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CASE REPORT

Successful ABO-incompatible heart transplantation in two infants

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Summary

In the pediatric age group shortage of donor hearts leads to mortality rates of 30–50% on the waiting list. Because of the immaturity of the immune system of infants, ABO-incompatible heart transplantation may be an option to increase donor availability. We transplanted two infants with blood type O at the age of 7 and 5 months, respectively, with complex congenital heart disease. Intraoperative plasma exchange was performed during cardiopulmonary bypass followed by standard immunosuppression. Both recipients received a blood type A donor organ. Plasma was exchanged up to six times until anti-A antibodies were eliminated. No hyperacute rejection occurred, ventricular function is excellent and there have been no acute rejection episodes up to 4 months after transplantation. Anti-A antibody titers remained low and eventually disappeared. ABO-incompatible cardiac transplantation shows good short-term results in young infants and appears to be a safe procedure to reduce mortality on the waiting list.

Introduction

Since September 2004 two infants of <12 months of age were listed in our department for urgent heart transplantation. Both patients had blood group O and isohemagglutinin titers of <1:4 and thus meeting the criteria for ABO-incompatible heart transplantation reported recently by West *et al.* [1].

Patient 1

The first patient was diagnosed with hypoplastic left heart complex with aortic stenosis, ASD and endocardiofibroelastosis at birth. She developed congestive heart failure (CHF) after an attempt for two ventricle repair by interventional cardiology with balloon valvuloplasty of the aortic valve and ASD device closure at the age of 2 months. She developed renal failure and had been on peritoneal dialysis for 2 months at the time of transplant. Having been on the waiting list for 2 months in high urgent status no appropriate donor was identified. Therefore she was listed for ABO-incompatible heart transplantation.

Patient 2

The second patient had restrictive hypertrophic cardiomyopathy with CHF and several periods of medical resuscitation. He was ventilated and on inotropes at the time of referral. After having been on the normal waiting list for 2 weeks he became borderline for mechanical circulatory support and was listed for ABO-incompatible heart transplantation.

We considered ABO-incompatible heart transplant because the Toronto group at the Hospital for Sick Children as well as the Freeman-Hospital in Newcastle had reported on several successful cases [1–3]. It was decided to adopt the Toronto protocol which was kindly provided

by Dr L. West. Informed consent for ABO-incompatible HTx was given by the parents. The chairman of the review commission of the German Medical Council approved the request. Consequently Eurotransplant International Foundation agreed to allocate an ABO-incompatible heart to our recipients provided that no other child in the respective blood group was waiting in a high-urgent status to receive an organ.

Methods

Blood group and isohemagglutinin monitoring

Donor blood groups were provided by the donor hospitals and conveyed by Eurotransplant. Standard reverse blood grouping hemagglutination methods were used to determine preoperative titers of isohemagglutinins (anti-A1/A2 and -B levels). 80 μl of patient plasma diluted with saline in a ratio ranging from 1:1 to 1:512 and 40 μl of a suspension of 3–5% test red cells of blood group A1, A2 or B were incubated at room temperature (21–23 °C) for 1 h. After centrifugation (1500/min, 30 s) agglutination was assessed microscopically. Titers of isohemagglutinins were assessed once per week while patients were on the waiting list. When a potential donor became available the test was repeated immediately. During transplantation it was repeated after plasma exchange. However, the test is too time-consuming to assess the necessity of further plasma exchange while on cardiopulmonary bypass.

Therefore a 'quick spin' was performed to allow for a rapid determination (within 15 min) of isohemagglutinin activity. Patient serum and test red cells of blood group A1, A2 or B was mixed and centrifugation (1500/min, 30 s) was performed immediately without prior incubation. Positive results were graded semiquantitatively from + to ++++.

After surgery the quantitative assay was performed daily for 2 weeks. If no antibody production occurred, subsequent testing was reduced to weekly for 2 months, and then performed monthly [1].

Perioperative management

Preparations of blood products

All red blood cells were from group O blood (recipient blood group) and therefore expressed no A or B antigens. Red cells were cytomegalovirus (CMV) negative, irradiated and only a few days old. All plasma products were from group AB blood thereby not including anti-A or anti-B antibodies. They were also tested CMV negative. Platelets from group AB (or from the blood group of the donor) were used in order to avoid the transfusion of anti-A or anti-B antibodies which would be included in blood group O platelet preparations (Table 1).

Table 1. Blood products during ABO-incompatible heart transplantation.

Donor	Recipient	Plasma	Red cells	Platelets
A	O	AB or A	O	AB or A
B	O	AB or B	O	AB or B
AB	O	AB	O	AB
AB	A	AB	O or A	AB
B	A	AB	O or A	AB
AB	B	AB	O or B	AB
A	B	AB	O or B	AB

Plasma exchange and surgical procedure

Priming of the cardiopulmonary bypass circuit included two red cell units of group O blood, two plasma units of AB blood and 3000 IU of heparin in addition to standard priming solutions. After cannulation a twofold plasma exchange was performed by drainage of 1000 ml of the infant blood during initiation of cardiopulmonary bypass through the venous line. Normovolemia was achieved by synchronous return of the prime volume trough the aortic return line. The infant's blood was separated into plasma and red cell fractions by means of an intraoperative autologous transfusion system (C.A.T.S. Fresenius, Bad Homburg, Germany). The blood was diluted with saline in a 1:2 ratio, washed and centrifuged; diluted plasma was then discarded. A plasma elimination of up to 99% can be achieved with the system [4]. Before retransfusion of the red cells a 'quick spin' (see above) was performed for the detection of reminiscent anti-A or anti-B antibodies.

After the first plasma exchange isohemagglutinins in the circulating blood were determined again ('quick spin') and the procedure was repeated until concentrations of antibody levels were below detection. Orthotopic heart transplantation was performed according to standard surgical techniques. Reperfusion of the transplanted heart was not started until a negative test for isohemagglutinins had been confirmed.

Immunosuppression

Induction therapy consisted of antithymocyte globulin (ATG) 3 mg/kg as a bolus followed by 2 mg/kg/day, adjusted to reach a lymphocyte count of 200–400/μl until sufficient plasma levels of tacrolimus were achieved. Intravenous methylprednisolone (30 mg/kg) was given before release of the aortic cross-clamp, then tapered down to 3 mg/kg/day for 2 days, 2 mg/kg/day for 2 days, 1 mg/kg/day for 2 days and maintained at 0.5 mg/kg/day.

Maintenance immunosuppression consisted of tacrolimus (0.01 mg/kg/day i.v., later 0.1–0.3 mg/kg/day p.o.) aiming at target levels of 10–12 ng/dl and mycophenolate mofetil (20–40 mg/kg/day i.v., later p.o.) aiming at trough

Table 2. Anti-A1/A2 antibody titres of patients 1 and 2.

Patient no.	Recipient blood group	Donor blood group	Preoperative titres		After plasma exchange		First postoperative day		1 week (postoperative)	
			anti-A1	anti-A2	anti-A1	anti-A2	anti-A1	anti-A2	anti-A1	anti-A2
1	O	A1	1:4	1:4	1:1	0	1:1	0	1:1	0
2	O	A1	1:4	0	0	0	0	0	1:1	0

levels of 2–5 ng/dl. Rejection monitoring included daily echocardiography, clinical assessment and cyto-immunological monitoring [5]. Endomyocardial biopsy is not performed in children on a routine basis in our department.

Results

The patients were 7 and 5 months old at the time of transplantation. In both recipients positive antibody titres to A and B antigens were present (Table 2). Anti-A antibody titres were borderline (1:4) according to the inclusion criteria of the Toronto group, possibly because of homologous blood transfusions.

In patient 1 a suitable donor organ of blood group A1 became available 2 weeks after she had been put on the Eurotransplant waiting list for ABO-incompatible transplantation. A sixfold plasma exchange was performed during cardiopulmonary bypass. After the fourth plasma exchange the quick spin showed low level (+) agglutination and anti-A1 levels in the quantitative assay of 1:1. These findings did not change by further exchange runs. Nevertheless, hyperacute rejection did not occur after release of the aortic cross clamp (ischemic time 224 min). Anti-A1 levels have become undetectable since day 7 after transplant until now while an anti-B antibody titre of 1:1 was only detected in one assay 4 weeks after surgery. Although urine production improved after transplant for a few days the patient remained on peritoneal dialysis which had to be switched to intermittent hemodialysis because of abdominal problems of the dialysis catheter. Tacrolimus dosage was reduced to improve kidney function, therefore daclizumab 6 mg i.v. was given after ATG was terminated on day 5. Eventually the girl was extubated on day 15; ventricular function is excellent on echocardiography and no rejection episodes have been detected up to 4 months after transplant. However, for the time being she remains anuric and on peritoneal dialysis.

In patient 2 a suitable donor organ of blood group A1 became available 1 week after ABO-incompatible listing. A fivefold plasma exchange allowed to reduce anti-A1 levels to undetectable levels both in the quick spin and the quantitative assay. Reperfusion (ischemic time 223 min.) and weaning from cardiopulmonary bypass were uneventful. Anti-A1 levels became again detectable on day 3 (titre 1:1) without any impairment of hemodynamic function. The patient was extubated on p.o. day 2 and is doing fine since then. He is free from rejection episodes 16 weeks after transplant although anti-A1 titres remained detectable at 1:1 for 2 months. Three months after transplant they totally disappeared. An anti-B titre of 1:1 was detected for 4 weeks after surgery and then disappeared. Tacrolimus levels have been within the therapeutic range since day 4 after transplant.

Discussion

The ABO compatibility has been considered a prerequisite for successful heart transplantation because the binding of preformed natural antibodies against donor A or B antigens may lead to hyperacute rejection through activation of the complement cascade [6].

However, in recent years ABO incompatible organ transplantation has been successfully performed especially in the field of living related kidney transplantation [7]. In Japan the use of organs from living related donors has often been the only option in transplantation medicine. In this context the technique of preoperative antibody removal by double filtration plasmapheresis (DFPP) with and without splenectomy was evaluated [8] with good long-term survival. The Japanese protocol requires circulating antibodies to be removed below a 1:16 titre before renal transplantation is performed (Ishida, personal communication).

However, in cadaveric organ transplantation of ABO-incompatible grafts the 1 year-survival rate of 64% remains inferior compared with 83% for ABO-compatible grafts [9].

Reports on ABO-incompatible heart transplantation in adults are rare and mostly result from erroneous allocation of donor organs [6]. In a series described by Cooper only one of eight patients survived for more than 1 year; in other case reports urgent retransplantation with ABO-compatible grafts had to be performed [10,11]. Therefore, ABO-incompatible heart transplantation remains a procedure which is considered unsafe in the adult age group [9].

Because of the immaturity of the immune system in small children the outcome is supposed to be more

successful in this age group. In newborns isohemagglutinins cannot be detected until several months after birth [12]. Four years ago West *et al.* [1] published a series of 10 infants, 4 h to 14 months old, who underwent ABO-incompatible heart transplantation at the Hospital of Sick Children, Toronto. Plasma exchange was performed during cardiopulmonary bypass until circulating antibody titres were reduced to undetectable levels. Hyperacute rejection did not occur in any case, overall survival rate was 80%. Two patients died 24 and 29 days after transplant presumably unrelated to ABO-incompatibility. A follow-up study 3 years later revealed donor-specific B-cell tolerance in 13 children aged 2 days to 14 months at the time of ABO-incompatible heart transplantation [2].

Both of our recipients had blood group O which leads to a longer waiting time and mortality on the waiting list. Usually they can only receive a group O donor heart while recipients of blood groups A, B or AB may also be transplanted with a group O donor heart in addition to their respective blood group.

Driven by this lack of donor organs especially in blood group O we started a program of ABO-incompatible heart transplantation in infants. We combined intraoperative plasma exchange during cardiopulmonary bypass with an immunosuppressive protocol representing our standard protocol in infant heart transplantation. In addition to standard agglutination tests to determine anti-A and -B antibodies only the 'quick spin' isohemagglutinin test needed to be established and made available during the transplantation procedure (which usually takes place at night). In the first case an anti-A1 titre of 1:1 remained detectable despite an exchange of the total plasma volume for six times. After reperfusion of the transplanted heart the antibody titre disappeared but returned at a 1:1 titre on day 2. Nevertheless, hyperacute rejection did not occur and delayed rejection was not seen up to 4 months after transplant. Therefore a remaining antibody titre of <1:4 may be acceptable before release of the aortic cross-clamp.

The autotransfusion device in the OR turned out to be safe to remove all circulating isohemagglutinins during the washing procedure. Isohemagglutinin titres were undetectable in all samples that were taken from washed red cells (data not shown). Therefore it appears unnecessary to send the drained blood to the Department of Transfusion Medicine for separation into red cells and plasma.

The critical preoperative isohemagglutinin antibody titre and/or age for ABO-incompatible heart transplantation is still intriguing. West *et al.* [1] successfully transplanted one 14 month old child with a 1:128 anti-A titre while Rao *et al.* [3] did not exceed a maximum titre of 1:8. Their oldest recipient was 21 months old at the time of transplant and survived. Both of our patients had 1:4 anti-A titres and were well below the typical recipient age of less than a year.

In summary the study suggests that isohemagglutinin titres may represent a more sensitive predictor of postoperative outcome than infant age. Postoperatively donor-specific (anti-A1) isohemagglutinins disappeared 7 days and 3 months after transplant, respectively, while anti-B titres disappeared after 4 weeks in both infants. Consequently we cannot prove the development of donor-specific tolerance as the absence of both anti-A and -B isohemagglutinin titres may be due to nonspecific systemic immunosuppression. However, conventional immunosuppression usually does not suppress isohemagglutinin development, and anti-B titres in the serum of A to O infant recipients can remain negative for several months after transplant although the development of tolerance is under evolution [2].

Our early postoperative results reconfirm that ABO-incompatible heart transplantation in the infant age group is safe provided that preoperative isohemagglutinin titres are limited and intraoperative plasma exchange is performed until titres are almost undetectable.

In conclusion we are encouraged to proceed with the program in order to reduce mortality on the waiting list as shown by the Toronto group and others [3,13].

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