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ORIGINAL ARTICLE

No changes in infliximab levels in blood stored for preoperative autologous blood donation

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Abstract Rheumatoid arthritis (RA) patients requiring total joint arthroplasties under administration of infliximab, which may remain in donated blood if preoperative autologous blood donation (PABD) is undertaken for the surgery, may risk infection. We clarified infliximab hemokinetics in blood stored for such patients. A 20-ml blood sample was obtained from each of the ten RA patients receiving infliximab at just after administration and at 2 and 4 weeks following the administration of infliximab, mixed with 2.8 ml citrate-phosphate-dextrose-adenine (CPDA-1) and stored at 4–6°C. Plasma levels of infliximab in the stored blood were measured just after-mixture with CPDA-1, and at 2 and 4 weeks following the start of storage. Serum levels were also measured just before infliximab administration and at each phlebotomy. The plasma infliximab levels in the stored blood remained close to their original serum levels at the time of each corresponding phlebotomy, only somewhat influenced by dilution of CPDA-1, and sustained for 4 weeks following the start of storage, unlike in vivo, where levels decreased. This suggests that in order to prevent side effects, the later after infusion of infliximab the phlebotomy occurs, the better, and that the amount of stored blood transfusion should be consistent with that of blood loss.

Keywords Arthroplasty · Infection · Infliximab · Preoperative autologous blood donation · Rheumatoid arthritis

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Introduction

With the recent wide use of infliximab for rheumatoid arthritis (RA), total joint arthroplasties must sometimes be performed under its administration.

Preoperative autologous blood donation (PABD) is the mainstay perioperative procedure against blood loss for total joint surgery in Japan, so the possibility arises here that infliximab may remain in donated blood. If infliximab levels in stored blood are high, its transfusion could cause adverse agent-related events, including infection. Nevertheless, infliximab hemokinetics in stored blood are not known, while those in serum are well known.

Using a PABD model, the authors measured infliximab level changes in stored blood and previously reported a two-case pilot study: the infliximab levels in the stored blood did not change substantially, remaining almost the same as they were at the time of the phlebotomies [1]. This indicated that both the timing of donation and total amount of blood obtained per phlebotomy may be key for preventing post-surgical infection. However, as the subject number in that study was very small, we reached no firm conclusions.

Against this background, we measured infliximab levels in stored blood, again using the PABD model, to clarify infliximab hemokinetics in stored blood for a larger number of RA subjects.

Table 1 Patients' characteristics ($n = 10$)

	Average ± SD	Range
Age (years)	55.3 ± 12.2	34–70
Disease duration (years)	8.3 ± 5.9	0.9–19
Timing of the trial (sequence number of administration of infliximab)	4.6 ± 1.6	3–7
Dosage of methotrexate (mg/week)	9.3 ± 3.0	4–13
Dosage of prednisolon (mg/day)	5.8 ± 1.4	4–8.75

Materials and methods

The subjects for our study were ten females who met RA classification criteria of the American College of Rheumatology. All were being treated with infliximab (3 mg/kg) in combination with methotrexate, and all were also receiving prednisolone. Methotrexate and prednisolone dosages remained unchanged during the study. The study received Teikyo University School of Medicine ethics committee approval and patients' written consent. Clinical and demographic characteristics are shown in Table 1.

Serum sampling

Serum samples were obtained just before, just after, at 2 and 4 weeks after infliximab administration.

Phlebotomy model

Patients donated 20 ml of blood with the same sterile procedures as in PABD at just after administration and at 2 and 4 weeks following the administration of infliximab; each sample was immediately and gently mixed with 2.8 ml citrate-phosphate-dextrose-adenine (CPDA-1) (Kawasumi Laboratories, Tokyo), and then stored, as in PABD, in a 50-ml polypropylene conical tube (Becton Dickinson, Franklin Lakes, NJ, USA) at 4–6°C in a blood transfusion service refrigerator in our hospital.

Plasma sampling from stored blood

Plasma was sampled from the stored blood at just after-mixture with CPDA-1, and at 2 and 4 weeks following start of storage.

Measurement of infliximab levels

Infliximab levels were measured by enzyme-linked immunosorbent assay, as described by Maini et al. [2] (actual measurements performed by Tanabe R&D service, Osaka, Japan).

Statistical analysis

All numerical data were expressed as mean ± SD. The changes in serum infliximab levels and the corresponding plasma infliximab levels over time in stored blood were evaluated using one-way analysis of variance (one-way ANOVA) for each donation; statistical significance was set at $P < 0.05$.

Results

Serum infliximab levels peaked at just after infusion, and thereafter decreased: 5.5 ± 6.7 μg/ml at just before-, 75.1 ± 21.3 μg/ml at just after-, 17.5 ± 7.4 μg/ml at 2 weeks after-, and 7.1 ± 4.6 μg/ml at 4 weeks after infliximab administration. Plasma infliximab levels in the stored blood, however, remained close to the original serum levels at the time of each phlebotomy, although they did decrease somewhat with CPDA-1 addition. Furthermore, no additional infliximab level changes were observed over time in the stored blood; they were sustained for the full observed 4 weeks following start of storage (Fig. 1). Statistical analysis confirmed that there were no significant differences among the serum infliximab levels and plasma infliximab levels following storage for each donation.

Discussion

Infliximab demonstrates dose-dependent pharmacokinetics in vivo, with an estimated 8–9.5-day elimination half-life at the 3 mg/kg dose [3]. It has been imperfectly understood what serum level of infliximab is minimally required for its clinical response. In an in vitro study, 4 μg/ml of infliximab was reported to be a sufficient level for preventing the biological effects of TNF [4]. In contrast, clinical response in vivo reportedly declines rapidly after serum infliximab drops below 1 mg/l [5]. Consistently, agent-related incidence of infection also varies among investigators. Hanauer [6] reported a 21% infection rate in infliximab versus 11% in placebo. On the other hand, several large clinical trials demonstrated that the risk of serious infection was similar between a group receiving infliximab plus methotrexate and a group receiving methotrexate alone [7–10]. To date, based on these trials, the risks associated with infliximab treatment are generally thought to be comparable to those of methotrexate treatment alone. Still, Lipsky et al. [8] reported in their recent trial that certain adverse

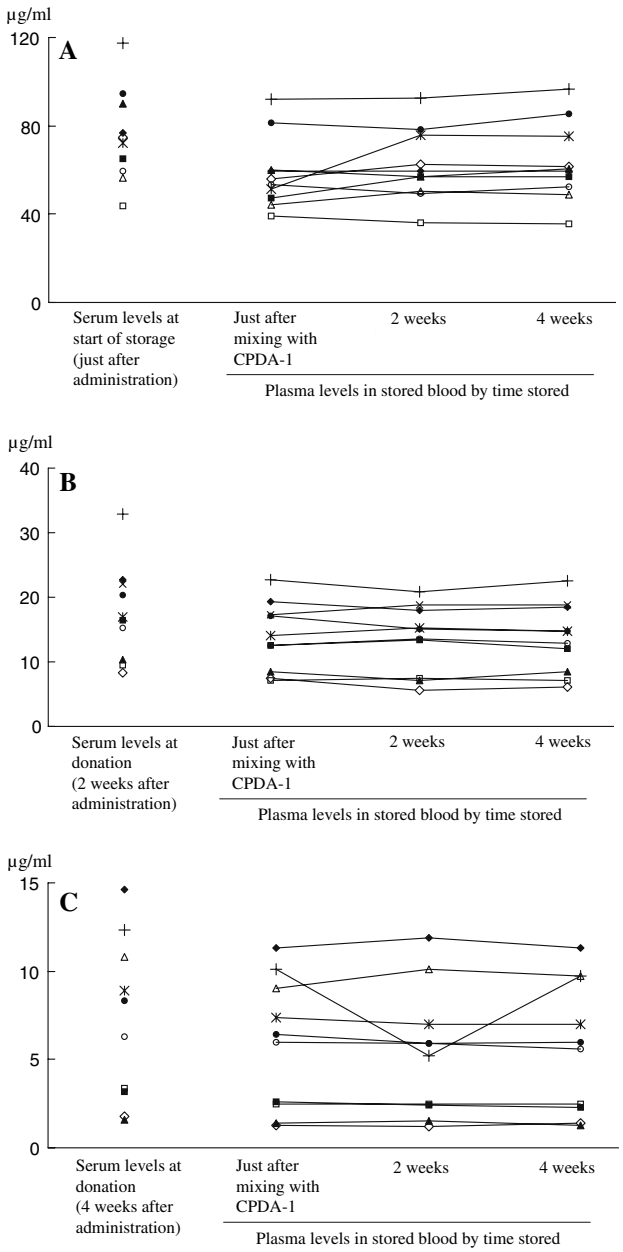

Fig. 1 Individual serum infliximab levels at phlebotomy and changes with time of corresponding plasma infliximab levels in stored blood for ten patients, represented by each *symbol*. Plasma infliximab levels in the stored blood remained close to the serum level for each donation for the full observed 4 weeks following start of storage, although they did decrease somewhat with CPDA-1addition. No other infliximab level changes were observed. There were no statistically significant changes among the serum levels and plasma infliximab levels with time for any phlebotomy. **a** Phlebotomy at just after infliximab administration. **b** Phlebotomy at 2 weeks after infliximab administration. **c** Phlebotomy at 4 weeks after infliximab administration

events including upper respiratory tract infections, sinusitis and pharyngitis tended to occur more frequently in the infliximab plus methotrexate group than in the methotrexate only group, although the difference of total infection rates was not significant. Further, it must be noted that those trials elucidated no data of infectious complications related to surgical events.

From the surgical event standpoint, few studies have addressed risk of infectious complication after orthopedic surgery in infliximab-treated RA patients. No increased risk was found in foot and ankle surgeries [11] or in total joint arthroplasties [12]. A recent retrospective study reported that perioperative continuation of anti-tumor necrosis factor (TNF) therapy does not seem to be an important risk factor for surgical site infection [13]. To the contrary, Giles et al. [14] reported that prescription of TNF inhibitor therapy was significantly associated with the development of a serious post-operative infection. Thus, whether anti-TNF therapy, including infliximab, is related to increased risk of infection during orthopedic surgery may still be unknown. It is, further, important to remember that, to date, no fundamental solution has been developed for infected total joint prosthesis.

Against this background, it would seem reasonable that any total joint surgery should be undertaken as long as possible after the latest infliximab administration, without impacting RA, and that subsequent administration be delayed until after complete surgical wound healing [15]. Still, a question remains about timing of PABD for surgery.

In our standard PABD program, with 50 kg body weight and hemoglobin over 11.0 g/dl, 400 ml of autologous blood would be obtained at each phlebotomy and stored in a sterile bag (Kawasumi Laboratories, Tokyo) including 56 ml CPDA-1 at 4–6°C. A total of 800 ml of blood is usually prepared for a total joint surgery. The current study phlebotomy model was designed on this basis.

Our results showed no substantial infliximab level changes over time in stored blood. They remained almost the same as at time of phlebotomy, influenced only by the dilution of CPDA-1. These results coincide with our previous two-case study [1]. That is, the results did not change even with a larger number of subjects.

These data suggest that the key to determining phlebotomy timing is not only the serum infliximab level at donation but also the total amount of blood to be stored.

For example, with total of 400 ml blood donated in case 10 (61 kg body weight; symbol filled diamond in Fig. 1) at 2 weeks after latest infliximab administration, the donated blood would contain a total of 7.4 mg of infliximab, as average plasma infliximab level in the stored blood donated at that time is approximately 18.6 μg/ml. Consequently, transfusing this blood would cause serum infliximab to rise by approximately 1.6 mg/l, if total circulating plasma volume were 4.7 l. At the same time, the serum level would also be influenced from perioperative blood loss. If 400 ml of blood were lost, it would contain about 1.2 μg/ml or less because of dilution

by fluid transfusion. Thus, finally, temporary elevation of serum infliximab level after PABD may reach 0.4 mg/l or a little more. Thus, it would seem that transfusing the stored blood would not strongly impact the serum level. However, when the phlebotomy timing is closer to the latest infliximab administration, or when the hemorrhage volume is smaller than the total amount of stored blood, we should note that transfusing such stored blood may cause the serum infliximab level to elevate higher than that scenario. Especially, the latter might occur because, usually, all stored blood is transfused back in the general PABD system, even if the hemorrhage volume is smaller than the amount stored. Moreover, serum infliximab levels may be also influenced by hepatic and renal function or dosage of methotrexate. In this manner, under these various situations, the elevated amount alone can easily exceed the 1 mg/l minimum serum infliximab level where clinical response may occur [5].

Recently, Mochizuki et al. [16] measured serum levels of infliximab in RA patients receiving infliximab who underwent total knee arthroplasty with PABD. The results obtained showed that the serum levels did not elevate appreciably after transfusing the stored blood, and that all serum levels measured 1 day after transfusion of stored blood were below 4 μg/ml, a level at which in vitro action of infliximab was reported to be effective. They thus concluded that there is little influence from PABD on the risks of infliximab. However, it is debatable whether the level of 4 μg/ml cited from in vitro data can be applied for clinical cases. Assuming it to be true, moreover, six patients in our study showed that their serum levels were still over 4 μg/ml at 4 weeks after the administration of infliximab. In those cases, transfusion of stored blood containing infliximab, even 400 ml, may trigger infectious complications.

Another point we should note in their study is that no elevation of serum infliximab level was observed at 1 day after the stored blood transfusion. Two reasons may be given for this: (1) elevation of serum level was diminished by the corresponding blood loss, and (2) the serum level had already decreased somewhat through 1 day after the transfusion. The latter reason may be uncertain, because no study has been performed on daily changes of serum infliximab level during that period. However, based on the fact that the serum level has decreased by 20 μg/ml at 24 h following the infliximab administration [17], it seems reasonable to suppose that temporary elevation of serum level by PABD may decrease through 1 day even during the perioperative period as far as the clearance of infliximab is normal.

Because the current study was designed as an in vitro model, changes of the serum infliximab level after stored blood transfusion in clinical cases would need to be substantiated by in vivo study. In other words, their actual changes are thought to be influenced not only by timing of phlebotomy or surgery but also by many other factors as mentioned above: i.e., blood loss volume, fluid, and blood transfusion volumes, hepatic and renal functions, dosage of methotrexate and so on. Consequently, we must carry out further detailed in vivo studies taking these factors into consideration.

Hitherto, there have been no specific quantitative reports on the relationship between serum infliximab level and incidence of adverse reaction. Furthermore, recall that no fundamental solution has been developed for infected total joint prosthesis. Orthopedic surgeons, thus, must take extra care in surgery using PABD in patients treated with infliximab.

In conclusion, infliximab levels in donated blood mirror serum levels at phlebotomy, and do not change during storage. This suggests that in order to prevent adverse effects, the later the better for phlebotomy after infliximab infusion, and also the amount of blood transfusion should be kept consistent with that of blood loss.

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