Specific Removal of Anti-A and Anti-B Antibodies by Using Modified Dialysis Filters

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Removal of anti-A and anti-B blood group antibodies from human blood has been shown to facilitate cross-matched kidney transplantation by preventing hyperacute rejection. Patients in these studies had anti-A and anti-B antibodies removed by using plasmapheresis, followed by immunoadsorption onto packed bead columns. We conducted a study to assess the feasibility of selectively removing anti-A and anti-B antibodies directly from blood by using modified dialysis filters. An anti-A and anti-B specific antigen was covalently attached to the lumenal surfaces of hollow fibers within selected commercial dialysis modules. The filters were able to reduce the anti-A and anti-B titers of 300 ml of blood to 2 or below. A low molecular weight fraction of our antigen system was found to have no antibody binding capacity. The standard antigen was purified by removal of the low molecular weight fraction and a dialysis filter was modified by using the purified antigen. This filter displayed a six-fold higher capacity than a dialysis filter modified with the same mass of standard antigen. We conclude that selective blood group antibody removal by antigen modified dialysis filters is feasible and may be a simpler system than plasmapheresis followed by immunoadsorption. *ASAIO Journal* 2000; 46: 702–706.

Renal transplantation across ABO blood groups leads to hyperacute rejection of the graft mediated by preexisting host ABO antibodies. In the human kidney, A and B antigens have been found in the vascular endothelium, convoluted distal tubules, and collecting tubules.[1] After ABO incompatible renal transplantation, host anti-A and anti-B antibodies bind to graft A and B antigens and the resulting antigen-antibody complexes activate the complement system.[2] Neutrophils infiltrate the graft and release lytic enzymes, destroying the graft endothelial cells and providing a surface of injured tissue to which platelets can adhere. Massive blood clots form within the graft capillaries and prevent vascularization. This reaction occurs within 24 hours of transplantation.

Relaxing the requirement of donor and recipient ABO compatibility would greatly expand the living donor kidney pool. Based on the frequencies of ABO blood groups in the United States, an estimated 36% of potential living donor-recipient pairs are ABO incompatible.[3,4] Recipients without ABO-compatible living donors must receive a cadaveric kidney. The 3 year graft survival rate for living donor kidney transplant is 81–85%, compared with only 70% for cadaveric kidney transplant.[5]

ABO incompatible transplantation may be facilitated by removing host ABO antibodies before transplant. Techniques used to remove ABO antibodies include plasma exchange combined with intravenous administration of soluble A and B antigens,[6] plasmapheresis followed by plasma immunoadsorption by using packed bead columns[7–9] or red blood cells,[10] and double filtration plasmapheresis (DFPP).[7,8] In a single center study, 52 patients received living donor ABO incompatible kidneys after one or two sessions of DFPP and three or four sessions of column immunoadsorption.[7,8] Both the IgM and IgG anti-A and anti-B hemagglutination titers of each patient were reduced to below 16 before transplant. The authors found a 3 year living donor ABO incompatible graft survival rate of 79%. This graft survival rate was not statistically different from reported living donor ABO compatible 3 year graft survival rates (81–85%). Because the 3 year cadaveric graft survival rate is only 70%, a living donor ABO incompatible kidney transplant performed after removal of host ABO antibodies may be preferable to a cadaveric ABO compatible transplant.

In a single case study, hyperacute rejection after accidental ABO incompatible renal transplant was reversed by using plasmapheresis followed by immunoadsorption with red blood cells.[10] The patient retained normal renal function for at least 20 months after the transplant. Hence, methods for removing anti-A and anti-B antibodies are clearly useful for pretreatment of the recipient to facilitate an ABO incompatible living donor kidney transplant, and for reversal of hyperacute rejection that may occur due to accidental ABO incompatible transplant. Additionally, a recent study has shown that patients receiving a kidney transplant live an average of 10 years longer than patients who remain on dialysis, further emphasizing the importance of novel methods of kidney transplant facilitation.[11]

The methods for ABO antibody removal described above involve initial separation of plasma from whole blood, followed by an additional procedure to remove the anti-A and anti-B antibodies from the plasma. The objective of this study was to assess the feasibility of removing anti-A and anti-B antibodies directly from whole blood by using modified dialysis filters. Selected commercial dialysis modules were modified by covalent attachment of an anti-A and anti-B specific antigen to the lumenal surfaces of the hollow fibers. Initial antibody capture experiments were performed to compare the amount of antibody removed by the antigen modified filters

with that removed by bovine serum albumin (BSA) modified control filters. Capacity of the antigen modified filters was then assessed and improved by purification of the antigen to remove a low molecular weight fraction found to have no antibody binding capacity.

Methods

Dialyzer Modification

Centrysystem 400 HG dialyzers (Cobe Laboratories, Inc., Lakewood, CO), comprised of Hemophan hollow fiber membranes with a molecular weight cut-off of approximately 10,000 Da (GAMBRO Healthcare Renal Care Products, Lakewood, CO, personal communication), were modified based on the procedure developed by Vallar and Rivat[12] Each dialyzer contained approximately 7,742 fibers of 185 mm length and 200 μm inner diameter, providing a blood contacting surface area of 0.9 m^2 and blood compartment priming volume of 47 ml.[13] The blood and dialyzing fluid compartments of each dialyzer were connected in series during all steps except the protein coupling. The dialyzer was first flushed with 2 L of distilled water. Cellulose mercerization (swelling of cellulose under basic conditions) was performed by recirculation of 3.5 L of 0.2 N NaOH at 4°C for 5 hours, after which the dialyzer was rinsed with 3.5 L of 0.1 M, pH 8.5 sodium bicarbonate buffer. An activating solution of 16 g CNBr in 200 ml 0.2 N NaOH was recirculated through the dialyzer at 4°C for 1.5 hours. The pH of the activating solution was maintained above 11.0 by addition of 10 N NaOH. The dialyzer was then flushed with 3.5 L of cold distilled water and 3.5 L of cold pH 8.5 sodium bicarbonate buffer.

During the protein coupling steps, the dialyzing fluid compartment was filled with sodium bicarbonate buffer and closed. A 7 mg/ml protein coupling solution, prepared by using either the anti-A and anti-B specific antigen (Neutr-AB, Dade Behring, Switzerland) or bovine serum albumin (BSA) and sodium bicarbonate buffer, was recirculated through the blood compartment at room temperature overnight. Three successive washings of the modified dialyzer were performed by recirculation of 500 ml of sodium bicarbonate buffer. The mass of protein bound to the modified dialyzer was calculated by subtracting the mass of protein recovered by the washings, the mass of protein in the coupling solution after the coupling step, and the mass of protein in the dialyzing compartment after the coupling step from the mass of protein initially in the coupling solution. The mass of protein in each solution was calculated by using the protein concentration, determined by measuring the absorbance at 265 nm with a UV/VIS spectrometer (Perkin Elmer, Norwalk, CT), and the solution volume. The modified dialyzers were stored at 4°C until use.

Antigen Purification

Aqueous gel permeation chromatography (GPC) revealed that our anti-A and anti-B specific antigen contained two significant protein fractions with molecular weights of approximately 35,000 and 6,000 Da. The components of a 34 mg/ml solution the antigen having molecular weight below approximately 12,000 Da were removed by using Spectra/Por dialysis tubing with a 12,000 molecular weight cut-off (Thomas Scientific, Swedesboro, NJ). The antigenic qualities of the purified

antigen and standard antigen were quantified by measuring the amount of each antigen required to reduce the anti-A and anti-B titers of 1 ml of human plasma from 16 and 16 to 0 and 0, respectively.

A dialyzer was modified by using a 3 mg/ml protein coupling solution of purified antigen in sodium bicarbonate buffer. A capacity experiment was performed as described below, in which the capacity of the purified antigen modified filter was compared with the capacities of antigen and BSA modified filters with approximately the same mass of bound protein.

Blood Preparation

Human type O fresh blood was drawn from healthy consenting donors over age 18 with the approval of the University of Pittsburgh Institutional Review Board. Fresh blood was drawn no more than 2 hours before initiation of the antibody capture experiment. Outdated or overfilled bags of type O banked whole human blood were obtained from the Central Blood Bank of Pittsburgh and stored at 4°C until use. Plasma for blood typing, antibody quantification, and crossmatching was obtained by centrifugation of blood samples. Blood was typed by using standard blood bank procedures.[14] For experiments requiring more than 1 unit of blood, all units were also crossmatched by using standard blood bank procedures.[14] Type A1 and B reagent red blood cells, Coombs control cells, and rabbit anti-human globulin were purchased from Immucor, Inc. (Norcross, GA).

Antibody Quantification

Anti-A and anti-B antibody titers were measured by using plasma serial dilutions and the standard blood bank hemagglutination assay.[14] By using this method, the titer was assumed to reflect the concentration of IgM ABO antibodies only. The titer was taken as the reciprocal of the largest dilution that yielded a positive hemagglutination reaction. A titer of 0 indicates a negative hemagglutination reaction with no dilution of the plasma sample.

In Vitro Capture Loop

In vitro experiments were conducted by using the simple flow loop shown in **Figure 1**. The loop consisted of a blood reservoir, a Masterflex peristaltic pump (Cole-Parmer Instru-
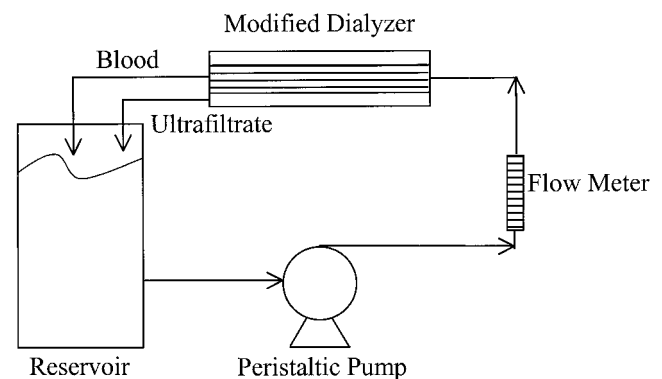

Figure 1. Schematic of the flow loop used for *in vitro* antibody capture experiments.

ment Company, Vernon Hills, IL), a glass bead rotameter (Cole-Parmer Instrument Company) for flow rate measurement, and the modified dialyzer. The components of the loop were connected in series by using tygon tubing. The dialyzer was oriented horizontally as shown in **Figure 1**. Flow was driven through the blood compartment of the dialyzer only. Ultrafiltrate was collected from the dialysate compartment and returned to the reservoir. The entire loop, including both the blood and dialysate compartments of the modified dialyzer, was flushed with 0.9% saline before each experiment. Air bubbles were removed from the system during priming with blood.

Initial Capture Experiments

Our first capture experiments were designed to determine whether anti-A and anti-B antibodies can be selectively removed directly from whole blood by using antigen modified dialyzers. The selectivity of the removal was assessed by comparing the percent reduction of anti-A and anti-B titers effected by the antigen modified dialyzers to that effected by control dialyzers. Each experiment used a modified dialyzer with anti-A and anti-B specific antigen covalently attached to the hollow fiber membranes. Control filters were either unmodified dialyzers, or modified dialyzers with BSA substituted for the anti-A and anti-B specific antigen. Antigen modified and control dialyzers were tested under identical experimental conditions. A 100 ml sample of fresh or banked whole blood was circulated through the dialyzer at 100 ml/min. Aliquots were taken from the reservoir every 15 min and the anti-A and anti-B IgM hemagglutination titers were measured. Each sample was circulated for 30 minutes. Blood samples used with the antigen modified and control dialyzers were from the same donor.

Dialyzer Capacity Experiments

Our next series of experiments was designed to estimate the capacity of dialyzers modified by using the antigen and a purified form of the antigen. Capacity experiments were performed by sequentially processing multiple 100 or 150 ml samples of banked whole blood. Each sample was circulated through the modified dialyzer at a flow rate of 100 ml/min. When the anti-A and anti-B titers of a given blood sample dropped to 2 or below, the entire flow loop was drained, including both the blood and dialysate compartments of the modified dialyzer, and a new blood sample was processed. The dialyzer was not washed between successive blood samples to avoid measurement of an artificially high capacity caused by removal of some of the bound antibodies during the washing. This procedure was repeated until the titer of a newly introduced blood sample was not reduced. The capacity of the modified dialyzer was taken as the volume of blood processed before titer reduction ceased.

Results

Anti-A and anti-B antibodies were effectively removed from 100 ml of whole human blood by a commercial dialysis module modified by covalent attachment of approximately 100 mg of an anti-A and anti-B specific antigen (**Figure 2**). Antibody removal by a BSA modified control filter subjected to
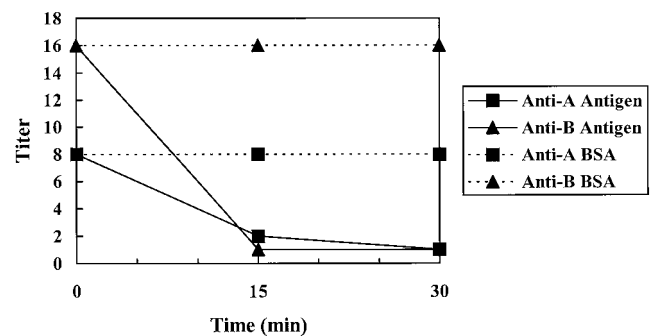
Figure 2. Anti-A and anti-B antibody removal from 100 ml of whole human blood by a commercial dialysis module modified by covalent attachment of approximately 100 mg of an anti-A and anti-B specific antigen.

identical experimental conditions is also shown. After 30 min, the antigen modified filter reduced the anti-A titer from 8 to 1 and the anti-B titer from 16 to 1. The BSA modified filter caused no titer reduction. This experiment was repeated five times, using a different blood donor for each trial. The results of each trial are summarized in **Table 1**. By using a paired t-test, the degree of anti-A and anti-B titer reduction caused by the antigen modified filters was statistically greater than the degree of reduction caused by the control filters ($p < 0.005$).

The percent reductions of the anti-A and anti-B titers of six 100 ml blood samples processed during a capacity experiment are shown in **Figure 3**. A dialyzer modified with approximately 100 mg of anti-A and anti-B specific antigen was able to significantly reduce the anti-A and anti-B titers of 300 to 400 ml of average to high titer type O blood. The anti-A titers of the first three blood samples were reduced from 64 to 1 (98%), 64 to 1 (98%), and 64 to 2 (97%), respectively. The anti-A titer of the fourth sample was reduced from 16 to 4 (75%), and no titer reduction occurred after introduction of the last two samples. The anti-B titers of the first four samples were reduced from 8 to 0 (100%), and those of the last two samples were reduced from 8 to 2 (75%) and 8 to 1 (88%). The BSA modified control filter caused no titer reduction (data not shown).

Figure 4 shows the effect of purification of the antigen before dialyzer modification on the anti-A and anti-B antibody capacities of an antigen modified dialyzer. Purification of the antigen by removal of the low molecular weight nonantigenic protein fraction increased the modified filter capacity by a factor of six. The anti-A and anti-B antibody capacity of a dialyzer modified with approximately 40 mg of purified antigen is compared with the capacity of a filter modified with approximately 40 mg of standard antigen. The purified antigen-modified dialyzer reduced the anti-A and anti-B titers of each of six 150 ml blood samples by 75 to 100%. The standard antigen-modified dialyzer reduced the anti-A and anti-B titers of the first sample by 75 and 88%, respectively, and caused no anti-A or anti-B titer reduction of the other five samples. A control experiment was also performed by using a BSA modified control filter. The BSA modified filter caused no anti-A or anti-B titer reduction in any blood sample (data not shown). The experiment was terminated after the sixth sample due to lack of additional blood to process.

Table 1. Results of Initial Capture Experiments*

Trial	Blood Source	Control Filter	Initial Anti-A Titer	Initial Anti-B Titer	Final Anti-A Titer (AMF)	Final Anti-B Titer (AMF)	Final Anti-A Titer (Control)	Final Anti-B Titer (Control)
1	Fresh Type O	BSA	8	16	1	1	8	16
2	Fresh Type O	BSA	16	8	8	2	16	4
3	Banked Type A	BSA	N/A	8	N/A	0	N/A	4
4	Fresh Type O	unmodified	16	8	2	0	16	4
5	Fresh Type O	unmodified	8	8	4	0	8	8
6	Banked Type O	unmodified	16	16	4	1	16	8

* AMF, antigen modified filter; BSA, bovine serum albumin; N/A, not applicable.

Discussion

Selective removal of anti-A and anti-B antibodies directly from whole blood is achievable by using commercial dialyzers modified by covalent attachment of an anti-A and anti-B specific antigen to the lumenal surfaces of the hollow fiber membranes. Most previously reported methods for selective removal of anti-A and anti-B antibodies from blood involve initial separation of plasma from whole blood, followed by perfusion of the plasma through an immunoadsorption column to remove the antibodies. This procedure is both expensive and complex; hence, a method for ABO antibody removal directly from whole blood may be preferable to removal from plasma. Immunoadsorption columns have been rendered more blood compatible by application of a thin coating of collodion,[15] thus presenting the possibility for the columns to be used directly with whole blood. However, removal of ABO antibodies from whole blood by using a modified commercial dialyzer may be a simpler solution. The modification we have proposed involves only covalent attachment of the antigen to the lumenal surfaces of the hollow fiber membranes, and the operational complexity and blood compatibility of the modified dialyzers would likely be unchanged from that of traditional dialyzers.

A dialyzer modified by attachment of approximately 100 mg of anti-A and anti-B specific antigen was able to significantly reduce (by 75 to 98%) the anti-A and anti-B titers of 300 to 400 ml of average to high titer type O blood. This suggests that at least 10 modified dialyzers would be required to significantly reduce the anti-A and anti-B titers of the entire blood volume of an average adult patient. Clearly the capacity of the standard antigen-modified dialyzers is insufficient. However, purification of the antigen by removal of the low molecular weight, nonantigenic component increased the capacity of the modified dialyzers by a factor of six. A dialyzer modified by attachment of 100 mg of purified antigen may be able to significantly reduce the anti-A and anti-B titers of 1.8 to 2.4 L of average to high titer type O blood. Consequently, only two to three dialyzers modified by using purified antigen may be required to significantly reduce the anti-A and anti-B titers of the entire blood volume of an average person. We will test this prediction as our study continues.

We have assumed that the antibodies are replaced by the patient much more slowly than they are removed and that the patient does not have a large amount of anti-A and anti-B antibodies sequestered outside the bloodstream. In the single center study discussed earlier, patients who received ABO
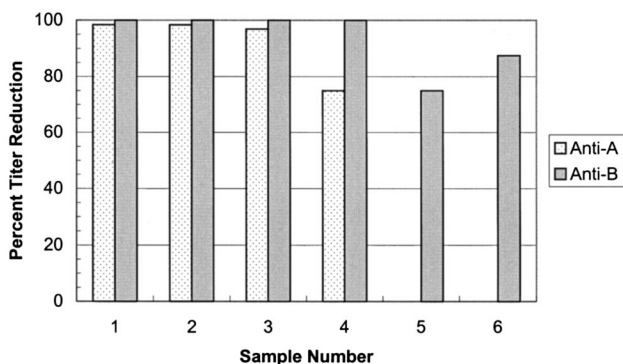

Figure 3. Anti-A and anti-B capacity of a dialysis filter modified with approximately 100 mg of antigen, assessed by sequentially processing 100 ml samples of banked human blood until the titer of newly introduced blood was not reduced. Zero titer reduction is indicated by the absence of a bar.
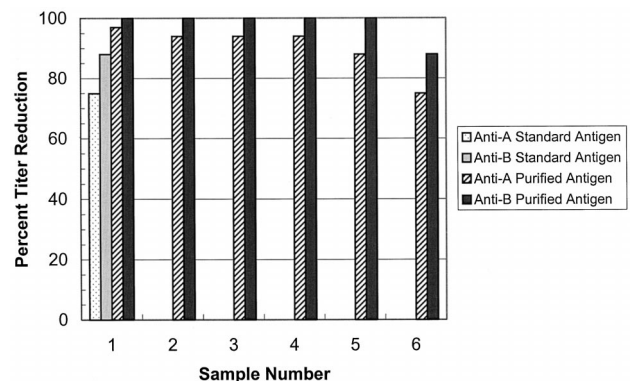

Figure 4. Anti-A and anti-B capacities of two dialysis filters, modified with approximately 40 mg of purified antigen and 40 mg of standard antigen, respectively, assessed by sequentially processing six 150 ml samples of banked human blood. Zero titer reduction is indicated by the absence of a bar.

incompatible kidney transplants underwent one or two sessions of double filtration plasmapheresis and three or four sessions of column immunoadsorption before transplant.[7,8] The ABO antibody titers rose slightly between immunoadsorption sessions, suggesting that either the patient replaced some portion of the removed antibodies or antibodies sequestered outside the bloodstream had reentered. We estimated that only two to three dialyzers modified by using purified antigen may be required to significantly reduce the anti-A and anti-B titers of the entire blood volume of an average person. Because this estimate is based on *in vitro* experiments, which do not account for the above mentioned effects, it is most likely an underestimate.

The capacity of the modified dialyzers may be further increased by more complete purification of the antigen. Ideally, every protein molecule bound to the hollow fiber membranes should be antigenic, thus, maximizing the capacity of the modified dialyzer. In addition, to ensure the maximum number of antigen molecules bound to the dialyzer, each molecule of antigen should possess minimal mass as well as the epitope. This additional mass should be limited to a spacer arm required to place the epitope sufficiently far from the surface of the hollow fiber membrane that antibody binding is possible. Further efforts toward antigen purification and optimization may contribute greatly to increasing modified dialyzer capacity.

An estimated 36% of potential living donor-recipient pairs are ABO incompatible.[3,4] Recipients without ABO compatible living donors must receive a cadaveric kidney. The 3 year graft survival rate for ABO incompatible living donor kidney transplant facilitated by removal of host ABO antibodies (79%)[7,8] is higher than that for cadaveric kidney transplant (70%).[5] Application of this procedure could allow ABO incompatible donor-recipient pairs to proceed with an ABO incompatible transplant, thus giving the recipient the increased chance of graft survival associated with living donor kidney transplants. Additionally, a recent study found that renal transplantation increases life expectancy dramatically compared with long-term dialysis, providing further encouragement to develop novel methods of transplant facilitation.[11]

Acknowledgment

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