Clinical Biochemistry 43 (2010) 559–565

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Effect of different calcineurin inhibitors on AOPP and TAS after kidney transplantation

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ARTICLE INFO

Article history:
Received 2 November 2009
Received in revised form 30 December 2009
Accepted 7 January 2010
Available online 18 January 2010

Keywords:
Renal transplantation
Cardiovascular mortality
Oxidative stress
Advanced oxidation protein products
Total antioxidant status
Cyclosporine A
Tacrolimus

ABSTRACT

Objectives: Little is known about the influence of calcineurin inhibitors on advanced oxidation protein products (AOPP) and total antioxidant status (TAS) after renal transplantation.

Design and methods: AOPP and TAS were evaluated in transplanted patients on different calcineurin inhibitors. Thirty-five patients were treated with cyclosporine A (group A) and 33 with tacrolimus (group B).

Results: Over 6 months, the mean levels of AOPP in group A decreased from 205.9 ± 125.7 to 140.9 ± 78.9 μmol/L and TAS from 1.89 ± 0.30 to 1.75 ± 0.27 mmol/L. In group B, the mean levels of AOPP decreased from 196.5 ± 123.9 to 129.6 ± 63.8 μmol/L and TAS from 1.80 ± 0.39 to 1.78 ± 0.23 mmol/L.

Conclusion: No significant differences in AOPP and TAS were found with respect to treatment. The only exception was the higher mean concentration of AOPP at month 1 in group A ($p = 0.026$).

Introduction

It is well known that enhanced atherosclerosis and subsequent cardiovascular complications are the leading cause of death in patients with chronic kidney disease (CKD). Recent findings have confirmed that oxidative stress (OS) is a potentially important mechanism of atherosclerosis and associated morbidity and mortality not only in patients with advanced chronic kidney disease but also in earlier stages of kidney disease with a mild decrease in glomerular filtration rate (CKD stages 1–2). One possible explanation of this could be the observation that the early stages of kidney disease with subnormal glomerular filtration are characterized by enhanced activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and other prooxidant enzymes and decreased activity of superoxide dismutases (SODs) and other antioxidant enzymes [1].

In renal transplant recipients, 50% to 60% of deaths are directly attributable to cardiovascular disease [2]. Besides common risk factors for coronary heart disease such as hypertension, diabetes mellitus, increasing patient age, elevated serum cholesterol, and cigarette smoking, a number of other factors have been found to correlate with cardiovascular complications of kidney transplantation [3]. Potential risk factors contributing to accelerated atherogenesis include hyperhomocysteinemia, hyperfibrinogenemia, elevated levels of lipoprotein(a), modification of low-density lipoproteins (LDL), elevated C-reactive protein levels, and decreased graft function [4].

In recent years, substantial attention has been paid to the possible role of OS as a nontraditional risk factor for high cardiovascular morbidity and mortality among transplant recipients [5]. Oxidative stress can be defined as an imbalance between the formation of reactive oxygen species (ROS) and antioxidant defense capacity [1,6]. The predominance of ROS over the cellular antioxidant defense results in the oxidative damage of cellular proteins, lipids, and nucleic acids; tissue damage; and organ dysfunction [7]. Oxidative stress is one of the main factors for enhanced atherosclerosis and is frequently observed in patients with end-stage renal disease. Although restoration of renal function after successful transplantation resolves many metabolic abnormalities associated with uremia, the effects of kidney transplantation on OS are incompletely understood.

Advanced oxidation protein products (AOPP) are protein biomarkers for oxidative stress in the uremic milieu and were first described by Witko-Sarsat et al. in 1996 [8]. Plasma proteins are generally extremely susceptible to oxidative stress. The levels of AOPP are elevated in patients with chronic kidney disease in comparison with healthy subjects. Plasma concentrations of AOPP increase with the progression of chronic renal failure and are closely related to

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0009-9120/$ – see front matter
doi:10.1016/j.clinbiochem.2010.01.003

advanced glycation end products (AGEs) [9]. Advanced oxidation protein products are able to trigger the synthesis of inflammatory cytokines in neutrophil leukocytes and monocytes and seem to act as inflammatory mediators in CKD patients [10]. A study in a remnant kidney animal model confirmed that the administration of AOPP upregulates the expression of the proinflammatory chemokine MCP-1 and fibrogenic growth factor TGF-β1 and results in a marked increase in interstitial fibrosis, glomerulosclerosis, and proteinuria, as well as deterioration of renal function [11].

Cyclosporine A (CyA) and tacrolimus (Tac) are calcineurin inhibitors, fundamental for the currently used immunosuppressive protocols after transplantation. Both drugs are effective in reducing the incidence of acute rejection after renal transplantation, but they differ as to the mechanism of action, safety profile, toxicity, and side effects [12]. With regard to nontraditional risk factors contributing to high cardiovascular morbidity and mortality in renal transplant patients, there is a paucity of data examining the impact of different calcineurin inhibitors on AOPP and total antioxidant status (TAS) after kidney transplantation [13]. The aim of this study was to describe and to compare the influence of CyA and Tac on AOPP and TAS in a group of stable patients after kidney transplantation.

Patients and methods

This was a prospective, randomized, 6-month, single-center study conducted according to ICH GCP guidelines in the University Hospital Olomouc. The trial was approved by the local ethics committee, and all patients gave written informed consent before inclusion in the study. The study aimed at the assessment of the impact of CyA and Tac on AOPP and TAS over a 6-month period after kidney transplantation.

Recipients of kidney transplants from deceased donors followed up for a minimum of 6 months were eligible for the study. The causes of end-stage renal disease in the study population were as follows: chronic glomerulonephritis ($n=25$; 37%), chronic tubulo-interstitial nephritis ($n=17$; 25%), autosomal dominant polycystic kidney disease ($n=6$; 9%), diabetic nephropathy ($n=5$; 7%), reflux nephropathy ($n=3$; 4%), obstructive nephropathy ($n=2$; 3%), Alport syndrome ($n=2$; 3%), nephrosclerosis ($n=2$; 3%), and an unknown cause ($n=6$; 9%). No patients had active viral hepatitis B or C. Excluded were female patients who were pregnant or breastfeeding, patients under 18 years of age, patients undergoing systemic immunosuppressive therapy for reasons other than kidney transplantation, patients with malignant disease or significant, uncontrolled concomitant infections, and those taking vitamin supplements containing folic acid and vitamin C or E.

The clinical cohort was randomized into two groups receiving different well-established triple-drug maintenance immunosuppressive regimens. No induction immunosuppression with anti-thymocyte globulin was administered. Patients in the first group (group A, $n=35$) were treated with CyA (Sandimmune/Neoral) combined with mycophenolate mofetil (CellCept) and corticosteroids (Prednisone). The initial daily dosage of Sandimmune/Neoral was 3 mg/kg, divided into two doses. The target CyA blood trough levels were 200–300 ng/ml at month 1 and 100–200 ng/ml by month 6. Cyclosporine A levels were measured using fluorescence polarization immunoassay (Abbott Diagnostics). Patients in the second group (group B, $n=33$) were treated with tacrolimus (Prograf) combined with mycophenolate mofetil (CellCept) and corticosteroids (Prednisone). The initial daily dosage of Prograf was 0.1 mg/kg, divided into two doses. The target Tac blood trough levels were 5–15 ng/ml at month 1 and 4–10 ng/ml by month 6. Tacrolimus levels were measured using microparticle enzyme immunoassay (Abbott Diagnostics). The daily doses of CellCept in both groups were 20 mg/kg. Prednisone was progressively tapered to reach daily doses of 20 mg by day 15 and 15 mg by month 3 and a 5-mg maintenance dose by month 6. The dose given to each patient to reach the required serum levels can be considered for standardized as well as the duration of the therapy, which was equal for each subject.

The control group comprised 32 healthy blood donors (19 males and 13 females) with a mean age of 49 ± 11 years (range, 22–64 years) who did not take folic acid, vitamin C or E, or any other antioxidants. The mean AOPP level in the control group was 81.3 ± 28.7 μmol/L.

The following parameters were studied: AOPP, TAS, urea, creatinine, glomerular filtration (MDRD), uric acid, total protein, albumin, hemoglobin, C-reactive protein (CRP), total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. The blood sampling were collected in pre-defined time points after overnight fasting and assessed before kidney transplantation and at days 1, 7, 30, 90, and 180 after transplantation, for which the adherence was 100%.

Determination of AOPP was based on a spectrophotometric assay according to Witko-Sarsat [8]. One milliliter of blood serum diluted 1:5 with phosphate-buffered saline (PBS), 1 ml of chloramine-T (0-100 μmol/L) for calibration, and 1 mL of PBS as blank were placed in corresponding tubes. Fifty microliters of 1.16 mol/L potassium iodide and 100 μl of glacial acetic acid were added and absorbance at 340 nm was measured immediately. AOPP levels were expressed in micromoles of chloramine-T equivalents per liter of plasma (μmol/L).

Total antioxidant status was determined using a commercial kit (Randox Laboratories Ltd., UK). The principle of the assay is based on suppression of blue–green color of the ABTS radical cation (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate$^{\cdot+}$]) by antioxidants in the added sample. The degree of the suppression of this color production is proportional to the antioxidant concentration. The assay was carried according to the kit manufacturer's instructions. The normal value range is 1.30–1.77 mmol/L.

The other described biochemical parameters were measured in a certified clinical chemistry laboratory on the Hitachi 917 analyzer (Roche).

All results are expressed as the mean ± standard deviation (SD). The data were statistically analyzed using the Wilcoxon paired test, Spearman correlation test, and Mann–Whitney *U*-test. Values at a ≤5% level ($p\leq0.05$) were considered statistically significant.

Results

A total of 68 patients with an improvement in kidney function after renal transplantation were enrolled in the study, of which 37 (54%) were males and 31 (46%) females. Their mean age was 54.9 ± 10.9 years (range, 29–72 years). All patients completed the 6-month study and over this post-transplant period, the graft function was stable in all of them. Demographic data of the patient population and baseline characteristics are summarized in Table 1. The table demonstrates that the two groups were similar with no statistical differences in basic characteristics.

The levels of AOPP and TAS as well as selected biochemical and hematological parameters before transplantation and 6 months after transplantation in both groups of patients are summarized in Table 2.

After kidney transplantation, there was an improvement in renal function and all patients had a well-functioning and stabilized graft. The mean serum urea concentration decreased from 15.4 ± 6.9 mmol/L at baseline to 8.1 ± 2.3 mmol/L at month 6 in group A and from 15.9 ± 5.2 mmol/L at baseline to 7.6 ± 1.4 mmol/L at month 6 in group B. The mean serum creatinine decreased from 621.5 ± 171.5 μmol/L to 119.3 ± 26.0 μmol/L at month 6 in group A and from 643.2 ± 140.5 μmol/l at baseline to 114.2 ± 21.6 μmol/L at month 6 in group B. The glomerular filtration rate increased from 0.15 ± 0.06 ml/s at baseline to 0.94 ± 0.17 ml/s at month 6 in group A and from 0.14 ± 0.04 ml/s at baseline to 0.96 ± 0.21 ml/s at month 6 in group B. No differences in the graft function, age, and others laboratory parameters were found between the two groups.

In group A, the mean levels of AOPP and TAS decreased from 205.9 ± 125.7 μmol/L to 140.9 ± 78.9 μmol/L and from 1.89 ±