The Feasibility and Clinical Efficacy of *In Vivo* Adsorption of Isohemagglutinins with Fresh Frozen Plasma (FFP) Infusion in Major ABO-incompatible Allogeneic Stem Cell Transplantation

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Background: We evaluated the efficacy and feasibility of performing prolonged donor type fresh frozen plasma (FFP) infusion for the in vivo adsorption of isohemagglutinins (IHGs) in major ABO-incompatible allogeneic stem cell transplantation.

Methods: Forty-five patients underwent allogeneic stem cell transplantation. Major ABO incompatibility was observed in 23 patients. 18 patients of these 23 patients had IHGs directed towards the donor ABO antigens and they received donor type FFP; in 5 patients, the bone marrow grafts were minor incompatible; in 17 patients, the grafts were compatible.

Results: The engraftment times of the granulocytes and platelets and the transfusion requirements for red blood cells in the FFP-transfused recipients of the major ABO-incompatible allografts were not different from those of therecipients of ABO-compatible allografts ($P>0.1$) and these factors were not different from those for the FFP-treated recipients of the major ABO-incompatible allografts. The median duration of FFP infusion and the number of FFP units were 23.5 days (range 8～39) and 47 units (range 16～78), respectively. The median IgG titers decreased from 1 : 64 to 1 : 4 over a median of 22.5 days (range 8～36) in the FFP-treated groups, compared with a median of 61 days (range 19～116) in the non-FFP-treated groups.

Conclusion: The infusion of donor-type FFP with red cell depletion represents a more feasible, effective alternative strategy to achieve in vivo immunoadsorption of IHGs and to prevent late immunohematologic complications. ***(Korean J Hematol 2005;40:254-260.)***

Key Words: Fresh frozen plasma, Isohemagglutinins, Major ABO incompatible bone marrow transplantation

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INTRODUCTION

Major ABO-incompatible hematopoietic stem cell transplantation (HCT) has the risk of severe hemolytic transfusion reaction. Immediate hemolysis might occur as a result of interaction of donor-derived red cell mass with recipient plasma isohemagglutinins (IHGs) directed against donor RBC antigens. The reaction can be prevented by removal of IHGs from the recipient plasma or in vitro red cell removal from the donor marrow. Depletion of IHGs can be achieved through the use of plasma exchange, ex vivo immunoadsorption of antibody using A or B antigen-containing columns, or in vivo adsorption by infusion of donor-type RBC or fresh frozen plasma (FFP).[1-8] Red blood cells may be removed from donor marrow through density sedimentation or centrifugation using an automated cell separator.[9-14]

Although both methods of RBC depletion and IHGs removal prior to transplantation are very effective in preventing immediate hemolysis, significant immunohematologic complications could be occurred in the post-transplant period in major ABO-mismatched HCT, such as increased red cell transfusion requirement,[10,11,15-19] delayed erythroid engraftment,[12,15] delayed hemolysis,[12,20] or red cell aplasia.[21,22] These risks are associated with post-transplant rise and persistence of high isohemagglutinin titers after BMT, cyclosporine administration for graft-versus-host disease (GVHD) prophylaxis, and in recipients of red cell depleted marrow than in those of performing plasma exchange.[12,21] It suggests that combining the RBC depletion from the marrow with the removal of isohemagglutinin and reducing IHGs for more prolonged periods may be of value to decrease the frequency of delayed complications. Plasma exchange and ex vivo immunoadsorption of IHGs have several drawbacks, including citrate toxicity, the risk of disease transmission, thrombocytopenia, and sometimes followed by rebound of IHGs in the post-transplant period, leading to severe delayed hemolysis in some cases.[4,5,20] Webb et al.[8] reported that infusion of donor type FFP infusion was effective to neutralize IHGs and to prevent adverse consequences of major ABO incompatibility in the setting of T-lymphocyte depleted allogeneic BMT.

We analyzed the effects of prolonged donor type FFP transfusion as an in vivo immunoadsorption of IHGs, irrespective of recipient's pretransplant ABO IHG titers, in the setting of major ABO-mismatched allogeneic stem cell transplantation.

MATERIALS AND METHODS

1. Patients and transplantation procedure

We investigated 45 patients who underwent HST from July 1997 to May 2004 at the Chonnam National University Hospital, retrospectively. All patients received bone marrow or peripheral stem cell transplantation from HLA-identical sibling or unrelated donors. In 23 patients, major ABO-incompatibility was observed, of whom 18 received FFP infusions and five patients received red cell-depleted bone marrow with or without plasma exchange to adsorb IHGs; in 5, bone marrow grafts were minor incompatible; and in 17, the grafts were compatible. Conditioning regimens varied according to the standards. Patients with leukemia, chronic myeloid leukemia or myelodysplastic syndrome were conditioned with cyclophosphamide (60mg/kg/day for 2 days) and total-body irradiation (fractionated 1.5×2Gy/day on four consecutive days) or busulfan (4mg/kg, four times for 4 days). Patients with severe aplastic anemia were conditioned with cyclophosphamide (50mg/kg/day for 4 days) and anti-thymocyte globulin, or cyclophosphamide and total-nodal irradiation. As prophylaxis for graft-versushost disease (GVHD), the patients were given cyclosporine with short-course methotrexate and intravenous immunoglobulins.

2. Prevention of hemolysis

Patients with IHGs directed at donor ABO antigens received donor type FFP 1 unit twice daily to adsorb antibodies from the day after first conditioning infusion till achieving the IHG titer of 1 : 1 or the lowest stable titer, regardless of recipient's pre-transplant ABO IHG titers. Red blood cells of donor marrow were depleted using the Fenwal CS 3,000 continuous flow cell separator. In cases of major and minor ABO mismatch, combined red cell and plasma depletion of marrow was done.

3. Measurement of ABO isohemagglutinins

Anti-A or anti-B isohemagglutinins were determined by incubating test red cells with serial dilutions of the patient's serum. IgM titer was measured by saline agglutination of type-specific cells at room temperature (Chonnam National University Hospital). IgG titer were determined by indirect Coombs test at 37°C after incubation of serum with anti-IgG (Korea University Hospital). IgM or IgG titers were performed twice weekly until the lowest stable titers were achieved.

4. Blood products support

All recipients of major ABO-incompatible marrow received O type leukocyte filtered packed red cell units to maintain a hemoglobin more than 8.0gm/dL and single donor apheresis to maintain a platelet count of 20,000/uL. Blood group O patients were transfused donor blood type platelet and blood group A or B patients received AB type platelet. All blood products were irradiated with 25Gy prior to transfusion.

5. Statistical analysis

Discrete or continuous variables were compared in cases with or without ABO incompatiblity using Fisher's exact test, respectively. $P<0.05$ was considered statistically significant. All calculations were performed using SPSS statistical software.

RESULTS

A total of 45 patients were evaluated and the details concerning ABO group and stem cell transplantation characteristics were given in Table 1.

Table 1. Pre-transplant patient's deomographics

	Major ABO-incompatibility, not FFP-treated, N=5 (%)	Major ABO-incompatibility, FFP-treated, N=18(%)	Minor ABO-Incompatibility, N=5 (%)	No ABO-Incompatibility, N=17 (%)
Age, median (range)	38 (25～43)	34 (16～46)	34 (20～43)	35 (18～44)
Sex Male/Female	1/4	10/8	2/3	12/5
Primary disease				
Acute leukemia	2 (40.0)	12 (66.7)	3 (60)	9 (53)
SAA	2 (40.0)	6 (33.3)	2 (40)	4 (23.5)
CML	0	0	0	2 (11.8)
Others	1 (20.0)	0	0	2 (11.8)
Conditioning regimen				
CyTBI	2 (40.0)	11 (61.1)	2 (40)	11 (64.7)
CyATG	2 (40.0)	6 (33.3)	2 (40)	4 (23.5)
BUCy	1 (20.0)	1 (5.6)	1 (20)	2 (11.8)

Abbreviations: FFP, fresh frozen plasma; SAA, severe aplastic anemia; CML, chronic myelogenous leukemia; CyTBI, cytoxan+total body irradiation; CyATG, cyclophosphamide+antithymocyte globulin; BUCy, busulfan+cytoxan.

Table 2. Clinical outcomes of engraftment and transfusion requirements

	Major ABO-incompatibility, not FFP-treated	Major ABO-incompatibility, FFP-treated	Minor ABO-incompatibility	No ABO-Incompatibility
Number of patients	5	18	5	17
Days to ANC>500/μL Median (range)	15 (14~19)	14.5 (11~21)	15 (13~18)	16 (9~18)
Days to ANC>1,000/μL Median (range)	16 (15~20)	16.5 (13~36)	18 (15~21)	17 (12~30)
Days to Platelets>20,000/μL Median (range)	19 (12~23)	18 (13~30)	19 (16~21)	13 (0~30)
Days to Platelets>50,000/μL Median (range)	22 (20~39)	24 (12~35)	25 (23~28)	22.5 (12~39)
RBC units transfused Median (range)	10 (6~34)	6 (0~18)	5 (2~6)	4 (0~8)
Platelet units transfused Median (range)	11 (4~29)	5 (2~12)	9 (6~10)	6 (3~11)

Table 3. Comparison of ABO-incompatible allografts

	Not FFP-treated (N=5)	FFP-treated (N=18)
Initial IgG titer, Median (range)	1 : 128 (1 : 2~1 : 256)	1 : 64 (1 : 4~1 : 1,024)
Final IgG titer, Median (range)	1 : 8 (1 : 1~1 : 32)	1 : 2 (1 : 1~1 : 64)
Days between initial, final titers Median (range)	61.0 (19~116)	22.5 (8~36)
Number of FFP units transfused Median (range)	N/A*	47 (16~78)
Days of FFP treatment Median (range)	N/A*	23.5 (8~39)
Delayed hemolysis (%)	2 (40)	1 (5.6)

*Not analyzed.

The pretransplant characteristics of each group were similar with respect to age, sex, primary disease, and conditioning regimens.

The engraftment times of granulocyte and platelet, as well as the red cell and platelet transfusion requirements were presented in Table 2. The median follow-up duration was 36.6 months (range, 1.7~82.9). The median days of absolute neutrophil count recovery to $\geq$500/uL and platelet recovery to $\geq$20,000/uL in FFP-treated major ABO-incompatible recipients were 14.5 days, and 18 days, respectively. The engraftment of granulocyte and platelet in FFP-treated major ABO-incompatible recipients were not significantly different from those in ABO-compatible allografts ($P>0.1$). The transfusion requirements of red blood cell and platelet in FFP-treated group were similar to requirements in ABO-compatible allografts. In contrast, there was a trend that FFP-transfused recipients of major ABO-incompatible allografts were less transfused red cell and platelet than not FFP-treated recipients of major ABO-incompatible allografts ($P=0.71$), but there were no differences of engraftment times of granulocyte and platelet between the two groups.

Initial and the lowest IHG titers in FFP-treated recipients of major ABO-incompatible a11ografts were shown in Table 3. The median durations of FFP infusion were 23.5 days (range, 8~39). Median IgG titers decreased from 1 : 64 to 1 : 4 over a median of 22.5 days (range 8~36) in FFP-treated groups, but took over a median of 61 days (range 19~116) in not FFP-treated groups. Similar changes were noted in IgM IHG titers. No

acute hemolysis was observed. Delayed hemolysis occurred in one patient with ABO- and Rh-incompatibility in FFP-treated groups and in two patients with delayed in erythroid engraftment in not-FFP treated groups. Manageable fever and skin rash at FFP infusion developed in 7 patients.

DISCUSSION

Transfusion hemolytic reaction at the time of major ABO-incompatible marrow infusion was prevented by decreasing the titer of patient's isohemagglutinins,[1-6] or by removing the red cells from the marrow product.[9-14] Despite these methods late immunohematologic complications may occur in the post-transplant period, such as increased red cell transfusion requirements, delayed erythropoiesis, delayed hemolysis, and red cell aplasia, especially in recipients of red cell depleted marrow than in those having plasma exchange.[10-12,15-22] Therefore, some investigators recommend combining the removal of IHGs from the recipient with the RBC depletion from donor marrow.[8,9] One approach is the removal of IHG by plasma exchange from recipients before infusion of RBC-depleted marrow.[9] Another approach utilizes RBC depletion and daily infusions of donor-type FFP for in vivo adsorption of IHGs.[8]

In the present study, none had evident hemolysis at the time of marrow infusion. Three patients with major ABO incompatibility experienced a delayed in erythroid engraftment. However, there was no significant differences in time to myeloid and platelet engraftment as well as red cell transfusion requirements between FFP-treated major ABO-incompatible allograft recipients and recipients of ABO-compatible bone marrow. This is consistent with the experience of FFP infusion in the setting of major ABO incompatible T-lymphocyte depleted allogeneic BMT by Webb et al.[8]

Only one patient who received FFP infusion during the pretransplant period experienced overt delayed hemolysis. In this case, hemolysis occurred at a time when IHGs were increased again. This patient was managed with the transfusion of Rh-negative group O red cell and corticosteroid. None of other 17 patients who received continued FFP infusion has a delayed hemolysis or red cell aplasia. This finding supports the role of prolonged FFP infusion for in vivo adsorption of IHGs.[26]

Depletion of IHGs can be achieved through the use of plasma exchange or ex vivo adsorption of antibody using synthetic immunoabsorbed columns.[1-6] These strategies are labor intensive and have several drawbacks. Plasma exchange and ex vivo immunoadsorption require continuous flow cell separator, technical expertise, and large bore apheresis catheterization, and usually carried out daily over 3 to 4 days prior to ABO-incompatible marrow infusion. Extracorporeal circulations carry the risks of citrate toxicity, hypotension, thrombocytopenia, and hemorrhage.[3-5] It is more problematic in the patients with thrombocytopenia, such as severe aplastic anemia, myelodysplastic syndrome, or relapsed acute leukemia. The use of donor-type RBC transfusions with forced alkaline diuresis to in vivo adsorb isohemagglutinins necessitates the use of intensive care unit resources for monitoring and treating potential hemolytic complication.[7]

FFP is known to contain 1 percent A and/or B IHGs, which were agglutinated by recipient antibody.[24] FFP infusion in major ABO incompatibility requires no specialized equipment or training. The safety and effectiveness of ABO-incompatible FFP infusion was confirmed in the previous studies.[8,25] Potential complications associated with FFP infusion include volume overload, febrile transfusion reactions, transfusion-related acute lung injury, and transfusion-related infections. FFP is acellular and cannot transmit cytomegalovirus but cannot be free from the risk of viral transmission. However, the risk of viral transmission may be present in plasma exchange, which also requires the infusion of multiple units of FFP, human albumin, or plasma protein fraction.

In our series, there were no complications associated with FFP infusion.

In the present study, we confirmed that the prolonged infusion of donor-type FFP with red cell depletion might represent a feasible and effective alternative strategy to neutralize IHGs and prevent late immunohematologic consequences of major ABO incompatibility in the setting of allogeneic stem cell transplantation.

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