Significant Decrease in Plasma N-Acetyl-seryl-aspartyl-lysyl-proline Level in Patients with End Stage Renal Disease after Kidney Transplantation

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Received December 25, 2013; accepted March 7, 2014.

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*N-Acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous peptide released from its precursor (thymosin-β4) by prolyl oligopeptidase. AcSDKP is a natural inhibitor of pluripotent hematopoietic stem cell proliferation and is normally found in human plasma. AcSDKP has been shown to be a potent angiogenic factor and to suppress renal fibroblast proliferation. Impairment of renal function has been suggested to have a significant impact on plasma AcSDKP level. The aim of this study was to assess whether improvement of renal function after kidney transplantation has an impact on plasma AcSDKP-like immunoreactive substance (IS) level. Fourteen patients with end stage renal disease (ESRD) who were scheduled to undergo the first kidney allograft transplantation were enrolled. Plasma AcSDKP-IS levels were measured before and 3, 7, 10, 14, 21, 30, 60 and 90 d after kidney transplantation. Plasma AcSDKP-IS level decreased significantly from day 3 after kidney transplantation compared to before kidney transplantation. Creatinine clearance increased significantly from day 7 after kidney transplantation. A significant negative correlation was observed between creatinine clearance and plasma AcSDKP-IS level from before transplantation to 90 d after kidney transplantation. These results suggest that recovery of kidney function after kidney transplantation may lead to a decrease in plasma AcSDKP level in patients with ESRD, and that plasma AcSDKP level may depend largely on renal function.

Key words N-acetyl-seryl-aspartyl-lysyl-proline; kidney transplantation; plasma; end stage renal disease

N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous peptide released from its precursor (thymosin-β4) by prolyl oligopeptidase (POP). AcSDKP is a natural inhibitor of pluripotent hematopoietic stem cell proliferation and is normally found in human plasma. AcSDKP has been shown to be a potent angiogenic factor and to suppress renal fibroblast proliferation. Impairment of renal function has been suggested to have a significant impact on plasma AcSDKP level. The aim of this study was to assess whether improvement of renal function after kidney transplantation has an impact on plasma AcSDKP level. If this is the case, improvement of renal function should lead to a decrease in plasma AcSDKP level. Therefore, the aim of this study was to assess whether improvement of renal function after kidney transplantation has an impact on plasma AcSDKP-like immunoreactive substance (IS) level.

MATERIALS AND METHODS

Patients

The study enrolled patients with ESRD (creatinine clearance less than 15 mL/min) who were scheduled to undergo the first kidney allograft transplantation between January 2011 and November 2012 in the Department of Urology, Faculty of Medicine at Oita University. After kidney transplantation, all patients were followed for 90 d. Morning blood samples were collected in tubes containing ethylenediaminetetraacetate (EDTA) anticoagulant before and 3, 7, 10, 14, 21, 30, 60 and 90 d after kidney transplantation. All blood samples were centrifuged and plasma samples were frozen at −40°C within 2 h of peripheral venipuncture. The following clinical data was collected: gender; age; body weight; prescribed drugs; and laboratory data including white blood cell count, hemoglobin, C-reactive protein, serum creatinine and blood urea nitrogen. Creatinine clearance was calculated according to the Cockcroft–Gault equation. Level of angiotensin II was determined by an enzyme-linked immunosorbent assay (Abcam, Cambridge, U.K.). Before kidney transplantation, all patients were hemodialyzed three times a
week and received medications for hypertension (amlodipine, benidipine, olmesartan, telmisartan, carvedilol or bisoprolol) and hyperphosphatemia (lanthanum carbonate or precipitated calcium carbonate). After kidney transplantation, hemodialysis was discontinued in all patients. Patients were treated using a triple-therapy immunosuppression protocol, consisting of tacrolimus, mycophenolate mophetil and corticosteroids. Furthermore, all patients received induction therapy with basiliximab before and 4 d after kidney transplantation. None were administered recombinant human erythropoietin after kidney transplantation. Patients who had an episode of rejection during the study were excluded. This study was approved by the Institutional Review Board of Oita University Hospital. Each subject received information about the scientific purpose of the study, and gave written informed consent.

**Materials** Synthetic AcSDKP, N-succinyl-glycyl-prolyl-7-amino-4-methylcoumarin (Suc-Gly-Pro-AMC) and 7-amino-4-methylcoumarin (AMC) were purchased from Bachem (Bubendorf, Switzerland). Antiserum to AcSDKP (A03881) was purchased from Bertin Pharma (Paris, France). All other reagents were analytical reagent grade from commercial sources.

**Preparation of Plasma Extracts** Five hundred µL of methanol was added to 10 µL of plasma sample and vortexed. After centrifugation at 1500×g for 15 min at 4°C, the supernatant was decanted into a clean test tube, evaporated to dryness under a stream of nitrogen and stored at 40°C until assayed. The recovery of plasma AcSDKP-IS was greater than 95% using this extraction procedure.  

**Enzyme Immunoassay Procedure for AcSDKP-IS** Plasma AcSDKP-IS levels were measured using a highly sensitive enzyme immunoassay as described previously. The assay was performed by a delayed addition method. An immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark) coated with anti-rabbit immunoglobulin G (IgG) (55641, ICN Pharmaceuticals, Inc., OH, U.S.A.) was used to separate bound and free antigens. Glycyl-γ-amino butanoyl-seryl-aspartyl-lysyl-proline peptide, an AcSDKP derivative, was conjugated with β-β-galactosidase by N-(3-mercaptopropionyloxy)-succimide according to the methods of Kitagawa et al. The enzyme immunoassay for AcSDKP-IS was specific and highly sensitive, with a detection limit of 8.0 fmol/mL.

**POP Activity Assay** POP activity was measured using a modification of the method of Venäläinen et al. Briefly, 100 µL of plasma sample was preincubated with 0.1 µM sodium phosphate buffer (pH 7.0) for 30 min at 30°C. The reaction was initiated by adding 25 µL of substrate (4mM Suc-Gly-Pro-AMC) and incubated for 60 min at 30°C. The reaction was terminated by the addition of 500 µL of 1 µM sodium acetate buffer (pH 4.0). Blank readings were obtained from wells in which sodium acetate buffer was added prior to the plasma sample. The formation of AMC was measured fluorometrically using an SH-9000 microplate reader (Corona Electric, Ibaraki, Japan). The excitation and emission wavelengths were 360 and 460 nm, respectively. Activity was expressed as pmol of AMC produced per min per mL of plasma.

**Statistical Analysis** Data are expressed as mean±standard deviation (S.D.). Data before and after kidney transplantation were compared by the Dunnet test. Correlation between creatinine clearance and plasma AcSDKP-IS levels was analyzed by Pearson’s product-moment correlation coefficient. Multiple regression analysis was performed using plasma AcSDKP-IS level as dependent variable and creatinine clearance, blood urea nitrogen, POP activity and angiotensin II level as independent variables. A p value less than 0.05 was considered statistically significant. Statistical analyses were performed using Predictive Analysis Software (PASW) Statistics version 18 (SPSS Inc., IL, U.S.A.).

**RESULTS**

Fifteen patients signed the informed consent form for this study. Among 15 patients, one patient who had a rejection during the study was excluded. Finally, the data of 14 patients were analyzed. Table 1 shows the clinical data of the 14 patients with ESRD before and 30 and 90 d after kidney transplantation.

| Characteristic                        | Before transplantation | 30 d after transplantation | 90 d after transplantation |
|---------------------------------------|------------------------|-----------------------------|-----------------------------|
| No. of patients                       | 14                     | 14                          | 14                          |
| Males/females                        | 9/5                    | 9/5                         | 9/5                         |
| Cause of kidney disease              |                        |                             |                             |
| Glomerulonephritis                   | 6                      | 6                           | 6                           |
| Immunoglobulin A nephropathy         | 2                      | 2                           | 2                           |
| Thin basement membrane disease       | 1                      | 1                           | 1                           |
| Unknown nephritis                    | 5                      | 5                           | 5                           |
| Living/cadaveric kidney transplantation | 11/3                  | 11/3                        | 11/3                        |
| Age (year)                           | 45.7±10.8 [24–66]      | 55.7±11.4 [40.7–75.4]       | 55.6±10.9 [40.7–73.4]       |
| Body weight (kg)                     | 58.7±12.4 [44.7–83.1]  | 58.1±17.88 [2810–8630]      | 5221±1934 [2680–8820]       |
| White blood cell count (/µL)         | 6915±2139 [3290–9860]  | 5818±1788 [2810–8630]       | 5221±1934 [2680–8820]       |
| Hemoglobin (g/dL)                    | 11.8±1.7 [9.5–16.1]    | 10.4±1.4 [8.5–13.9]         | 10.6±1.6 [8.2–14.1]         |
| C-Reactive protein (mg/dL)           | 0.08±0.08 [0.01–0.27]  | 0.10±0.11 [0.01–0.37]       | 0.11±0.11 [0.01–0.29]       |
| Blood urea nitrogen (mg/dL)          | 48.3±21.9 [26.3–100.7] | 24.0±6.4 [12.7–38.5]        | 23.4±6.5 [13.3–37.5]        |
| Angiotensin II (pmol/L)              | 12.9±6.0 [8.6–32.3]    | 11.8±2.0 [8.6–14.8]         | 12.1±2.2 [8.8–15.9]         |
| Prolyl oligopeptidase activity (pmol/min/mL) | 122.6±17.7 [96.9–156.8] | 116.0±23.4 [86.3–173.0]    | 125.2±31.2 [81.5–182.9]    |

Data are expressed as numbers, or mean±S.D. [Range]. *p<0.01, vs. before kidney transplantation.
transplantation. As expected, blood urea nitrogen decreased significantly after kidney transplantation. On the other hand, no significant differences in POP activity and angiotensin II level were observed before and after kidney transplantation.

The changes in plasma AcSDKP-IS level and creatinine clearance over time after kidney transplantation in patients with ESRD are shown in Figs. 1a, b. Plasma AcSDKP-IS level decreased significantly from day 3 after kidney transplantation compared to before kidney transplantation, and remained elevated and almost stable thereafter. Creatinine clearance increased significantly from day 7 after kidney transplantation, and remained almost stable thereafter. Correlation analysis showed a significant negative correlation between creatinine clearance and plasma AcSDKP-IS level from before transplantation to 90 d after kidney transplantation ($r = -0.43$, $p < 0.0001$). Stepwise multiple regression analysis using the datasets before and 30 and 90 d after kidney transplantation identified creatinine clearance as the only significant independent factor associated with plasma AcSDKP-IS levels ($p < 0.001$).

**DISCUSSION**

In this study, we investigated the changes in plasma AcSDKP-IS level over time after kidney transplantation in patients with ESRD. We report for the first time a significant decrease in plasma AcSDKP-IS level in patients with ESRD after kidney transplantation.

Increases in plasma AcSDKP level have been reported in patients with various diseases, such as chronic renal failure, chronic heart failure and hematologic malignancy. In this study, no patient had chronic heart failure or cancers including hematologic malignancy. In addition, none were prescribed ACE inhibitor during this study. These suggest that the variation in renal function was the prominent pathological factor affecting plasma AcSDKP-IS level. To evaluate renal function, we used creatinine clearance according to Cockcroft–Gault equation because difference in body size may affect the elimination of AcSDKP. In fact, creatinine clearance was used to evaluate the correlation between renal function and plasma AcSDKP level in a previous study.

We reported previously that plasma AcSDKP-IS levels in patients with ESRD before kidney transplantation were higher than those of healthy subjects (1.29±0.56 vs. 0.29±0.07 pmol/mL), which was consistent with past reports of elevated plasma AcSDKP levels in patients with chronic renal failure. Plasma AcSDKP-IS level decreased significantly from day 3 after kidney transplantation compared to before kidney transplantation. The degrees of decrease in plasma AcSDKP-IS level were not different between living and cadaveric kidney transplant recipients (data not shown). On the other hand, creatinine clearance increased significantly from day 7 after kidney transplantation. These findings suggest that recovery of kidney function after kidney transplantation may lead to a decrease in plasma AcSDKP level in patients with ESRD. Furthermore, a significant negative correlation was observed between creatinine clearance and plasma AcSDKP-IS level and multiple regression analysis identified creatinine clear-

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**Fig. 1. Changes in Plasma N-Acetyl-ser-ly-syl-proline (AcSDKP) Level (a) and Creatinine Clearance (b) over Time after Kidney Transplantation in Patients with ESRD**

Data are expressed as means±S.D., $n=14$. *$p<0.05$, †$p<0.01$, vs. before kidney transplantation.
ance as the only significant independent factor associated with plasma AcSDKP-IS level, suggesting that plasma AcSDKP level may depend largely on renal function. In this study, plasma AcSDKP-IS level decreased earlier than serum creatinine after kidney transplantation. Change in serum creatinine varies after renal recovery or injury, with a time lag. Plasma AcSDKP may reflect promptly the change in renal function, and hence may be a more sensitive marker of renal function than serum creatinine. As other factors that potentially affect plasma AcSDKP level, POP that releases AcSDKP and ACE that hydrolyzes AcSDKP may be important. In this study, no significant differences in POP activity and angiotensin II level were observed before and after kidney transplantation and these were not identified as the significant independent factors associated with plasma AcSDKP-IS levels by multiple regression analysis, indicating that renal function was the only factor affecting plasma AcSDKP level in patients with ESRD after kidney transplantation.

AcSDKP is a natural inhibitor of pluripotent hematopoietic stem cell proliferation. Van der Meer et al. have reported that plasma AcSDKP level is significantly higher in the anemic chronic heart failure patients compared with non-anemic chronic heart failure patients and that plasma AcSDKP correlates negatively with proliferation of erythropoietic progenitor cells. Le Meur et al. have reported a relation between AcSDKP level and the weekly dose of recombinant human erythropoietin for the treatment of renal anemia. Their findings suggest an inhibitory role of AcSDKP on hematopoiesis. In the present study, in spite of the decrease in plasma AcSDKP-IS level after kidney transplantation, hemoglobin level did not change significantly after kidney transplantation. It is possible that decrease in hemoglobin level caused by bleeding or inflammation as a result of the kidney transplantation procedure continued during the present study, such that recovery of hematopoietic capacity after AcSDKP level decreases might not be reflected. The clinical significance of the decrease in plasma AcSDKP level after kidney transplantation and its role in hematopoiesis require further study.

In conclusion, plasma AcSDKP-IS level in patients with ESRD decreased significantly after kidney transplantation. It is possible that decrease in hemoglobin level caused by bleeding or inflammation as a result of the kidney transplantation procedure continued during the present study, such that recovery of hematopoietic capacity after AcSDKP level decreases might not be reflected. The clinical significance of the decrease in plasma AcSDKP level after kidney transplantation and its role in hematopoiesis require further study.

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