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Glucocorticoid resistance in dialysis patients reduces long-term graft survival after kidney transplantation

Gustavo Frezza, Leandro Machado Colli, Sergio R. De Antonio, Margaret De Castro *

Department of Internal Medicine, Ribeirao Preto School of Medicine, University of Sao Paulo, Brazil

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ABSTRACT

Glucocorticoid (GC) resistance has been observed in chronic kidney disease (CKD) patients on dialysis. It can be evaluated by binding assays based on the dissociation constant (Kd), which is inversely proportional to ligand affinity. CKD patients with GC resistance had increased number of acute rejection episodes. We followed up 26 patients that underwent kidney transplantation to observe whether GC resistance could affect the response to acute rejection episode pulse therapy and the long-term allograft outcome. Using Kaplan–Meier survival curve, GC resistant patients showed lower acute rejection-free survival ($p = 0.03$) and lower kidney allograft survival ($p = 0.008$). No difference was found regarding number of deaths. Multivariate logistic regression showed that high Kd value was an independent predictor of lower kidney allograft survival ($p = 0.001$). There was a negative Spearman correlation between Kd and kidney allograft survival ($r = -0.88$, $p = 0.03$). In conclusion, our findings indicate the usefulness of binding assay performed previously to kidney transplantation to define GC resistance. In addition, the dissociation constant (Kd) is a reliable and independent predictive marker of higher frequency of acute rejection episodes, lower rejection-free graft survival, poor response of acute rejection episodes to methylprednisolone pulse therapy, and lower kidney allograft survival in a long-term follow-up.

1. Introduction

Glucocorticoids (GCs) are essential in the maintenance of homeostasis and enable the organism to respond to both physical and emotional stresses. GCs interact with the cytoplasmic glucocorticoid receptor (GR), which is a member of the nuclear receptor superfamily. An appropriated response to GCs depends on the ability of the cell to receive and transduce the hormonal signal [1]. The presence of GR in an adequate number and an efficient response of receptor-mediated signal transduction are necessary for proper GC action [2].

GCs have been a mainstay of maintenance immunosuppressive regimen since the beginning of kidney transplantation. Although there are many attempts of GC withdrawal or avoidance, they are still used, particularly in patients at high immunological risk, and are the first line treatment of acute rejection episodes [3]. The occurrence of chronic allograft nephropathy is the leading cause of transplant failure in long-term follow-up and remains elevated and without adequate treatment [3]. Moreover, despite the use of GCs, some patients still have acute rejection episodes requiring specific treatment. Approximately 30% of these patients have no or an inadequate response to GC therapy alone and require therapy with anti-thymocyte globulin [4]. Of note, steroid resistant patients may show worse evolution of long-term kidney graft [5].

GC resistance has been extensively studied in patients with idiopathic nephrotic syndrome [6], asthma [7], rheumatoid arthritis [8], familial cortisol resistance [9], chronic kidney disease [10], and also in normal subjects [11]. Chronic kidney disease (CKD) has been described as a chronic systemic inflammatory disease [12] and the activation of the inflammatory response can lead to GC resistance. Several mechanisms, such as reduced renal clearance of proinflammatory mediators (tumor necrosis factor alpha and interleukin 6), accumulation of advanced glycoxidation end products, production of reactive oxygen species, oxidative damage, and chronic infection are likely to contribute to the activation of inflammatory response [13].

GC resistance in peripheral blood mononuclear cells (PBMC) of CKD patients undergoing dialysis and awaiting renal transplantation has been observed in several studies [10,14,15]. Binding assays have been used to evaluate GC resistance, based on the number of receptor sites per cell (Bmax) and the dissociation constant (Kd), which is inversely proportional to ligand affinity [14,15]. We previously demonstrated

Abbreviations: Bmax, number of receptor sites per cell; CKD, chronic kidney disease; DEX, dexamethasone; GR, glucocorticoid receptor; GCs, glucocorticoids; HLA, human leukocytes antigen; Kd, dissociation constant; PRA, panel-reactive antibodies; PBMC, peripheral blood mononuclear cells.

* Corresponding author at: Department of Internal Medicine, Ribeirao Preto School of Medicine, University of Sao Paulo, Av. Bandeirantes 3900, Ribeirao Preto, SP 14049-900, Brazil. Tel.: +55 16 3602 2940; fax: +55 16 3633 6695.
E-mail address: castrom@fmrp.usp.br (M. De Castro).

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that patients undergoing dialysis had increased number and decreased affinity of GR to dexamethasone (DEX) compared to healthy subjects. In addition, GC resistance negatively influenced kidney transplant short-term outcome with increased number of acute rejection episodes [10]. There are lacking studies that attempt to correlate GC resistance to long-term outcome after kidney transplantation. Therefore, in the present study, we followed up patients that underwent kidney transplantation for up to 10 years to observe whether GC resistance could also affect the long-term allograft outcome. We also assessed the response to acute rejection episode treatment in patients with different sensitivities to GCs.

2. Material and methods

2.1. Subjects

This retrospective study was approved by the Institutional Review Board for Human Research at the Clinical Hospital of the Ribeirao Preto School of Medicine — University of Sao Paulo (Proc. HCRP no. 4522/2003) and written informed consent was obtained from all subjects.

2.2. Binding assay

In a previous study, DEX-binding assay was performed in blood samples from CKD patients before receiving induction immunosuppressive therapy to renal transplantation [10]. Receptor sites per cell (Bmax), indicating GR-binding capacity, and the dissociation constant (Kd), indicating GR binding-affinity, were calculated by the Scatchard method. The Kd and Bmax upper limit 95% CI of mean of 40 healthy subjects were 9.1 nmol/L and 7.9 fmol/mg of protein, respectively [11]. Patients presenting Kd higher than 9.1 nmol/L value were classified as GC resistant. The mean ± standard error of the mean of Bmax and Kd in PBMC were higher in CKD patients compared to healthy controls (7.7 ± 2.3 and 4.1 ± 0.3 fmol/mg of protein; $p = 0.001$; and 12.8 ± 3.1 and 7.2 ± 0.9 nmol/L; $p = 0.02$, respectively).

2.3. Long-term allograft outcome

We have previously evaluated CKD patients in a short-term allograft outcome (18 months after transplantation) [10]. In the present study, all patients were followed up for up to 10 years and the occurrence of acute rejection, allograft loss by any cause, and death was determined. Allograft failure was defined by the need for long-term dialysis after transplantation or death. Biopsies were performed under clinical indication based on biological marker abnormalities of graft function (serum creatinine, urinary sediment, and Cockroft and Gault estimated creatinine clearance). The biopsies were blinded to the pathologist, and acute rejection episodes were biopsy proven diagnosed according to Banff criteria [16].

The first line treatment for acute rejection was GC pulses with methylprednisolone at 500 mg/day for 3 days. In the present study, the efficacy of this regimen was evaluated by the renal function recovery.

2.4. Data analysis and statistics

All results are expressed as the mean ± standard error of the mean and percentiles, when appropriate. Statistics were carried out using the nonparametric tests. Receptor assay data were analyzed by the method of Scatchard using computerized linear regression analysis. Uni- and multivariate logistic regressions were performed to exclude confounding factors. Survivorship curves were evaluated using the Kaplan–Meier technique and were compared using the Wilcoxon test. We used R software for statistical analysis. Significance was assumed when p is <0.05.

3. Results

3.1. Subjects

This study included 28 CKD patients undergoing dialysis (19 males and 9 females, ranging in age from 18 to 64 years), which short-term outcome has been previously published [10]. Exclusion criteria were previous use of immunomodulatory drugs, including GCs, for at least 3 months before the study and patients who would be submitted to kidney transplantation after 2200 h, due to technical difficulties. Two patients were excluded, one due to an acute vascular complication during the surgical procedure and another by no treatment compliance. In a previous study, the Kd was evaluated in 40 healthy subjects by binding assays [11]. Based on the Kd value upper 95% confidence interval (CI) of mean of normal subjects, patients were divided into two groups, GC sensitive and GC resistant (Table 1).

There were no differences regarding sex, age, race of recipient, and duration of dialysis between GC sensitive and GC resistant groups [10]. In addition, no difference was observed on induction, initial or long-term maintenance immunosuppressive therapies [10]. Twenty three out of 26 patients received a kidney transplant from deceased donors. Table 1 shows the causes of native kidney disease, kidney cold ischemia time, number of human leukocytes antigen (HLA) mismatches, and the serum levels of panel-reactive antibodies (PRA) of sensitive and resistant patients with chronic kidney disease (CKD) at the time of transplantation, which were also not different between groups. After short-term follow-up (18 months), Kd was an independent predictor of acute rejection in a multivariate logistic regression when analyzed with PRA >30, cold ischemia time >24 h, acute tubular necrosis, and the number of HLA mismatches >3. Indeed, there was a higher incidence of acute rejection episodes (55.6% vs 12.5%; $p = 0.03$) in CKD GC-resistant patients compared to CKD GC-sensitive patients [10]. However, allograft failure and number of deaths at 18 months of follow-up were not significantly different between both groups.

3.2. Long-term kidney allograft outcome

After renal transplantation, all patients were followed up for 10 years or until allograft lost or death, and the occurrences of acute rejection episodes were determined. At the end of 10 years, using Kaplan–Meier survival curve of cumulative risk, acute rejection-free survival was lower ($p = 0.03$) in GC resistant patients (Fig. 1A). GC resistant patients had a lower kidney allograft survival ($p = 0.008$) compared to GC sensitive patients (Fig. 1B). In addition, there is a negative correlation between Kd and kidney allograft survival ($r = -0.48$, $p = 0.01$; Fig. 2A). No difference was found regarding number of deaths ($p = 0.30$).

Multivariate logistic regression showed that high Kd value was an independent predictor of a lower kidney allograft survival ($p = 0.001$) when analyzed with age, presence of diabetes, immunosuppressive therapy, acute rejection episodes, the number of HLA mismatches, and cold ischemia time. Diabetic patients and patients with increased number of mismatches also negatively influenced the kidney allograft survival ($p = 0.04$). The presence of an acute rejection episode and higher cold ischemia time showed a trend ($p = 0.1$ and $p = 0.06$, respectively) of being an independent predictor of lower kidney allograft survival.

Six patients had a biopsy-proven acute rejection episode throughout the study; five of them were GC resistant. All six patients were treated with methylprednisolone. Four patients showed improvement of creatinine levels and were maintained without dialysis. Two patients did not recover kidney function despite treatment and required dialysis. Higher Kd before kidney transplantation predicted poorer response to methylprednisolone pulse therapy regarding graft survival after acute rejection episodes. Indeed, after methylprednisolone pulse therapy, patients presenting Kd values above 20 nM lost their graft in less than 2 years after transplantation. On the other hand, patients with normal or slightly elevated Kd maintained their graft for a long period (5 to 10 years). Fig. 2 shows the Spearman correlation coefficient between Kd and kidney allograft survival. There were a negative correlation in all CKD patients ($r = -0.48$, $p = 0.01$; Fig. 2A) and in patients that presented acute rejection episodes ($r = -0.88$, $p = 0.03$; Fig. 2B).

4. Discussion

In this study, we observed that in a long-term outcome after kidney transplantation, GC resistant patients showed higher incidence of acute rejection episodes, lower acute rejection-free survival, poor response of acute rejection treatment, as well as higher incidence of renal allograft loss, although no difference was found regarding patient mortality.

Lymphocyte resistance to GC has been described in several clinical conditions, including the CKD patients awaiting renal transplantation [6–8,10]. We previously demonstrated by DEX binding studies in PBMC of CKD patients undergoing dialysis an increased number and a decreased affinity of GR compared to healthy subjects [10]. The Kd was of clinical significance to predict GC resistance in CKD patients. In addition, high Kd was a reliable and independent predictive index for acute rejection episodes and also for chronic allograft nephropathy leading to a poor 18 months short-term outcome after renal transplantation without difference in kidney allograft survival [10]. It is important