse.

**Methods:** Thirty-three corticosteroid refractory patients with active UC received 10 weekly GMA sessions as remission induction therapy. Then, they were randomized to receive one GMA session every 4 weeks (True, n=11), extracorporeal circulation without the GMA column every 4 weeks (Sham, n=11), or no additional intervention (Control, n=11). Prednisolone (PSL dose, mg/day) was 11.3±6.8, 6.8±6.1, 13.9±13.3, respectively, and was to be tapered during maintenance therapy. Primary endpoint was the rate of avoiding relapse (AR) over 48 weeks; relapse meant clinical activity index (CAI) ≥5.

**Results:** At week 48, the AR rates in True, Sham, and Control were 40.0%, 9.1%, and 18.2%, respectively, all steroid free, yet statistically no significant difference was seen among the 3 arms. However, in patients who could taper their PSL dose to <20mg/day during remission induction therapy, the AR in True was better vs Sham (P<0.03) or Control (P<0.05).

**Conclusions:** GMA with the Adacolumn as a nondrug treatment intervention has an excellent safety profile, which is favoured by patients. Monthly GMA potentially should prevent UC relapse in patients who have achieved remission by weekly GMA, especially in patients on <20mg/day PSL at the start of maintenance therapy. Additional trials in large cohorts of patients are warranted to strengthen our findings.
INTRODUCTION

Ulcerative colitis (UC) together with Crohn’s disease (CD) are the major phenotypes of the idiopathic inflammatory bowel disease (IBD), which afflicts millions of individuals throughout the world with symptoms that impair quality of life (QoL) and ability to function [1]. Currently, the aetiology of UC is not well understood, but mucosal tissue oedema, increased gut epithelial cell permeability, and extensive infiltration of the colonic mucosa by leucocytes of the myeloid lineage are major pathologic features of this immune disorder. Accordingly, selective depletion of peripheral granulocyte and monocyte/macrophage by extracorporeal adsorption (GMA) with an Adacolumn has been applied as a non-pharmacologic treatment strategy to alleviate the inflammatory response in patients with active IBD [2,3]. The primary target of GMA is to deplete elevated/activated circulating myeloid leucocytes, which infiltrate the colonic mucosa in vast numbers during active IBD [2,4]. Further, weekly GMA has been accepted as a non-pharmacologic treatment option for IBD patients with an active flare while on conventional medications including high dose corticosteroid. Shimoyama et al. [5] were the first to carryout a multicentre trial, and show that steroid refractory UC patients with a severe acute flare could achieve remission and reduce their steroid dosage by combining 5 GMA sessions over a 5 week period. They also reported significantly less side effects for GMA vs prednisolone (PSL). The outcomes of this multicentre controlled trial in 2000 convinced the Japan Ministry of Health to approve GMA therapy for funding in the national health insurance scheme to treat steroid refractory UC patients with an acute flare. Since then, this treatment option has shown an excellent safety profile together with steroid sparing effect.

In clinical setting, there is a need to establish an effective therapeutic strategy for long-term maintenance of remission without compromising safety [6]. Further, it might be reasonable to expect a strategy that is effective as remission induction therapy to work as maintenance therapy as well. An extracorporeal leucocytapheresis system like GMA is expected to have the
potential to achieve this intention. There is evidence to support the clinical efficacy for monthly leucocytapheresis as an adjunct maintenance therapy in UC patients with steroid refractory background [7]. With this background in mind, in the present study, our objective was to design a prospective, single centre, randomized, sham controlled, double blind trial with three arms to see if monthly GMA can suppress UC relapse in a population of patients who had achieved remission with a series of weekly GMA sessions.

METHODS

General information

This prospective, single centre, randomized, sham controlled, double blind trial with three arms was conducted at the division of Lower Gastrointestinal Disease & IBD Centre, Hyogo College of Medicine, Japan between April 2004 and December 2009. The study protocol was reviewed and approved by the institutional ethics committee on first April 2004, and all patients provided both oral and written informed consent. The study was registered with UMIN.ac.jp (number UMIN000004242).

Patients

Eligible patients were men and women aged 12 to 75 years and body-weight ≥39kg with UC following remission induction intervention involving 10 weekly GMA sessions within 4 weeks prior to the start of this maintenance study. Remission induction therapy with weekly GMA was decided for moderate-to-severe UC in spite of receiving conventional medication. Active disease was defined as clinical activity index (CAI) ≥5, according to Lichtiger, et al. [8], and CAI ≤4 was considered clinical remission. Concomitant azathioprine (AZA) and PSL were allowed if had been started before the randomization at a stable dose, but were to be tapered during the trial.
Exclusion criteria included treatment with cyclosporine A, or tacrolimus \( \leq 4 \) weeks prior to the start of this study, infliximab \( \leq 8 \) weeks prior to the start of this study. Also, patients with granulocytopenia (neutrophil count \(<2,000/\mu l\)), serious heart or kidney disorders, coagulation abnormalities, history of hypersensitivity to heparin like heparin-induced thrombocytopenia, hypotension \((<90/65\text{mmHg})\) or uncontrolled hypertension \((>180/120\text{mmHg, despite medical therapy})\); anaemia, haemoglobin \(\leq 9.0\text{g/dl}\), and women being or wishing to become pregnant, were excluded.

**Study protocol**

Figure 1A shows the design of the present trial. Within 14 days following the last weekly GMA session as remission induction therapy, we obtained both oral and written informed consent and then the eligible patients were randomized to one of the three arms (True, Sham, or Control) of the study for maintenance therapy. Patients who were assigned to True received monthly GMA, patients in the “Sham” arm received monthly 1 hour extracorporeal blood circulation without the GMA column (circuit lines only; see Figure 1B), and patients in the Control arm remained on their ongoing conventional medications. Patients were randomized in a 1:1:1 ratio by a statistician at an independent organization. Since this was a pilot trial with small sample sizes, randomization was done blindly according to a computer-generated scheme with blocks of three (each three patients were randomly allocated to True, Sham, or Control). This was to minimize the risk of unbalanced group sizes. This trial was designed as proof-of-concept (of active intervention) as opposed to a definitive evaluation of the monthly GMA therapy. Therefore, the primary goal was to evaluate the likely effective size and appropriate candidate patients for this nondrug intervention for subsequent evaluation in a large definitive trial.

**Treatment**

GMA was done with the Adacolumn as previously described [2,3,5,6,9]. Briefly, the
Adacolumn is filled with specially designed cellulose acetate beads, which serve as the column adsorptive leucocytapheresis carriers [2]. The carriers selectively adsorb from the blood in the column about 65% of granulocytes, 55% monocytes/macrophages and a significant fraction of platelets, which bear the FcγR and complement receptors; lymphocytes are spared and subsequently increase [2,3]. The patients assigned to the True arm, each received one GMA session every 4 weeks for up to 48 weeks. The duration of one session was 60 minutes, at 30mL/minute (similar to the weekly GMA). An optimum dose of sodium heparin (2000units/session) as an anticoagulant was administered during both GMA and the sham procedures. Nafamostat mesilate (a common anticoagulant during leucocytapheresis in Japan) was avoided as this substance is associated with allergic reactions [11]. The sham GMA was based on the design by Sands et al. [12], having the circuit blood lines without the Adacolumn itself. The circulation time for the Sham GMA was 60 minutes, at 30ml/min (the same as in True). The circuit line was covered securely by a curtain to blind both the physician and the patients on the type of treatment. (Figure 1B)

Efficacy assessment

The primary endpoint of the study was a non-relapsing ratio (%AR) at week 48. Relapse was considered if a patient experienced a flare-up severe enough to warrant PSL administration or increase the ongoing PSL dose or give another set of GMA sessions during the follow up period, CAI≥5. Further, to evaluate mucosal UC activity prior to the start of the study, we compared colonoscopic findings between pre-and post-remission induction therapy with GMA. Also, patients who achieved sustained remission up to week 48 had colonoscopy at week 48.

Ethical considerations

GMA with the Adacolumn is a Japan Ministry of Health approved treatment option for patients with active IBD. Additionally, the investigation was carried out in accordance with the Principle
of Good Clinical Practice and the Declaration of Helsinki at all times. The study protocol was reviewed and approved by the Ethics Committee of Hyogo College of Medicine on the first of March 2004, and all patients provided informed consent. In the case of under age patients, consent from one of the patient’s parents was sought.

Statistics
Quantitative variables are compared by using a two-sided Mann-Whitney U test. The measures of long-term outcomes are assessed by the Kaplan-Meier survival analysis, while categorical data are analyzed by the 2-sided Fisher exact test. P<0.05 was considered statistically significant.

RESULTS
Patient characteristics and disposition
Thirty-three corticosteroid refractory UC patients, who were treated with a series of 10 weekly GMA sessions as remission induction therapy, agreed to participate in the present study. The demography of these 33 patients is presented in Table 1. Among patients in the 3 arms, there was no significant difference in demographic variables prior to beginning of either the remission induction therapy, or GMA maintenance therapy.

The overall clinical outcomes
In Figure 2, the overall clinical outcomes in the 3 arms of the study (True, Sham, and Control) are presented. One patient in True had to be excluded because this patient experienced a clinical relapse before receiving the 1st monthly GMA session. Similarly, 1 patient in the Sham arm willingly withdrew from the study 10 days after receiving the 1st monthly sham GMA session. In the Control arm, one patient was unable to attend, and one decided to sign up for another trial. At week 48, AR rates in True, Sham, and Control were 40.0%, 9.1%, 18.2%, respectively, yet
statistically no significant difference was seen between the 3 arms (Figure 3). Among our patients, there were many cases who could reduce their PSL dosage to <20mg/day during remission induction therapy (low dose PSL sub-group). The number of such patients in True, Sham, and Control were 7, 10, and 9, respectively. The low dose PSL sub-group had longer duration of UC (P=0.0451) in addition to the PSL dosage at the end of their remission induction therapy. There was no significant difference between these two sub-groups with respect to other demographic variables. Figure 4 shows the AR values in the <20mg/day PSL sub-group. The overall result of the low PSL sub-group in a log-rank test at the primary end-point was significantly higher in favour of True vs other 2 groups (P=0.0433). All patients who avoided relapse up to week 48 became PSL free. Accordingly, we believe that the monthly GMA should increase the long-term survival (without relapse) as indicated by the Kaplan-Meier survival analysis (P=0.0219, vs Sham; P=0.0439, vs Control). To see any contribution from AZA to the clinical efficacy associated with monthly GMA, we compared the results of patients on concomitant AZA with those without AZA in each of the 3 arms. However, the Kaplan-Meier survival analysis did not show significantly better remission maintenance in favour of concomitant AZA (data not presented).

There was no serious adverse side effect suspected of 30ml/min x 60min of extracorporeal circulation procedures in either True or the Sham arm. However, in the True arm, 2 patients complained of nausea several hours after completing a GMA session (see Figure 2). They decided to withdraw from this study in spite of the fact that their nausea symptom had disappeared without medication. Also, their CAI was maintained within remission level, ≤4 even with their PSL having been discontinued. In the Sham arm, one patient showed mild skin itchiness on both forearms without eruptions, appearing during the 2nd extracorporeal circulation session. Further, in the Sham arm, a 37 year old patient with left hemi-colicitis type of 14 months duration had to opt for surgery 3 months after starting maintenance sham GMA. This
was the only patient of this trial who received surgical treatment.

DISCUSSION
In this investigation, we were interested to see if monthly GMA has efficacy as maintenance therapy in patients who had just achieved clinical remission, induced with the same modality, but at a more frequent GMA sessions. From the viewpoint of remission maintenance, we believe that it is significant to use a strategy, which is effective in inducing remission of the active disease, and then the same strategy should be used as maintenance therapy. It is also important to state that all patients of this study had a corticosteroid refractory background prior to remission induction therapy with weekly GMA. Further, the authors like to acknowledge that this was a prospective exploratory study with a total of 33 patients, which limits the statistical significance level. Nonetheless, the clinical outcomes over 48 weeks were encouraging to us.

The % of patients who could maintain clinical remission up to week 48 in the three arms of this study was in the order: GMA > Control > Sham. Most notably, we could taper PSL and all patients who maintained remission were PSL free at week 48. Additionally, colonoscopy revealed mucosal remission in patients who maintained clinical remission. Overall, compliance was good, and there was no severe adverse side effect in any arm. Transient flushing and lightheadedness were seen in a small number of patients associated with extracorporeal circulation. These observations are in line with the reports in previous studies with GMA in patients with UC [3,5,13-18]. However, the safety profile of the Adacolumn GMA is in sharp contrast to pharmacologics which are often associated with serious adverse side effects that further complicate the ongoing IBD [4,19-21]. Further, a significant fraction of patients in each arm were on concomitant PSL or AZA and this enabled us to assess the contribution of these medications (albeit in small sub-groups) to the efficacy of monthly GMA as maintenance therapy. In reference to patients who had their PSL dose tapered to <20mg/day by the end of the
remission induction therapy with weekly GMA, the number of patients with steroid free remission was significantly in favour of GMA at week 48. This is to say that monthly GMA increased the probability of long-term remission in this population as indicated by the Kaplan-Meier survival analysis. To evaluate the contribution of AZA, we compared the results between patients with and those without concomitant AZA in each of the 3 arms. Unlike the situation with PSL, the Kaplan-Meier survival analysis did not show beneficial effect for concomitant AZA. This is not surprising given that only 6 patients were on AZA at the randomization (in Japan, AZA was officially approved for UC patients in June 2006).

Since the publication of the first clinical trial on GMA in patients with UC [5], a large number of papers, mostly from Japan [15,17,18,22-26], but also from Europe [13,14,16,27-30], and the USA [12,31-33] have reported varying efficacy outcomes ranging from an 85% [24,29] to a statistically insignificant level [12]. Except [5,12,22,29,30,34], all other studies did not include control arms. Both Shimoyama et al. [5] and Hanai et al. [21,34] used PSL in their control arms and in all these 3 studies GMA efficacy was better or equal to PSL. In contrast Maiden et al. [29] used GMA to suppress clinical relapse in one arm, while the control arm received no treatment. At the end of a 6-month follow-up, both the relapse rate and time to clinical relapse were significantly better in the GMA arm. In a randomized, double blind, controlled trial in patients with active UC by Sands and colleagues [12], patients in the control arm received the same volume of extracorporeal circulation without the Adacolumn (sham). In this study, the clinical outcomes between the two arms did not reach statistical significance. Nonetheless, sub-group analysis indicated a significant efficacy for GMA in patients with severe histological evidence of inflammation [12]. However, patients with deep colonic lesions and extensive loss of the mucosal tissue are reported to be very poor responders to GMA [25]. In spite of unmatched efficacy outcomes in the hitherto studies, currently, the clinical application of GMA with the Adacolumn is expanding in the EU and in Japan. One of the most unrivalled features of
GMA with the Adacolumn, which is very much favoured by the patients, is its safety profile; severe adverse side effects are very rare. Even in studies with poor efficacy outcome, its safety profile has been acknowledged [12]. Therefore, it is our intention to continue using GMA as a safe and effective therapeutic option for achieving sustained steroid free clinical remission.

In conclusion, GMA with the Adacolumn as a nondrug based treatment intervention has an excellent safety profile, it is very much favoured by patients and our impression is that the use of therapeutic GMA will expand rather than diminish. This small, but the first prospective randomized sham-controlled trial was to see if monthly GMA has efficacy as maintenance therapy in patients with quiescent for UC with a steroid-refractory background. All 33 UC patients of this study completed the remission induction therapy with GMA and monthly GMA appeared to increase the probability of avoiding relapse in the long term, especially in patients who could reduce their PSL dosage to <20mg/day at the start of maintenance therapy. Therefore, monthly intervention with GMA potentially should prevent UC relapse in patients who have achieved remission by weekly GMA. Additional trials in large cohorts of patients are warranted to substantiate our findings.

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REFERENCES


prospective randomized multicenter study shows very rapid remission of ulcerative colitis by intensive granulocyte and monocyte adsorptive apheresis as compared with routine weekly treatment. Am J Gastroenterol 2009, 104: 2990-2995.


Table 1. Demographic characteristics of the enrolled patients are shown. Data are presented as the mean ± SD values.

<table>
<thead>
<tr>
<th></th>
<th>Number of patients (n=33)</th>
<th>True (n=11)</th>
<th>Sham (n=11)</th>
<th>Control (n=11)</th>
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<td>Gender (Male/Female)</td>
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<td>3/8</td>
<td>7/4</td>
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<tr>
<td>Age (year)</td>
<td>32.6±8.9</td>
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<td>39.4±13.7</td>
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<tr>
<td>Duration of UC (months)</td>
<td>71.2±79.3</td>
<td>180.1±226.9</td>
<td>70.9±75.4</td>
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<tr>
<td>Location of UC (total/left-sided)</td>
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<td>4/ 7</td>
<td>3/ 8</td>
<td></td>
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<td>9/ 2</td>
<td>7/ 4</td>
<td></td>
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<tr>
<td>First onset (Yes/No)</td>
<td>0/ 11</td>
<td>0/ 11</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td>Use of AZA (Yes/ No)</td>
<td>3/ 8</td>
<td>1/ 10</td>
<td>2/ 9</td>
<td></td>
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</tbody>
</table>

At entry for the remission induction therapy with weekly GMA

|                               |                          |             |             |                |
| CAI score                     | 11.7±4.5                 | 9.2±4.3     | 11.5±2.7    |                |
| Dose of PSL (mg/day)          | 34.9±19.5                | 20.5±21.8   | 37.7±21.1   |                |
| CRP (mg/dl)                   | 0.94±1.39                | 0.69±1.28   | 2.32±3.77   |                |
| EI score                      | 8.6±1.1 (n=5)            | 9.7±1.2 (n=3) | 8.7±1.3 (n=6) |                |

At the end of the remission induction therapy with weekly GMA

|                               |                          |             |             |                |
| CAI score                     | 3.5±1.8                  | 3.7±1.2     | 3.5±1.4     |                |
| Dose of PSL (mg/day)          | 11.3±6.8                 | 6.8±6.1     | 13.9±13.3   |                |
| CRP (mg/dl)                   | 0.07±0.10                | 0.44±0.76   | 0.08±0.05   |                |
| EI score                      | 3.7±1.2 (n=3)            | 4.3±1.2 (n=3) | 3.7±1.2 (n=3) |                |

Abbreviations: AZA, azathioprine (0.5-1.0mg/day); GMA, granulocyte/monocyte adsorption; CAI, clinical activity index;^8^ EI, endoscopic index. ^13^ *Steroid refractory was defined as active disease in spite of an optimum dose of PSL for 14 days.*
Figure legends

Figure 1. (A) The study design showing patients treatment, the remission induction therapy course involving 10 once a week GMA session over 10 weeks, then patients were grouped for remission maintenance therapy with 1 GMA session every 4 weeks (True), sham GMA every 4 weeks (Sham) or no additional treatment (Control). Patients who completed a series of weekly GMA therapy were randomly assigned to one of the 3 groups as shown. (B) The blood flow circuit diagrams for both GMA (True) and sham GMA are shown. In the sham GMA, a bypass was added to the standard GMA circuit lines. Patients in the Sham group received the same volume of extracorporeal circulation via the Adacolumn circuit lines, similar to the sham design by Sands, et al. (12). Both patients and the physician were blinded by a curtain.

Figure 2: Treatment of patients and summary of the clinical outcomes.

Figure 3: The overall 1 year survival is shown (n=33). The probability of avoiding relapse (%AR) following a series of 10 weekly GMA tended to be higher in True at the primary end-point compared with the other 2 arms. However, a log-rank test did not reveal statistically significant difference between the True and the other 2 groups (P=0.2641). Also, the Kaplan-Meier survival analysis did not indicate significant difference between True vs Sham (P=0.1297) or Control (P=0.4240).

Figure 4: The %AR in the low (<20mg/day) PSL sub-group is shown. The %AR following the remission induction with weekly GMA was maintained by 57.1% of the patients at the primary end-point. A log-rank test indicated a significantly higher %AR in True vs other 2 groups (P=0.0443). Also, the Kaplan-Meier survival analysis indicated significantly higher prognosis for True vs Sham (P=0.0219) or Control (P=0.0439).
Figure 1

(A) Randomization at the ratio of 1:1:1

Remission induction therapy
One GMA session/week over 10 weeks

One GMA session: 1800ml/session
One sham session: 1800ml/session

Study period

4 weeks 4 weeks

Primary end-point (week 48)

True #1 #2 #12

Sham S S S

Control

Entry period: 14 days

(B) Bypass circuit

Blinding Curtain

*Processed blood volume: 1800ml (30ml/min x 60min) in both Sham and True arms
Anticoagulant: Heparin sodium: 2000U/session

Fukunaga K, et al. Figure 1
Figure 3

Log-Rank test $P=0.0443$

- True
- Control $P=0.0439$
- Sham $P=0.0219$

Patients avoiding relapse (%) vs. Time (day)