Abstract

Objectives: We conducted a desensitization program in our center in patients undergoing kidney transplant for end-stage renal disease. These patients had a living-donor either ABO incompatible and/or human-leukocyte antigen-incompatible. The safety and efficacy of this program were evaluated.

Materials and Methods: A pretransplant desensitization program relies on immunosuppressants and apheresis to remove detrimental antibodies. We chose immunoadsorption as the apheresis technique, and coupled this with hemodialysis in a tandem procedure.

Results: We report on the efficacy of this new method in 120 procedures performed in 20 patients (14 ABO incompatible, 6 ABO incompatible/human leukocyte antigen-incompatible). The tandem procedure was well tolerated, and saved time compared with conducting sequential immunoadsorption and hemodialysis (6 h vs 10 h). The tandem procedure was associated with significantly decreased isoagglutinin titers and donor-specific alloantibodies (assessed by mean fluorescence intensity). Dialysance was effective (183, 102-264). The biochemical and hematologic parameters were similar to those observed after a conventional hemodialysis session, with the exception of protidemia; this might be related to some degree of albumin loss during the immunoadsorption procedure. The posttransplant events included 1) one ABO incompatible/human leukocyte antigen-incompatible patient with vein thrombosis and ultimate kidney loss; 2) two patients with steroid-sensitive cellular acute rejection; and 3) two patients with acute antibody-mediated rejection, which was successfully treated with apheresis and steroid pulses, plus rituximab in one and eculizumab in the other.

Conclusions: We conclude that the tandem immunoadsorption-hemodialysis procedure is efficient at desensitizing patients with end-stage renal disease who are candidates for a living ABO incompatible and/or human leukocyte antigen-incompatible donor-kidney transplant.

Key words: Pretransplant desensitization, Tandem procedure, Dialysance, ABO incompatible kidney transplantation, Nursing time

Introduction

In our kidney transplant center at Toulouse University Hospital, France, we have a large number of patients awaiting kidney transplants (> 500); we can only perform around 180 kidney transplants per year. Three years ago, we implemented a living kidney transplant program, which now accounts for about a third of our kidney transplants a year. However, we often face the problem of ABO incompatibility (ABOi) and/or HLA incompatibility (HLAi). In the latter case, this is due to the presence of preformed donor-specific alloantibodies (DSAs) in the recipient. In our center, DSAs are detected by the Luminex technique.
In 2011, we implemented a pretransplant desensitization program to increase the ability to use ABOi and/or HLAI living kidney donors. Desensitization relies on pretransplant immunosuppression in association with apheresis to remove culprit antibodies: isoagglutinins in the setting of ABOi kidney transplants, and DSAs in the setting of HLAI kidney transplants. The goal of these procedures is to obtain isoagglutinin titers of ≤ 1/8, and DSA titers (expressed as mean fluorescence intensity) of below 3000 at the time of kidney transplant. Although in some cases these goals were not met, we have conducted kidney transplants with the assumption that the pretransplant desensitization would nonetheless show benefit posttransplant.

Because immunoadsorption, which is normally performed on its own, is cumbersome both for the patients and medical staff, we coupled immunoadsorption with hemodialysis in a tandem session. This saves time for the medical and nurse staff and avoids the need for a return visit to the hospital the following day for hemodialysis. Herein, we report on the efficacy and safety of 120 tandem immunoadsorption/hemodialysis sessions.

Materials and Methods

This single-center study included 20 consecutive hemodialysis patients who were referred to our department for ABOi and/or HLAI kidney transplant from a living donor. There were 14 ABOi patients and 6 ABOi/HLAI patients. An ABOi kidney transplant was only considered when the pre-desensitization titer of the specific isoagglutinins was ≤ 1/256. In such cases, the patient could begin the pretransplant desensitization procedure (Figure 1).

In the setting of ABOi kidney transplant, we used specific immunoadsorption (IA) with non-reusable columns able to remove either anti-A isoagglutinins or anti-B isoagglutinins (Glycosorb ABO, Glycorex Transplantation, Lund, Sweden). For HLAI kidney transplants, we used immunoadsorption with reusable semi-specific columns (Immunosorba, Fresenius, Bad Homburg, Germany).

This protocol included a single injection of rituximab (375 mg/m²) at 30 days pretransplant. On the basis of the initial titer of the specific isoagglutinins, specific IA sessions were started at pretransplant days 8 to 10. We scheduled 3 specific IA sessions on alternating days, with titration of the isoagglutinin levels before every IA session. The goal was to achieve a pretransplant titer of ≤ 1/8. In cases when isoagglutinin titers were not decreasing rapidly, we conducted a plasmapheresis session between 2 IA sessions. In some cases, we also performed a few plasmapheresis sessions between pretransplant days 5 to 2; plasmapheresis sessions were never conducted the day before transplant. Other immunosuppressants (tacrolimus [0.2 mg/kg/d], mycophenolate mofetil [1 g b.i.d.], and prednisolone [0.5 mg/kg/d]) were started at 12 days pretransplant. At the same time, we also started Pneumocystis jiroveci prophylaxis using sulfamethoxazole (400 mg) and trimethoprim (80 mg), scheduled for 1 year. During this period, the dosage of recombinant erythropoietin was increased to avoid the need for blood transfusions at pretransplant.

We used a different protocol in the setting of ABOi + HLAI kidney transplant (Figure 2). Intravenous immunoglobulin was given at 1 g/kg during the hemodialysis sessions once, 40 days pretransplant. Patients received 2 doses of rituximab (375 mg/m²) on pretransplant days 30 and 15. Semi-specific IA was started on pretransplant day 17. The number of IA sessions was determined on the basis of the intensity of the pre-sensitization DSA titer; if > 10 000 units of mean fluorescence intensity was measured, we planned at least 9 sessions. If the DSA mean fluorescence intensity was < 10 000, we planned 6 sessions (Figure 2). After every 3 sessions we assessed the DSA mean fluorescence intensity to enable us to plan the next block of IA sessions.

Immunoadsorption was performed in tandem, as previously described. This allowed us to perform the two procedures within half a day of each other. The tandem procedure also allowed us to stop one or the other procedure if necessary. All patients were first connected to the hemodialysis generator and

**Figure 1.** Pretransplant Desensitization Protocol in the Setting of ABO Incompatible Kidney Transplant

**Abbreviations:** d, day; IA, immunoadsorption; MMF, mycophenolate mofetil
then to the immunoadsorption generator. Patients were treated via a large vascular access, either an arteriovenous fistula or a jugular central venous catheter. Before and after each tandem procedure, we assessed the patient’s weight (using a single balance for the whole apheresis unit) and drew blood to enable analyses of the following parameters: Na⁺ (mmol/L), K⁺ (mmol/L), bicarbonates (mmol/L), protidemia (g/L), calcemia (mmol/L), creatinine (µmol/L), hemoglobin (g/dL), platelet count (×10³/mm³), fibrinogen (g/L), and isoagglutinin titers (anti-A and/or anti-B, according to the incompatibility).

**Results**

We have analyzed 120 procedures performed over an 18-month period. There were no technical problems during the procedures; all sessions were completed satisfactorily. We did not observe any allergic reactions. The tandem procedure saved time compared to conducting sequential immunoadsorption and hemodialysis (6h vs 10 h). The average weight loss during these procedures was 3 kg (range, 0.7–4.3). The dialysance was 183 units (range, 102–264) in the hemodialysis sessions.

Table 1 presents the biochemical and hematologic parameters following the tandem procedure. We observed no change at all with regard to natrema; conversely, hyperkalemia decreased from 4.4 (3.4–6.0) to 3.9 (3.1–5.2) mmol/L, and bicarbonates and calcemia increased from 28 (16–37) to 31 (25–35) mmol/L and from 2.0 (1.6–2.45) to 2.23 (1.85–3.0) mmol/L, respectively. We observed an increase in hemoglobin levels from 9.9 (7.8–13) to 10.8 (8.8–14.3), whereas protidemia decreased from 51 (36–79) to 47 (30–67) g/L. There was no change with regard to platelets counts and fibrinogen levels.

At the final follow-up, the patient-survival rate was 100% and graft-survival rate was 90%. One patient presented at day 1 posttransplant with a renal-vein thrombosis, which led to a transplantectomy. A second patient presented on day 2 posttransplant with a renal-vein thrombosis, but this was successfully treated by surgery. Nonetheless, over the following days he presented with delayed graft function, which had hidden an acute humoral rejection. By day 10 posttransplant, he had features of thrombotic microangiopathy (thrombopenia, schistocytic anemia); because of this, he underwent a kidney-allograft biopsy, which indicated typical features of acute humoral/vascular rejection despite the fact that he had an isoagglutinin titer of ≤ 1/4. He was treated with pulses of methylprednisolone ([10 mg/kg] on 3 consecutive days) and daily plasmapheresis (up to 9 sessions). Because these therapies did not improve his condition, he was treated successfully with eculizumab (1200 mg a week for 2 weeks, then 900 mg every 2 weeks for up to 3 months). He progressively regained renal function and serum-creatinine levels stabilized at ~300 µmol/L. However, within the next few months, his renal function slowly deteriorated and hemodialysis had to be resumed at 15 months posttransplant.

Two patients presented with acute T-cell mediated rejection, which occurred on days 10 and 15 posttransplant; they were successfully treated with 3 pulses of methylprednisolone (10 mg/kg each). Their last serum creatinine levels were 160 and 170 µmol/L, respectively. Another patient presented with acute humoral/vascular rejection on day 10 posttransplant, which was treated by daily plasmapheresis (6 sessions in total) plus one injection of rituximab (375 mg/m²) and 3 pulses of methylprednisolone (10 mg/kg each). The last serum creatinine measurement was 160 µmol/L.
Discussion

In this study we have demonstrated that IA and hemodialysis, performed in tandem, are safe and efficient. With regard to safety, all tandem procedures were completed satisfactorily; none needed to be interrupted for any reason. No adverse events were observed. The tandem procedure was much more convenient than the serial procedure: we could stop either the immunoadsorption or hemodialysis without affecting the other, if needed. This gave the medical staff more flexibility. These results are consistent with the findings from other studies which have also reported on the safety and efficiency of plasmapheresis performed in tandem with hemodialysis.\(^2,3,4\)

Weight loss per tandem session averaged 3 kg, although the IA procedure was associated with a mean weight gain of ~1 kg.\(^1\) Thus, before each tandem procedure, we anticipated the weight loss by taking the patient's dry weight into account as well as the weight delivered via the IA procedure.

The mean dialysance was efficient and was within the recommended values, an average of 183. In the setting of tandem procedure, with regard to electrolyte levels, natremia was stable at 141 mmol/L after the procedure and there was a decrease in kalemia from 4.4 to 3.9 mmol/L. We observed a slight increase in bicarbonates and calcemia; this is usually observed in conventional hemodialysis sessions. Because of the good dialysance we obtained, there was a large decrease in both urea (from 18.8 to 6.4 mmol/L) as well as serum creatinine (from 797 to 350 mmol/L). There was an increase in hemoglobin level, from 9.9 to 10.8 g/dL, which reflects the hemo-concentration. There was also a slight decrease in protidemia from 51 to 47 g/L, which may have been related to the loss of some albumin during the IA procedures. However, we cannot be certain of this explanation as albumin levels were not monitored during this study. Coagulation was not impaired as assessed by fibrinogen levels or platelets counts.

In performing an ABOi kidney transplant, the goal is to have an isoagglutinin titer of \(\leq 1/8\) immediately before transplant.\(^5\) Most recipients had only 1 ABO incompatibility, although 2 had both incompatibilities (an AB donor into an O-group recipient).

Isoagglutinin titers (anti-A and anti-B) were assessed before every tandem procedure. The tandem procedure reduced both isoagglutinin titers by 1 dilution; isoagglutinin anti-A was decreased, on average, from 1/32 to 1/16, and isoagglutinin anti-B was decreased, on average, from 1/8 to 1/4. These are values typically seen following an IA session. Because measuring DSA levels (as assessed by Luminex) is very expensive, we did not assess DSAs before and after every procedure.

This desensitization program enabled us to give 20 patients a living-donor kidney transplant. Patients' survival rates at the last follow-up (> 2 years) was excellent, at 100%. There were 2 renal-vein thromboses within the first day posttransplant; 1 led to transplantectomy, whereas, in the second case, the thrombus was retrieved. However, the second patient subsequently developed oliguria and delayed graft function. This condition had initially masked the clinical signs of acute rejection. He eventually developed thrombotic microangiopathy 10 days posttransplant which was related to humoral/vascular rejection. This acute rejection episode responded poorly to pulses of methylprednisolone and daily plasmapheresis. Thus, we had to implement eculizumab therapy. There have been a few reports on humoral/vascular rejection after ABOi kidney transplant that have been resistant to conventional therapy but were responsive to eculizumab therapy.\(^6,7\) Our patient, similarly, had a good clinical and biological response with eculizumab. However, because of sequelae, he continued to have poor renal function and eventually lost his allograft at 15 months posttransplant.

Two other patients (10%) presented with mild, reversible acute-rejection episodes; these were episodes of acute T-cell–mediated rejection. The reported acute cellular rejection rates after ABOi kidney transplant range between 5% to 12%.\(^8,9\) Our series is therefore within the expected rates.

From our study, we conclude that a tandem desensitization procedure of immunoadsorption

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\text{Table 2. Isoagglutinin Titers Before and at the End of the Tandem Procedures} \\
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& \text{Pretandem Procedure} & \text{Posttandem Procedure} \\
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\text{Anti-A} & 1/32 (1-1/128) & 1/16 (1-1/128) \\
\text{Anti-B} & 1/8 (1-1/256) & 1/4 (1-1/256) \\
\text{DSA} & \text{High} & \text{Low to medium} \\
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NB: We assessed both anti-A and anti-B isoagglutinins in every patient, even when ABO incompatibility was directed against a single isoagglutinin.
plus hemodialysis is safe and effective in the treatment of patients undergoing ABOi and/or HLAi kidney transplant for end-stage renal disease. Moreover, hemodialysis parameters, decreased isoagglutinin titers and DSAs indicate that this tandem procedure of immunoadsorption and hemodialysis is more efficient than its sequential counterpart.

References