Clinica Chimica Acta 453 (2016) 160–163

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Association of metabolic complications with plasma mid-regional pro-adrenomedullin level in stable kidney transplant recipients

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ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 10 December 2015
Accepted 14 December 2015
Available online 17 December 2015

Keywords:
Mid-regional pro-adrenomedullin
Metabolic complications
Hypertension
Hyperlipidemia
Kidney transplantation

ABSTRACT

Background: Recently, increased plasma mid-regional pro-adrenomedullin (MR-proADM) concentrations have been demonstrated in patients with chronic renal failure. In this study, we attempted to identify significant clinical factors associated with MR-proADM concentration in stable kidney transplant recipients.
Methods: Forty-seven Japanese kidney transplant recipients who underwent transplantation >180 days prior to the study were analyzed. To facilitate comparability of anti-hypertensive regimens across recipients taking different drugs, we calculated the treatment intensity score of anti-hypertensive drugs in each recipient. Morning blood samples were collected and plasma MR-proADM concentrations were measured using an enzyme immunoassay.
Results: Multiple regression analysis identified treatment intensity score for anti-hypertensive drugs, serum albumin, creatinine clearance and use of lipid-lowering agents as significant independent factors associated with plasma MR-proADM concentration. Adjusted coefficient of determination for this model was 0.46.
Conclusion: Apart from indicating lowered renal function, plasma MR-proADM concentration may be a useful biomarker for metabolic disorders, especially hypertension and hyperlipidemia, in stable kidney transplant patients.

1. Introduction

Advances in kidney transplantation in recent years have resulted in a significant increase in stable kidney transplant recipients. While survival outcome has improved, there are also increases in some complications after kidney transplantation. Especially, hypertension commonly appears in stable kidney transplant recipients, necessitating prescription of prescribed anti-hypertensive drugs [1]. Reports have indicated the involvement of various factors such as vascular disorder, damaged renal parenchyma, accumulated water or sodium ion, autonomic nervous system disorder, metabolic disturbance and adverse effects of prescribed drugs in the development of high blood pressure after kidney transplantation [2–5]. Functional decline of the transplanted kidney is involved in enhanced renin–angiotensin system, resulting in refractory hypertension. We have reported the relationship between plasma mid-regional pro-adrenomedullin (MR-proADM) concentration and resistance to anti-hypertensive therapy in stable kidney transplant recipients [6]. MR-proADM is derived from a larger precursor peptide (preproadrenomedullin) by posttranslational processing. This peptide is co-synthesized with adrenomedullin in equimolar amounts. Contrary to adrenomedullin that has a variety of physiologic functions such as immune-modulating activity, direct bactericidal activity, maintenance of renal homeostasis, and vasodilatory activity [7–11], MR-proADM has a longer half-life, no bioactivity and no protein binding [12]. Therefore, MR-proADM is a useful surrogate for adrenomedullin secretion. In patients with chronic renal failure, increased plasma MR-proADM concentrations have been demonstrated in non-dialysis [13,14] and dialysis patients [15].

Several studies have been reported that plasma concentration of adrenomedullin or MR-proADM is also increased in patients with metabolic syndrome other than hypertension, such as diabetes mellitus, hyperlipidemia and hyperuricemia [16–18]. MR-proADM is a powerful predictor of future cardiovascular events and survival [19–21]. Hence, it was important to know the plasma MR-proADM concentration in individual stable kidney transplant recipients.

2. Patients and methods

2.1. Patients

Forty-seven Japanese kidney transplant recipients who underwent transplantation >180 days prior to the study were analyzed. The study

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http://dx.doi.org/10.1016/j.cca.2015.12.016
0009-8981/

period was from July 2011 to November 2014. All recipients had no cardiovascular and infectious diseases, and none were hemodialyzed.

Morning blood samples were collected in tubes containing EDTA during follow-up visits at Oita University Hospital. 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 mass index (BMI); systolic and diastolic blood pressure; duration after kidney transplantation; prescribed drugs; and laboratory data including serum albumin, serum creatinine, blood urea nitrogen, total cholesterol, high-density lipoprotein, triglyceride, hemoglobin A1c (HbA_1c), uric acid, C-reactive protein and white blood cell count. Blood pressures were measured and recorded every morning by the patients themselves using electronic device, and the mean value for the period between two follow-up visits was calculated. Serum albumin was measured by a modified bromocresol purple method. Serum creatinine, total cholesterol, triglyceride and uric acid were measured by enzymatic methods. Blood urea nitrogen was measured by the urease–glutamate dehydrogenase method. High-density lipoprotein was measured by direct method. HbA1c was measured by HPLC. C-reactive protein was measured by latex particle enhanced immunoturbidimetric assay. White blood cells were counted by flow cytometry. Creatinine clearance was calculated according to the Cockcroft–Gault equation [22]. Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula [23]. Recipients were prescribed anti-hypertensive, anti-hyperlipidemic, anti-diabetic and anti-hyperuricemic drugs to control systolic blood pressure <130 mm Hg and diastolic blood pressure <80 mm Hg, LDL cholesterol below 120 mg/dL, HbA_1c <6.5% and uric acid <8.0 mg/dL, respectively.

To facilitate comparability of anti-hypertensive regimens across recipients taking different drugs, we calculated the treatment intensity score for each drug as follows: daily dose taken by the patient divided by the maximum recommended daily dose [24]. The maximum recommended daily dose for each anti-hypertensive drug was obtained from the December 2014 Monthly Prescribing Reference. For example, a recipient taking a 40-mg daily dose of an anti-hypertensive agent for which 200 mg was the maximum daily dose was considered to be taking 0.2 intensity unit. For each patient, treatment intensity score was calculated for each of the anti-hypertensive drugs taken, and summed to obtain the treatment intensity score of the anti-hypertensive regimen.

This study was approved by the Institutional Review Board of Oita University Hospital (approval number: B11-017). Each subject received information about the scientific purpose of the study, and gave written informed consent.

2.2. Materials

Synthetic human MR-proADM was purchased from Phoenix pharmaceuticals Inc. Anti-serum to MR-proADM (T-4843) was purchased from Peninsula Laboratories. All other reagents were of analytical reagent grade from commercial sources.

2.3. Preparation of plasma extracts

One milliliter of methanol was added to 200 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, concentrated by spin-vacuum evaporation, lyophilized, and stored at −40 °C until assayed. The recovery of plasma MR-proADM was >97% using this extraction procedure [25].

2.4. EIA procedure for MR-proADM

Plasma MR-proADM concentrations were measured using an enzyme immunoassay as described previously [25]. The assay was performed by a delayed addition method. An immunoplate (Nunc-Immuno Module Maxisorp F8) coated with anti-rabbit IgG (55641, ICN Pharmaceuticals, Inc.) was used to separate bound and free antigens. Fragment preproADM (83–94) was conjugated with β-D-galactosidase by *N*-(ε-maleimido-caproyloxy)-succimide according to the methods of Kitagawa et al. [26]. The enzyme immunoassay for plasma MR-proADM was specific and sensitive, with a detection limit of 0.08 nmol/L. Interassay and intraassay CV for samples of 0.2 and 2.0 nmol/L were 10.8% and 8.9%, and 3.9% and 7.8%, respectively [25].

2.5. Statistical analysis

Data are expressed as mean ± SD. Factors associated with plasma MR-proADM concentrations were analyzed by univariate and stepwise multiple regression studies to test various combinations of variables together. A $p < 0.05$ was considered statistically significant. Statistical analyses were performed using the R software ver. 3.1.1 (http://www.r-project.org), and the Predictive Analysis Software (PASW) Statistics ver 21.0 (SPSS Inc.).

3. Results

Table 1 shows the clinical data of the 47 stable kidney transplant recipients. Thirty-eight patients had impaired renal function (creatinine clearance < 60 mL/min). Anti-hypertensive drugs such as calcium

Table 1
Characteristics of patients in the study.

Characteristic	Value
Males/females	28/19
Age (y)	47.4 ± 14.1 [16–74]
BMI (kg/m^2)	20.4 ± 2.5 [14.6–26.7]
Duration after kidney transplantation (day)	1063 ± 1427 [183–7051]
Systolic blood pressure (mm Hg)	116.1 ± 9.1 [92–130]
Diastolic blood pressure (mm Hg)	70.0 ± 7.5 [52–80]
LDL cholesterol (mg/dl)	106.2 ± 30.5 [51.2–175.7]
HbA1c (%)	5.8 ± 0.7 [4.7–8.9]
Urinary acid (mg/dL)	6.4 ± 1.3 [2.7–9.8]
Serum albumin (g/dL)	4.2 ± 0.4 [3.3–5.0]
Creatinine clearance (mL/min)	43.7 ± 18.0 [10.8–80.8]
Blood urea nitrogen (mg/dL)	27.3 ± 9.7 [12.1–61.2]
C-reactive protein (mg/dL)	0.06 ± 0.07 [0.01–0.49]
White blood cell count (/μL)	6110 ± 2741 [1630–13,450]
No. of patients prescribed anti-hypertensive drugs	39
Anti-hypertensive drugs	
Amlodipine	14
Nifedipine	4
Benidipine	2
Imidapril	4
Enalapril	2
Olmesartan	17
Telmisartan	15
Candesartan	2
Losartan	2
Carvedilol	17
Bisoprolol	1
Furosemide	3
Treatment intensity score for anti-hypertensive drugs	1.00 ± 0.84 [0–2.90]
No. of patients prescribed anti-hyperlipidemic drugs	10
Anti-hyperlipidemic drugs	
Atorvastatin	10
No. of patients prescribed anti-diabetic drugs	3
Anti-diabetic drugs	
Insulin	1
Glimepiride	1
Repaglinide	1
Anagliptin	1
No. of patients prescribed anti-hyperuricemic drugs	26
Anti-hyperuricemic drugs	
Allopurinol	25
Benzbromarone	2

Data are expressed as numbers of subjects, or mean ± SD [range].