Thymoglobulin as an induction therapy: protection against ischemia and reperfusion injury

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ABSTRACT

Objective: To assess graft function and survival after one year in patients at high risk of acute tubular necrosis (ATN) and to define the impact of this lesion on allograft function in patients who utilized an induction protocol with Thymoglobulin (Thymo).

Methods: Thymo was utilized as an induction strategy. CD3+ cells counting was monitored. Graft function and survival was demonstrated.

Results: Seventy-eight patients who received kidneys from cadaveric donors utilized the referred protocol. Follow-up was 2 years. Cold ischemia time was 19.9 ± 4.8 hours. Thymo total doses was 7.48 ± 3.7. CD3+: 19.3 ± 22.3. Acute rejection occurred in 14.1% of cases, ATN in 66.6% and CMV infection in 55.1%. One-year graft survival non-censored by death was 95.9%, and serum creatinine was 1.56 ± 0.53 mg/dl. Patients who presented ATN had longer length of hospital stay (18.8 ± 6.1 vs. 10.4 ± 4.3 days, p < 0.001) and worse serum creatinine levels (1.66 ± 0.47 vs. 1.39 ± 0.6 mg/dl, p = 0.003). The only one variable related with graft function was donor age (when > 38 years, RR = 3.43, p < 0.001).

Conclusion: Thymoglobulin induction provided an excellent graft survival and a low rate of acute rejection and good graft function until 2 years after transplantation, despite high prevalence of ATN.

Keywords: Kidney tubular necrosis, acute; Ischemia; Reperfusion; Kidney transplantation; Antibodies, monoclonal/therapeutic use

RESUMO

Objetivo: Avaliar a função e a sobrevida do enxerto em pacientes com alto risco para NTA (necrose tubular aguda) e determinar o impacto dessa lesão na função do enxerto renal em pacientes utilizando protocolo de indução com timoglobulina.

Métodos: Timo foi usado com monitoramento da contagem de células CD3+. Avaliaram-se a sobrevida e a função do enxerto ao final de um ano.

Resultados: Setenta e oito pacientes que receberam rim de doador cadáver com o referido protocolo foram avaliados em dois anos de acompanhamento. Tempo de isquemia fria foi de 19,9 ± 4,89 horas. O total de doses de timoglobulina foi 7,48 ± 3,79. Contagem de CD3+: 19,3 ± 22,5. Ocorreu rejeição aguda em 14,1% dos casos, NTA em 66,6% e infecção pelo CMV em 55,1%. A sobrevida do enxerto em um ano foi de 95,9%, com creatinina de 1,56 ± 0,53 mg/dl. Pacientes que apresentaram NTA tiveram maior tempo de hospitalização (18,8 ± 6,1 x 10,4 ± 4,3 dias p = < 0,001) e pior creatinina sérica ao fim de um ano (1,66 ± 0,47 x 1,39 ± 0,6 mg/dl, p = 0,003). A única variável relacionada com a função do enxerto foi a idade do doador (> 38 anos, RR = 3,43 p < 0,001).

Conclusão: A indução com timoglobulina conferiu excelente sobrevida ao enxerto, com baixo índice de rejeição aguda e preservação da função do enxerto até o segundo ano após o transplante, a despeito da elevada prevalência de NTA.

Descritores: Necrose tubular aguda; Isquemia; Reperfusão; Transplante de rim; Anticorpos monoclonais/uso terapêutico
INTRODUCTION

Acute renal insufficiency of ischemic etiology is associated to adverse clinical outcomes, both in native and transplanted kidneys(1). The post-ischemia reperfusion is equally considered deleterious to the renal tissue, in such a way that it is now generally called ischemia and reperfusion injury (IRI)(2). Kidneys for transplant originated from cadaveric donors are kept out of the body for hours, in preservation solutions, and after this ischemic period they are submitted to reperfusion when arterial anastomosis is declamped during surgery(3). Clinical data have shown that this initial injury influences the graft survival and function both in the short- and long run(4-6). The manifestation of IRI after the transplant is delayed graft function, usually associated to acute tubular necrosis (ATN) in histopathological examination. The clinical picture includes oliguria, maintenance of high values of serum creatinine and dependence of dialysis after surgery, which, in most cases, is limited to the first post-transplant week(2).

Ojo et al. showed that ATN increases the risk of acute rejection and is, per se, an independent factor of a poorer renal prognosis(5). This study showed that the ATN has a higher deleterious impact when compared to the number of HLA compatibilities. Besides, the use of drugs that can potentially cause injuries to renal tubules, such as calcineurin inhibitors, can extend the time of ATN, hindering tubular regeneration. Since those drugs play an unquestionable role in the initial immunosuppressant therapy, clinical studies evaluated protocols that enable postponing their introduction in the immunosuppressive regimen(7-9), particularly during the period in which the graft remains without appropriate function.

On the other hand, experimental studies demonstrated that IRI is not limited to exclusively hemodynamic factors. Many studies assessed the participation of immunological factors on the injury pathophysiology, especially on the T-lymphocyte population, but specific mechanisms are still not completely elucidated(10). It is known that knock out mice for CD4/CD8 have a marked protection for the functional effects of IRI(11). Based on this idea, Yokota et al. showed that animals depleted of CD4/CD8 presented better evolution after IRI(12).

Polyclonal antibodies, such as thymoglobulin, have been used to prevent and treat acute rejection, including steroid-resistant rejection(13) and aim to delay the introduction of calcineurin inhibitors(7,9). Studies with non-human primates showed that thymoglobulin can cause quick depletion of T-cells in peripheral lymphoid tissue(7). It was demonstrated that thymoglobulin induces lymphocyte apoptosis and contains antibodies against surface and adhesion molecules(7). This feature could be responsible for attenuating IRI in transplanted kidneys. To this day, few studies showed that T-cell depletion, resulting from immunological induction, can reverse the effects of IRI in renal function in the short- and long- run. In this study, we show function and survival of the renal graft after one year of transplant with kidneys received from cadaveric donors, using thymoglobulin as an immunological induction strategy in a population with high incidence of ATN.

METHODS

This study assessed 78 patients submitted to kidney transplant from cadaveric donors, performed from May 2000 to July 2005, at the Hospital Israelita Albert Einstein. All patients were prospectively followed-up at the Transplant Outpatient’s Clinic. The induction protocol consisted of 1.5 mg/kg of thymoglobulin® during the intraoperative period, as described by Goggins et al.(8) The sequential doses were of 1 mg/kg and administered according to the induction target: CD3+ count less than 20 cells(10).

The initial immunosuppression therapy used mycophenolate mofetil (MMF), 1 g twice a day, and prednisone, 0.5 mg/kg/day. The MMF dose was adjusted according to side effects: leukopenia or gastrointestinal complaints. The triple immunosuppressant therapy was completed with cyclosporine (54%), tacrolimus (36%) or sirolimus (6%), depending on the choice of the local team. Three patients (4%) only used prednisone and MMF. The calcineurin inhibitor was introduced when serum creatinine levels were below 3.0 mg/dl.

Acute tubular necrosis, clinically characterized by a delay in the graft function, was defined by the need of dialysis during the first week after transplant, with subsequent return to function. All acute rejection episodes were confirmed by histology and treated with solumedrol or orthoclone, according to the service indication. Thymoglobulin was discontinued when patients presented graft function. On the other hand, all patients that did not present graft function after the first week were submitted to biopsy and thymoglobulin was administered until reaching the maximum dose: 14 doses in the period between the beginning of the protocol until 2004; 6 doses from then on. This change was based on recently published results obtained by the group, showing that there are no benefits in extending the induction period for longer than six doses(14).

The graft function was assessed by serum creatinine levels and creatinine clearance estimate according to the Cockcroft Gault formula, at hospital discharge, and after 1, 3, 6 and 12 months. All patients were followed up for
at least one year, and the mean follow-up was 24 ± 12 months. The primary endpoint was the graft survival, missing data for death. The graft function and survival after one year according to the presence of ATN were assessed as secondary endpoints. The relevance variables related to the patients and transplant were submitted to statistical analysis to identify the risk factors related to loss of graft function at the end of the first year of follow-up. All patients followed up for one year were included, totaling up 68 patients, who were divided into two groups, according to the estimated clearance: > 50 ml/min (total of 40 patients) and < 50 ml/min (total of 28 patients). For the univariate and multivariate risk analysis, the following parameters were considered: age (> 43 years) and sex (male) of the recipient, donor age (> 38 years), cold ischemia time (CIT > 20 hours), presence of ATN, type of calcineurin inhibitor (using cyclosporine as reference), acute rejection episode, chronic nephropathy of the graft or cytomegalovirus (CMV) infection.

Demographic data were summarized using descriptive statistics and shown by mean ± SD (standard deviation) or by median (minimum-maximum), as indicated. The variables were analyzed by suitable tests: t-test or Mann-Whitney, for continuous variables, and chi-square or Fisher's test for category variables. Graft survival was calculated by the Kaplan-Meier method and the multivariate analysis by the Cox regression method. The value of p < 0.05 was considered for statistical significance. The software used for calculations were SPSS, SigmaStat or SigmaPlot.

**RESULTS**

Demographic data are summarized in table 1. The mean age of recipients was 43.1 ± 14.2 years, and 56.4% were males. Among donors, the mean age was 34.8 ± 13.2 years. Cold ischemia time was 19.9 ± 4.89 hours, and 66.6% of patients presented ATN. The mean number of thymoglobulin doses was 7.48 ± 3.79 doses and the target for CD3+ counting was reached: 19.3 ± 22.5 cell. The patients were hospitalized for 15.6 ± 7.4 days. Eleven patients (14.1%) presented at least one acute rejection episode, and all recovered graft function. Three patients (4.5%) progressed with graft loss and three patients (4.5%) died. One death was caused by cardiovascular disease and the patient maintained preserved graft function. The other two deaths occurred due to *Staphylococcus aureus* infection on the vascular anastomosis. The two latter received the graft from the same donor. No death was attributed to use of thymoglobulin. At the end of the follow-up, at least 15.3% of patients were diagnosed with graft chronic nephropathy by renal biopsy. The indications for biopsy varied according to services. Graft survival after one year, missing data for death, was 95.9% (figure 1A). The mean serum creatinine level was 1.56 ± 0.53 mg/dl, with an estimated clearance of 57.5 ± 18.4 ml/min.

Figure 2 shows the evolution of graft function in up to two-year follow-up. In the first three months there was significant improvement in renal function, and creatinine clearance varied from 23.9 ± 16.1 ml/min in the first month, to 54.6 ± 18.0 ml/min in the third month (p < 0.001). From then on, the renal function remained stable and there were no drops in the values after the first year.

Fifty-two patients (66.6%) developed ATN with a mean dialysis time of 10.7 ± 6.6 days. Comparing patients who developed ATN with those who did not (table 1), it was observed that the cold ischemia time was significantly longer in patients with ATN (21.0 ± 4.5 versus 17.5 ± 4.8 hours, p = 0.002); moreover, they had a longer hospital stay: 18.8 ± 6.1 days versus 10.4 ± 4.3 days (p < 0.001). One year later, the serum creatinine levels of patients with no ATN were 17% better than in others and this difference was significant (1.39 ± 0.6 versus 1.66 ± 0.47 mg/dl, p = 0.003) (Figure 3). Graft survival with missing data for death was also better in the group with immediate renal function, however this difference did not reach statistical significance: 100% versus 93.8% (p = 0.15) (figure 1B).

The most frequent infectious complication was CMV, present in 56.5% of patients, with median diagnosis time of 53 (25-410) days. The count of CMV-infected cells (antigenemia) was, in average, 37 cells (0-1213). Two patients were diagnosed as having CMV by gastric mucosa biopsy, with negative antigenemias. CMV infection was not related to higher risk of acute rejection (RR = 1.14, p = 0.23, 95% CI [0.69-1.88]) neither to graft renal function lower than 50 ml/min at the end of one-year follow-up (RR = 1.20, p = 0.46, 95% CI [0.76-1.87]). The demographic data on patients who developed CMV infection and of those who did not are compared in table 2, and there was no significant difference in the variables tested: recipient age and sex, CIT, ATN, acute rejection, transplant chronic nephropathy and creatinine after one year.

The main variables related to patient and transplant were analyzed, as described, to assess the risk of presenting worse graft function at the end of one-year follow-up. In the univariate analysis, the only variable that interfered in the graft function was donor age: donors aged over 38 years had a relative risk of 3.43 for creatinine clearance lower than 50 ml/min after one year (p < 0.001; 95% CI [1.76-6.66]). When the same variables were submitted to multivariate analysis, only donor age presented risk of worse function: relative risk of 2.33 (p = 0.20 95% CI [1.14-4.77]).
DISCUSSION

In this study we evaluated an immunological induction protocol with thymoglobulin in a population of transplanted patients with kidneys received from cadaveric donors, presenting a high prevalence of ATN, obtaining excellent graft survival, preserving function in up to two-year follow-up. Acute tubular necrosis is an important risk factor for loss of renal graft function in the long run. This effect not only occurs because of increased incidence of acute rejection, but also independently. Today it is known that the T-cell and its adhesion molecules are related to IRI. T-cells were found in human kidneys after ischemic injury and cytokines related to these lymphocytes are significantly expressed. However, specific IRI immunological mechanisms are not clear.

In an IRI experimental model, Rabb et al. demonstrated that mice with low T-cell CD4/CD8 count had intense functional protection against IRI, when compared to controls, and concluded that activated lymphocytes played an important role in the functional evolution of post-reperfusion kidneys. Burne et al. also observed this characteristic of the IRI and went beyond, demonstrating that T-cell deficient mice, when reconstituted with a preparation of clones of these same cell types presented responses similar to the controls. Hammer et al., in turn, proved that IRI increases the adhesion of leukocytes in the microvasculature of muscles using experimental models of cynomolgus primates. In the same study, the use of polyclonal antilymphocyte antibodies prevented adhesion, suggesting that the preparation...
containing antibodies against surface molecules could improve IRI.

The protective effect of T-cell depletion in IRI was assessed in murine models. Yokota et al. (12), using wild-type mice demonstrated that T-cell depletion resulted in protection against renal IRI. Thymoglobulin causes depletion of T-cells, and therefore, could improve IRI in transplanted kidneys. The treatment with thymoglobulin in a non-human primate model resulted in a significant reduction in the lymphocyte population in peripheral blood (22). Furthermore, clinical studies have already shown that T-cell depletion with polyclonal antibodies in transplants of cadaveric organs is related to decreased acute rejection, even when compared to living transplants (7).

In the present study we evaluated 78 cases of renal transplant with kidneys from cadaveric donors, using thymoglobulin as an immunological induction protocol. Graft survival, missing data for death, was 95.9% and comparable to the results of the UNOS (23) and of other protocols using polyclonal antibodies (24). In our protocol we chose to use an intraoperative dose of the antibody, based on a previous publication in which this practice was related to ATN reduction and to graft function improvement in short term (8). In the study mentioned above, the incidence of ATN was 14.8%, with CIT of 15 hours. In our study, with a mean CIT of 20 hours, the ATN prevalence was approximately 67%. Despite this difference, graft function was similar in both studies.

Therefore, we considered the ATN frequency to be very high, although compatible with data found in publications in the national literature (25). In a multicenter study published by Azevedo et al. (26), compiling data from six important Brazilian centers, the mean ATN prevalence was 55.6%, varying between 42.4% and 81.5%. Although it is frequent to find heterogeneity in the classification, the rate found in Brazilian groups is higher than others found in publications from centers abroad (27-28). The reasons for this fact are not totally explained, but one probable explanations would be the high mean CIT. Knowledge that ATN causes a negative impact in the graft evolution has been well established. Shoskes et al. (6), studied 27096 transplants from cadaveric donors and observed that there was an increase in acute rejection and in impact on graft survival secondary to initial ATN. Similar to the results presented here, they showed that donor age also influenced in graft function. Ojo et al. established that ATN is a predicting factor for graft loss within five years, regardless of other variables (5). In that study, the relation between ATN and CIT was also well established, with an increase of 23% in the risk of developing ATN for every 6 hours of CIT. We believe that, in centers that present high CIT means, such as the Brazilian centers, this protocol should be considered as an option to improve the results related to graft survival and function.

The most common infectious complication was CMV (55.1%). Ozaki et al. analyzed a population that used mono and polyclonal antibodies in Brazil, followed it up with sequential antigenemia and found positive results in 80% (29). The prophylactic use of ganciclovir can reduce this complication from 38% to 8%, in patients on thymoglobulin. Our population did not systematically receive ganciclovir as a prophylactic regimen, and these results agree with the national publications. Graft survival and function, as well as patient survival, were not influenced by CMV infection.

The frequency of acute rejection was considered satisfactory. In the publication by Ojo et al. (5) the frequency of acute rejection was 37% in patients with ATN and 20% in those with no ATN. Shoskes et al. (6) reported a frequency of 25% in patients with ATN and 8% in those with no ATN. In the present study, we observed a frequency of 16.9% in the group who developed ATN and 8% in the group who did not, with a mean of 14.1%. We relate this reduced rate, especially among patients with ATN, to the use of thymoglobulin.

Another important feature of this protocol is the delay in introducing calcineurin inhibitor. Silva et al. (25)
studied a population that received the organ with a mean CIT of 20 hours and an incidence of delayed graft function (classified in the study as “slow graft function” and “delayed graft function”) between 15% and 60%. Their protocol did not use induction and there was immediate introduction of calcineurin inhibitor. Graft survival after one year was 78.4% and acute rejection occurred in 22% of patients with ATN (delayed graft function). Using an induction protocol, introducing calcineurin inhibitor after improvement of ATN, we reached a survival rate of 95.9% (when considering survival with no missing data for death, the rate was 91%) and acute rejection occurred in 14.1% of cases, as mentioned before.

CONCLUSION

Therefore, we demonstrated that the induction protocol with thymoglobulin gave an excellent graft survival in patients receiving kidneys from cadaveric donors, comparable to studies with shorter CIT and lower prevalence of ATN than ours. We also demonstrated that graft function evolves with progressive improvement up to one year, and remains stable up to the second year of follow-up. The acute rejection rate was satisfactory. We concluded that thymoglobulin, with appropriate lymphocyte depletion, measured by CD3+ cells, can reverse the effects of IRI in transplanted kidneys.

REFERENCES