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ORIGINAL ARTICLE

Single shot of alemtuzumab as induction therapy after kidney transplantation is sufficient

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Summary

In an earlier study, we were able to show that Tac monotherapy following 2 × 20 mg alemtuzumab induction is at least as effective as Tac-based triple-drug immunosuppression in cadaveric renal transplantation. We were interested to learn whether 1 × 30 mg of alemtuzumab is as effective as 2 × 20 mg. Patients of the initial study group (group A) received 20 mg alemtuzumab on days 0 and 2, and tac monotherapy from day 2 on. This group acted as control group for the new arm (group C), where patients were given only 1 × 30 mg alemtuzumab on day 0 followed by Tac monotherapy from day 2 on with the same target levels as in the control group. Frequency of rejection at 6 months was 15% in the control group compared to 6% in the study group and 20% at 12 months in group A versus 6% in group C ($P = 0.034$). Time to rejection was 4.9 months in group A and 0.8 in group C. One-year patient survival was 98.5% in both groups, graft survival 96.9% in group A, and 98.5% in group C. Safety profile was similar in both groups apart from more viral and bacterial infections in group C. Single shot alemtuzumab induction of 30 mg is as effective as 2 × 20 mg in cadaveric renal transplantation.

Introduction

Alemtuzumab is a monoclonal anti-CD52 antibody that produces profound and long-lasting depletion of most immunocompetent cells including T- and B-lymphocytes. It has primarily been used as induction agent in organ transplantation with the aim of allowing steroid-free and calcineurin-sparing maintenance immunosuppression [1].

We have demonstrated in a prospective randomized trial that alemtuzumab induction together with tacrolimus monotherapy is at least as efficient in renal transplantation as is a tacrolimus-based triple-drug regimen with a similar safety profile [2]. Alemtuzumab induction, followed by cyclosporine monotherapy, was first reported by Calne *et al.* [1]. In their series, the antibody was given on day 0 and day 1 at a dosage of 20 mg. Most of the groups that followed similar protocols also used two doses of alemtuzumab on two different days [3–5]. Alemtuzumab is commercially available in vials containing 30 mg. Following Calnes protocol, our patients were given two times 20 mg on day 0 and day 1. Although alemtuzumab with regard to the cost aspect compares very favorably with other lymphocyte-depleting agents, some money could be saved by using one dose of 30 mg instead of two times 20 mg, the more so, as 10 mg of each vial had to be discarded. We therefore decided to answer the question

whether one dose of 30 mg is as efficient as two doses of 20 mg by creating a third arm at a single center with the immunosuppressive protocol being the same as in our previous study group except for the antibody dosage, and to compare the outcome with the original study group.

Patients and methods

Patients

After the amendment of the study protocol had been approved by the institutional review board, recipients of a first cadaveric kidney transplant aged 18–65 years and having given written consent were eligible to participate. Exclusion criteria were the same as in the original study: A positive cross-match against donor cells, more than 25% panel-reactive HLA-antibodies, HIV-positivity of recipient or donor, previous treatment with alemtuzumab, the use of other investigational agents within 6 weeks, autoimmune hemolytic anemia and a history of anaphylaxis following exposure to humanized monoclonal antibodies. Pregnant or breast-feeding women were also excluded as were recipients of a live donor transplant.

Immunosuppressive protocol

A total of 66 consecutive patients of the Innsbruck transplant centre, who met the inclusion criteria between April 2006 and May 2007, were accepted and given 250 mg of methylprednisolone intravenously immediately after completion of surgery, followed 1 h later by 30 mg alemtuzumab intravenously over 6–8 h (study group C). No immunosuppression was given on day 1. Tacrolimus monotherapy was begun on day 2 at a dosage of 0.05 mg/kg bodyweight twice daily, aiming for trough levels of 8–12 ng/ml for the first 6 months. It was tried not to let the tacrolimus level fall below 10 ng/ml. For the following 6 months, targeted trough level was 5–8 ng/ml.

Patients of the original study group (group A) had received 20 mg of alemtuzumab on days 0 and 1 after pretreatment with 250 mg of methylprednisolone. They were given tacrolimus at a dose of 0.05 mg/kg twice daily beginning on day 2. Trough levels of 8–12 ng/ml were aimed for during the first 6 months and 5–8 ng/ml thereafter. Centers were asked to prevent trough levels from falling below 10 ng/ml in the first 3 months. Steroids were given according to the center's standard regimen: at three centers, 500 mg methylprednisolone on day 2, and from day 3 on prednisolone orally with a rapid taper to 25 mg on day 10. At the remaining center, prednisolone at a dosage of 200 mg was prescribed at day 2 and tapered to 20 mg at day 10 and 5 mg at the end of the first year, as were all patients of the control group at the other three centers. In the control group (B), 1–1.5 g mycophenolate mofetil had been given in addition and adjusted on the basis of clinical signs of toxicity.

Biopsy-proven rejections were treated with 3×500 mg of methylprednisolone, steroid-resistant rejections with antilymphocyte preparations.

Infection prophylaxis consisted of trimethoprim–sulfamethoxazole twice daily three times a week for 2 months and oral gancyclovir or valgancyclovir for 3 months in patients with EBV+ and/or CMV+ donors.

The primary endpoint of the study was the proportion of patients with a first histologically confirmed rejection during the first 6 months of transplantation. Rejection was defined as any episode with relevant clinical and laboratory signs and symptoms. All clinically suspected episodes of rejection had to be confirmed by core biopsy. Biopsies were assessed locally and later reread by a single expert. Rejections were classified according to the Banff 97 grading system. For patients who underwent more than one biopsy during a single rejection episode, the highest grade was used for analysis.

Secondary endpoints included biopsy-proven acute rejection episodes during the first post-transplant year, time to first biopsy-proven rejection, patient and graft survival, incidence of corticosteroid rejections, serum creatinine as well as clearance at 1 year and adverse events. Graft loss was defined as the need to resume chronic hemodialysis, for retransplantation, transplant nephrectomy or death. Every change in immunosuppression because of steroid-resistant rejection was considered treatment failure. Creatinine clearance was performed with an enzymatic assay. For safety and tolerability assessment, the overall rate of adverse events, laboratory tests such as hematology, biochemistry and urine analysis as well as vital signs were recorded on days 0, 7, 14, 28 and at month 3, 6 and 12.

Patients with normal total cholesterol and/or triglyceride levels at baseline, but who had more than 200 mg/dl total cholesterol or more than 150 mg/dl triglycerides at month 6 and/or 12 were considered hyperlipidemic.

The necessity to take any oral hypoglycemic medication or insulin for more than 2 weeks between day 15 and the end of the first year was counted as new-onset diabetes.

Statistical analysis

Statistical testing was performed by means of appropriate techniques depending on data distribution (Mann–Whitney *U*-test, *t*-test, Pearson's chi-square test, Fisher's exact test).

The rate of acute rejection at month 6 and month 12 was analyzed with a one-sided chi-square test at a level of 5%. Freedom from rejection and patient and graft survival were analyzed with Kaplan–Meier survival procedures. The analyses were made with SPSS 17 (Chicago, IL, USA).

Results

Demographic data of donors and recipients of all three groups are depicted in Table 1. Patients in group C were better matched for CMV than patients in the other two groups. The difference, however, was statistically not significant. Patients of group A were better matched for HLA-AB and -DR in comparison with patients of group C ($P = 0.004$ and $P = 0.039$, respectively), and group B patients were better matched for HLA-DR than were group C patients ($P = 0.013$). Otherwise, there were no apparent differences among the three groups in any baseline or demographic characteristics.

Rate of rejection, time to rejection/freedom from rejection and histological severity are depicted in Table 2 and Figure 1, respectively. Frequency of biopsy-proven rejection at 6 months was 15% (10/65) in group A and 6% (4/66) in the study group. At 12 months, a total of 13 (20%) rejections were recorded in group A and four (6%) in group C. The difference between group A and C was significant ($P = 0.034$). Time to rejection at 1 year was 4.93 months in group A and 0.79 months in group C. The difference between the control group and the study group was significant ($P = 0.034$). Most rejections were mild (Banff I).

Overall, two grafts were lost in group A and two in the study group: one due to a C4D-positive Banff II rejection

Table 1. Demographic data of donors and recipients.

	Campath Group A	Control Group B	Campath Group C	*P*-value	Significance	*P*-value	Significance
Donor							
Age (years), mean (SD)	50 (13.1)	45 (14.9)	48 (10.7)				
Male, *n* (%)	37 (57)	34 (52)	33 (50)				
CIT (h), mean (SD)	15.7 (4.9)	16.4 (6.1)	14 (5)				
CMV neg, *n* (%)	22 (34)	28 (42)	22 (34)				
Recipient							
Age (years), mean (SD)	50 (10.6)	49 (12.7)	49 (18.4)				
Male, *n* (%)	38 (58)	50 (76)	43 (65)				
Primary disease							
Chronic glomerulonephritis	14	13	16				
Polycystic kidney disease	13	10	14				
Nephrosclerosis	4	4	4				
Interstitial nephritis	6	2	5				
Diabetic nephropathy	2	4	2				
Other	25	30	19				
Unknown	1	3	6				
Dialysis (month), mean (SD)	55 (27.0)	55 (32.7)	44 (32.2)				
CMV mismatch (%)	24 (36.9)	25 (39.1)	7 (21)	A/C: 0.696	No	B/C: 0.665	No
HLA mismatch, mean							
A + B	1.48	1.89	2.03	A/C: 0.004	Yes	B/C: 0.412	No
DR	0.74	0.70	0.97	A/C: 0.039	Yes	B/C: 0.013	Yes

Table 2. Rejection: details.

	Campath Group A	Control Group B	Campath Group C	*P*-value	Significance
Biopsy-proven rejection 6 months	10	19	4		
Median time to rejection	4.9	0.4	0.79		
Biopsy-proven rejection 12 months	13	21	4	A/C: 0.034	Yes
Median time to rejection	4.9	0.4	0.79		
Histopathology					
Borderline	0	3	1 (Mo 1)		
Mild (Banff I)	11	10	1 (Mo 1)		
Moderate (Banff II)	1	7	1 (Mo 1)		
Severe (Banff III)	1	1	0		
C4D positive + Banff II	0	0	1 (Mo 1)		

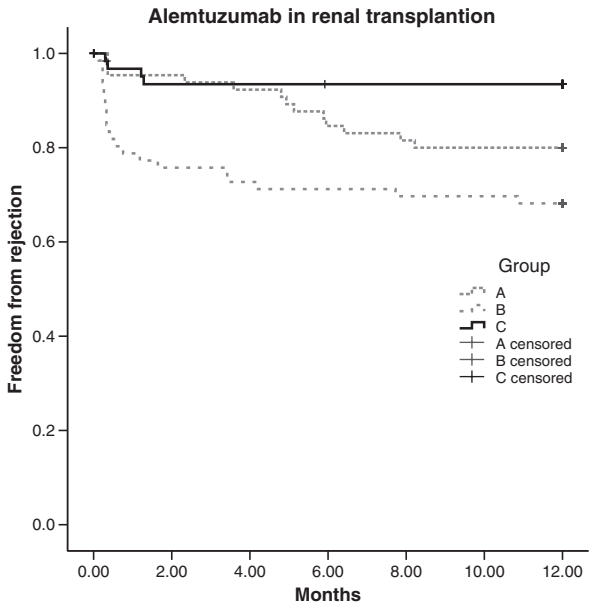

Figure 1 Freedom from rejection.

Table 3. Causes of graft loss.

Graft loss	Campath Group A	Control Group B	Campath Group C
Surgical	0	2	0
Rejection (Banff III)	1	1	0
Death with functioning graft	1	0	1
Recurrent glomerulonephritis	0	2	0
Hemolytic uremic syndrome	0	1	0
Rejection C4D-positive + Banff II	0	0	1
Total	2	6	2

Table 4. Reasons for change in immunosuppression.

Reasons for change in IS	Campath Group A	Control Group B	Campath Group C
Rejection proven/not confirmed	5	3	4/2
Polyoma virus infection	2	1	0
CMV infection	1	1	1
Tacrolimus toxicity	4	2	1
ATN	2	1	0
Diarrhea/vomitus	1/1	2/0	0/0
DM	0	3	0
Leukopenia/anemia	0	3/0	2/1
HUS	0	0	0
Focal seg. GN/mesangioprol. GN	1/0	0	0/1
Proteinuria	1	0	0
Sclerosing peritonitis	0	0	2
Tremor/polyneuropathy	0	1/0	0/1
FK drug fever	0	0	1
Total	19	17	16

Table 5. Complications.

	Campath Group A	Control Group B	Campath Group C
Infections total	Excl CMV-Ag+: 30		Severe: 29
Viral: non-CMV	16	15	21
CMV	18 (no invasive)	8 (3 invasive)	16 (1 invasive)
Bacterial	17	29	58*
Fungal	7	9	10
Cardiovascular	13	14	14
Gastrointestinal	30	30	34
Hematologic	49	48	(92) 75**
Metabolic			
Hyperlipidemia	19	18	5
New-onset DM	2	2	3
Malignancies	0	0	1

*In two patients recidivating UTI with graft reflux and ileum augmented bladder respectively recidivating peritonitis in 2 patients with sclerosing peritonitis after CAPD.
**Excluding recidivating events in the same patient.

and the other one due to patient's death (Table 3). When censored for death (one in group A and one in group C), 1-year graft survival was calculated to be 96.9% in group A, 90.9% and 98.5% in group C. The difference was statistically not different.

One patient had died in each group, which gives a survival of 98.5% for each group. The reason was intracerebral hemorrhage in group A and lymphoma in group C.

Mean serum creatinine concentration at 1 year was 1.58 mg/dl in group A and 1.72 mg/dl in group C. Mean creatinine clearance at 12 months was 61.7 ml/min for group A and 61.2 ml/min for group C.

At 1 year, 46/65 (71%) patients in group A and 50/66 (76%) patients in group C were on tacrolimus monotherapy, whereas 49/65 patients of group B were on their initial immunosuppressive regimen. Acute rejection was the reason to change immunosuppression in five patients in group A and four patients in group C. The other patients were changed because of adverse events or infectious complications (Table 4).

Adverse events are summarized in Table 5. Adverse events in group C were similar to those that were observed in group A patients. In particular, the incidence of CMV infection was about the same. There were, however, more other viral and bacterial infections and hematologic disorders in group C patients as were in the control group, but fewer cases of hyperlipidemia.

Discussion

The usefulness of an immunosuppressive regimen with alemtuzumab induction, followed by tacrolimus monotherapy for maintenance has already been demonstrated

[2–7]. However, it has not been shown that one single shot of 30 mg of the antibody is as effective as two doses of 20 mg, which implies waste of 2 × 10 mg of the drug. This strategy has been applied in two series of patients, reported by Ortiz *et al.* and Tan *et al.* [6,7]. One of them was a retrospective analysis of cadaveric and living donor kidney transplants, and the other a prospective observation study of living donor kidney transplants [6,7].

The shortcoming of this study is that a third group was compared with the study group of an earlier trial [2]. It is probably difficult to finance and conduct a new trial to address this question. It has to be mentioned, however, that all patients of this new arm were treated at one institution and compared with the study arm of the initial trial, of which more than half of the patients were recruited at the same center. Efficacy of this ''low dose'' alemtuzumab regimen is at least as good as in the ''high dose'' alemtuzumab group with a similar safety profile, except for the higher number of viral and bacterial infections observed in the ''low dose'' alemtuzumab arm. One explanation could be that we see our patients frequently and every event is carefully recorded.

Although none of the CMV infections were tissue invasive and none of the infections represented a major clinical problem, the fact remains and is difficult to explain [8]. A higher incidence of CMV infections following alemtuzumab induction has been reported by Walker *et al.* [8]. The authors therefore recommend routine prophylaxis particularly for high-risk patients. It has to be mentioned, however, that other groups did not report a single CMV infection in a large series of living-related transplants [7].

Interestingly, all four rejection episodes in the ''low dose'' group occurred during the first month and none thereafter, which is in contrast to group A where patients received the same immunosuppression apart from the antibody dosage. Patients were similarly depleted of lymphocytes on day 1 and down to 3.08% (2–3.38%) at the end of the first month as compared to 1% in group A patients. At the time of rejection, however, two patients had tacrolimus levels of 1 ng/ml and 7 ng/ml, which are below the targeted trough level of 8–12 ng/ml. A third patient had 8 ng/ml and the remaining patient 12 ng/ml. Low-tacrolimus levels may thus have been causative of rejection at least in three of the four episodes.

We were not able to demonstrate a longer lasting effect of the higher alemtuzumab dosage as lymphocytes were 15% at month 6 in group A and 17% in group C. Interestingly, at the end of the first year, lymphocytes were 34% in group A, but only 15% in group C. Even if the earlier occurrence of rejection might be due to the lower alemtuzumab dosage, which is very unlikely when considering lymphocyte count and tacrolimus level at the time of rejection, it certainly had no impact on graft and patient survival in the long-term. In addition, patients of the low alemtuzumab group were at somewhat higher immunological risk than patients of the high alemtuzumab group due to their worse HLA-matching.

The overall number of rejections was somewhat lower in the ''low dose'' group than in the ''high dose'' group, but was about the same as seen in group A patients of our center (four in 29 patients, 13.8%) only.

Immunosuppression had to be changed in 16 patients of group C, which is comparable to group A. Eleven patients were given steroids in addition to tacrolimus, three patients MMF and four were switched from tacrolimus to cyclosporine A for neurological, hematological and infectious reasons, respectively, and drug fever. Two patients were converted to sirolimus for sclerosing peritonitis. The main reason to change therapy in the study group was rejection in four and clinically suspected but not histologically confirmed rejection in two. Still, 76% of group C patients freedom from rejection were on tacrolimus monotherapy and 83% steroid-free at the end of the first year.

Even if we take into account the nonprospective randomized nature of the study, it seems appropriate to assume that a single shot alemtuzumab induction of 30 mg is as effective as 2 × 20 mg, which makes this regimen even less expensive and more simple [9,10].

Authorship

CB: collection and analysis of data, writing the manuscript. MS: analysis of data. AP: collection of data. JK: collection of data. FM: collection of data. AS: analysis of data. RM: study design, writing of the manuscript.

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