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Original Paper

Renin-Angiotensin System Inhibitors Reduce Serum Asymmetric Dimethylarginine Levels and Oxidative Stress in Normotensive Patients with Chronic Kidney Disease

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Key Words

Chronic kidney disease · Renin-angiotensin system inhibitor · Asymmetric dimethylarginine · Oxidative stress

Abstract

Background/Aims: The purpose of our study was to elucidate the relationship between asymmetric dimethylarginine (ADMA) and intrarenal lesions and to determine the effect of renin-angiotensin system inhibitors (RAS-Is) on serum ADMA levels, nitric oxide (NO) synthesis and oxidative stress in normotensive patients with chronic kidney disease (CKD). **Methods:** This study included 23 normotensive patients with chronic glomerulonephritis and normal or mildly impaired renal function who underwent renal biopsy. We evaluated the relationship between serum ADMA levels and intrarenal lesions, and examined renal function, urinary protein excretion, ADMA levels, NO synthesis, oxidative stress and blood pressure (BP) before and 3 months after starting the treatment with RAS-Is. **Results:** Serum ADMA levels were correlated only with arterial intimal fibroplastic thickness. Despite comparable renal function and BP, serum ADMA levels and excretion of urinary protein excretion significantly decreased, and urinary NO metabolite excretion significantly increased after starting the treatment with RAS-Is. Oxidative stress markers also tended to be reduced by the treatment. **Conclusion:** These findings suggest that RAS-Is improve the NO system and decrease oxidative stress in normotensive patients with CKD. In addition, ADMA may be associated with intrarenal lesions and can be a useful marker for the effects of treatment in the early stages of CKD.

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Introduction

It is well known that chronic kidney disease (CKD) is a very important risk factor associated with cardiovascular events and mortality [1]. Therefore, its management is essential for improving patients' prognoses. Renin-angiotensin system inhibitors (RAS-Is) including angiotensin-converting enzyme inhibitor and angiotensin receptor blocker (ARB) can not only reduce urinary protein excretion and slow the decline of renal function but also prevent the progression of cardiovascular disease (CVD) in patients with CKD [2, 3]. Furthermore, basic and clinical reports have demonstrated that RAS-Is can suppress the activity of glomerular nephropathy. For example, an *in vivo* study showed that RAS-Is increase the permeability and size-selective functions of the glomerulus [4]. Furthermore, a clinical study of patients with IgA nephropathy demonstrated that RAS-Is significantly improve renal survival in proteinuric patients with normal or moderately reduced renal function [5]. Another study has reported that RAS-Is effectively reduce proteinuria and improve serum albumin in patients with lupus nephritis [6]. To summarize, RAS-Is are effective for the treatment of various types of CKD.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase, has been suggested to be a marker of endothelial dysfunction and atherosclerosis [7]. ADMA is also associated with the production of oxidative stress [8]. A previous study has reported that serum ADMA levels are increased in patients with CKD and are associated with renal function and proteinuria [9]. In addition, both experimental and clinical studies have demonstrated that RAS-Is decrease serum ADMA levels and improve endothelial dysfunction [10].

Although many studies have demonstrated the effect of RAS-Is in patients with CKD and hypertension, to the best of our knowledge, detailed data in normotensive patients with CKD are lacking. In the present study, we examined the effects of RAS-I treatment on oxidative stress and the NO system in normotensive patients with CKD.

Subjects and Methods

Study Population

Thirty-one normotensive patients with chronic glomerulonephritis who were hospitalized in our institution between January 2008 and December 2009 were enrolled in the present study. All patients met the criteria for normotensive subjects according to the guidelines of the Japanese Society of Hypertension. Patients who were taking RAS-Is or an immunosuppressant or who had a history of smoking, hypertension, diabetes mellitus, hyperlipidemia, overt infection, malignancy, inflammatory disease or CVD were excluded. Of the 31 patients, 8 were excluded from this study because of the discontinuation of prescribed medication, transfer to another facility or insufficient clinical data. For the 23 remaining patients, renal biopsies were performed after enrolment, and RAS-Is were administered for at least 3 months (losartan, n = 11; olmesartan, n = 6; valsartan, n = 5, and enalapril, n = 1). The patients did not take any other antihypertensive or renoprotective drugs, and there was no change in the medication dose during the study period. To compare the histological findings, 5 subjects with mild proteinuria whose renal histological findings showed only minor glomerular abnormalities were evaluated as controls. In addition, as for serum ADMA levels, 20 young healthy volunteers were evaluated. For the study patients, we prospectively performed blood and urinary examinations, renal histological analyses and blood pressure (BP) measurement before and 3 months after starting treatment with RAS-Is. The experimental protocols were approved by the appropriate institutional review committee and performed in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Table 1. Patients' baseline characteristics (n = 23)

Age, years	48 ± 4
Males/females	15/8
Systolic BP, mm Hg	115.6 ± 3.1
Diastolic BP, mm Hg	67.6 ± 2.3
Cr, mg/dl	0.99 ± 0.07
eGFR, ml/min/1.73 m ²	65.6 ± 4.1
BUN, mg/dl	15.2 ± 1.0
TP, g/dl	6.74 ± 0.20
Albumin, g/dl	3.73 ± 0.12
ADMA, nmol/ml	0.46 ± 0.01
U-8-OHdG, ng/mg•Cr	99.1 ± 14.4
U-NOx/U-Cr	1.29 ± 0.16
U-protein, g/g•Cr	1.22 ± 0.32

Cr = Creatinine; eGFR = estimated glomerular filtration rate; BUN = blood urea nitrogen; TP = total protein; U-8-OHdG = urinary 8-OHdG; U-Cr = urinary creatinine; U-protein = urinary protein.

Table 2. Renal histological findings

	Control (n = 5)	CKD (n = 17)	p value
Global sclerosis, %	0	12.3 ± 3.4	0.038
Interstitial fibrosis, %	0	13.4 ± 3.7	0.042
Hyalinosis, %	0	5.3 ± 4.0	0.439
Arterial intimal fibroplastic thickness, %	7.6 ± 0.3	22.0 ± 3.4	0.036
8-OHdG-positive cell score	42.1 ± 6.7	84.3 ± 3.8	0.032

Renal Histological Analysis

Of the 23 study patients, renal biopsy specimens of 17 patients were available for histological analysis. We evaluated the extent of glomerulosclerosis, interstitial fibrosis, arteriolar hyalinosis and intimal fibroplastic thickness of interlobular arteries in a blinded manner. The number of globally sclerotic glomeruli was counted to determine the extent of global sclerosis. Furthermore, formation of 8-hydroxydeoxyguanosine (8-OHdG) was assessed with anti-8-OHdG monoclonal antibodies raised in humans (NOF Corp., Tokyo, Japan). 8-OHdG-positive cells in all glomeruli were counted, and the average was used as the 8-OHdG-positive cell score. The following formulae were used to calculate global sclerosis, interstitial fibrosis, arteriolar hyalinosis and arterial intimal fibroplastic thickness, respectively:

global sclerosis (%) = 100 × (number of globally sclerotic glomeruli/total number of glomeruli),

interstitial fibrosis (%) = 100 × (area of fibrosis/total area of the specimens),

arteriolar hyalinosis (%) = 100 × (number of arterioles with hyalinization/total number of arterioles), and

arterial intimal fibroplastic thickness (%) = 100 × (a + b)/od, where a and b = intimal thickness of interlobular arteries and od = outer diameter.

Serum and Urine Measurements

Before and 3 months after starting treatment with RAS-Is, venous blood was collected from study patients in the morning following overnight fasting. Urinary NO metabolites (U-NOx) and 8-OHdG excretion were determined using ELISA kits (NOx: Dojindo Laboratories, Kumamoto, Japan; 8-OHdG: Japan Institute for Control of Aging, Shizuoka, Japan). Serum ADMA

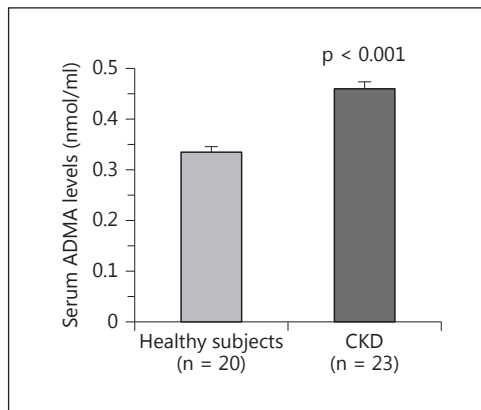


Fig. 1. Comparison of serum ADMA levels between healthy subjects and patients with CKD.

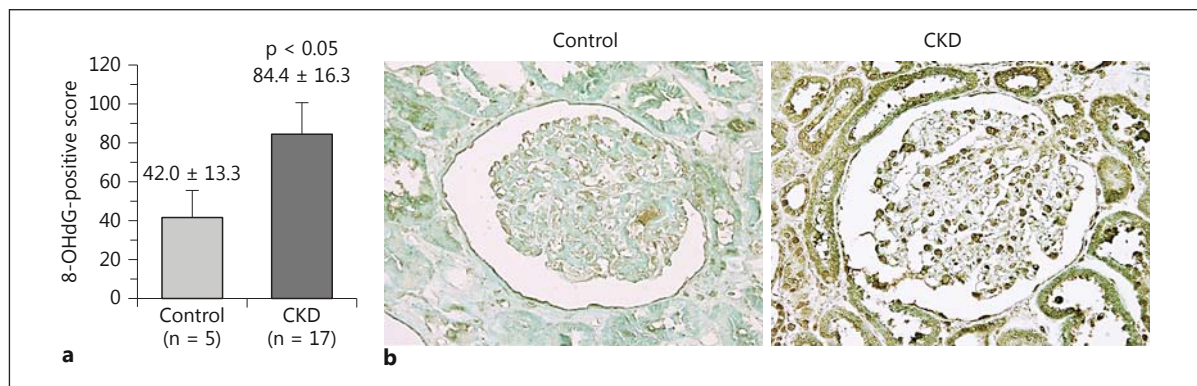


Fig. 2. Histological examination of the kidney sections for 8-OHdG. **a** 8-OHdG-positive cell scores were significantly higher in the patients with CKD than in the control subjects. **b** Immunostaining for 8-OHdG is indicated by brown reaction products.

levels were determined using a novel high-performance liquid chromatography method. Other laboratory tests were conducted using standardized clinical laboratory methods.

Statistical Analysis

We used the computer software application StatView 5.0 (SAS Institute, Cary, N.C., USA) for all statistical analyses. Values are presented as mean ± SEM. For continuous variables, the Mann-Whitney U test was used to analyze the significance of the differences between the 2 groups. Pearson's correlation coefficient was used to analyze the relationship between the variables. $p < 0.05$ was considered to be statistically significant.

Results

Patients' Characteristics and Renal Histology

The baseline characteristics of the study patients are shown in table 1. Serum ADMA levels in the study patients were higher than those in the healthy subjects (fig. 1). Renal histological analysis revealed more severe lesions in the study patients compared with the control subjects (table 2). Furthermore, the 8-OHdG-positive cell score was significantly higher in the patients with CKD than in the control subjects (fig. 2).

Table 3. Relationship between serum ADMA levels and clinical characteristics

	r	p value
Age	0.362	0.117
Sex	0.298	0.203
Systolic BP	0.013	0.933
Diastolic BP	0.165	0.272
Cr	0.274	0.065
eGFR	-0.336	0.024
BUN	0.375	0.010
TP	0.050	0.833
Albumin	0.015	0.950
U-8-OHdG	0.219	0.163
U-NO _x /U-Cr	-0.160	0.313
U-protein	0.275	0.064

Cr = Creatinine; eGFR = estimated glomerular filtration rate; BUN = blood urea nitrogen; TP = total protein; U-8-OHdG = urinary 8-OHdG; U-Cr = urinary creatinine; U-protein = urinary protein.

Table 4. Relationship between serum ADMA levels and renal histological findings

	r	p value
Global sclerosis	0.205	0.415
Interstitial fibrosis	0.309	0.212
Hyalinosis	0.092	0.910
Arterial intimal fibroplastic thickness	0.573	0.013

Correlation of ADMA with Clinical Characteristics and Renal Histology

We evaluated the correlation of serum ADMA levels with the patients' clinical characteristics (table 3) and renal histological findings (table 4). Serum ADMA levels were significantly correlated with estimated glomerular filtration rate and tended to be correlated with the degree of urinary protein excretion. Among the renal histological findings, we found that only arterial intimal fibroplastic thickness was significantly related to serum ADMA levels.

Changes of ADMA, NO and 8-OHdG by RAS-Is

Although BP and renal function did not differ significantly during the study period, the degree of urinary protein excretion was significantly reduced 3 months after starting treatment with RAS-Is (fig. 3). In addition, serum ADMA levels and U-NO_x and urinary 8-OHdG excretion were improved 3 months after starting treatment with RAS-Is compared with the respective values at baseline (fig. 4).

Discussion

Our study demonstrated that serum ADMA levels were significantly higher in the normotensive patients with CKD than in the control subjects, that arterial intimal fibroplastic thickness was significantly correlated with serum ADMA levels and that RAS-Is lowered the elevated serum ADMA levels and oxidative stress and increased NO production.

The mechanisms underlying abnormalities in CKD are complicated, and several factors contribute to their pathogenesis. Of these factors, oxidative stress is considered to play a key

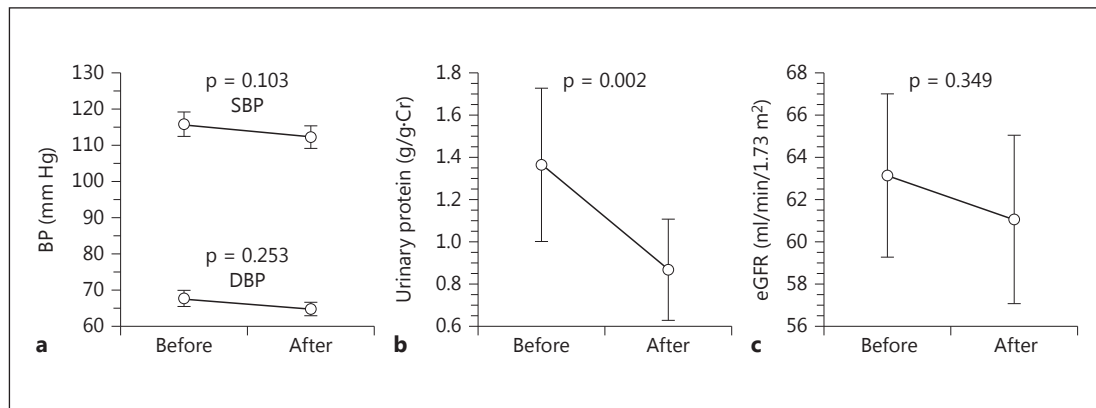


Fig. 3. Changes in systolic (SBP) and diastolic BP (DBP) (a), urinary protein excretion (b) and renal function after starting treatment with RAS-Is (c). eGFR = Estimated glomerular filtration rate; before = before treatment; after = after treatment.

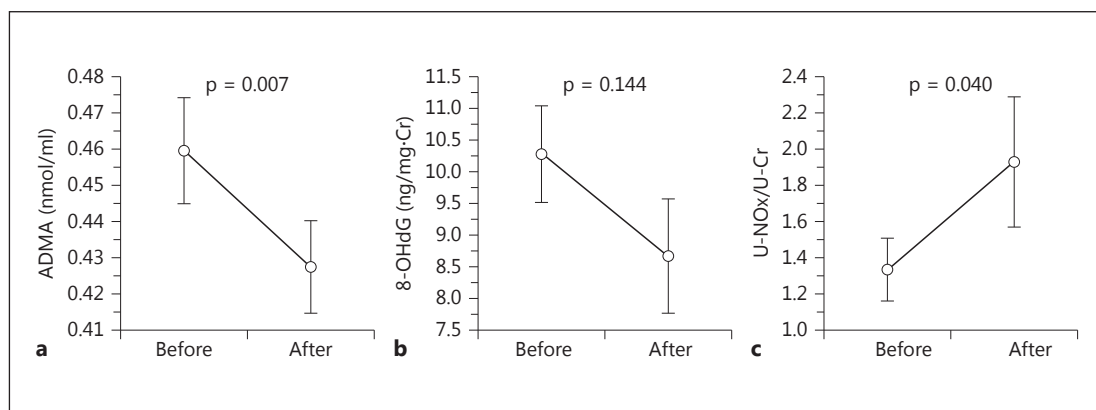


Fig. 4. Changes in ADMA (a), U-NOx (b) and oxidative stress (c) after starting treatment with RAS-Is. U-Cr = Urinary concentration of creatinine.

role in the progression of CKD [11, 12]. In the present study, compared with healthy subjects, systemic and intrarenal oxidative stress increased in normotensive patients with CKD. These findings suggested a relationship between oxidative stress and the progression of CKD in such patients.

ADMA is an endogenous competitive inhibitor of NO synthase, and NO is a potent anti-atherosclerotic molecule [7]. Therefore, increased ADMA levels lead to a decrease in NO synthesis and progressive damage due to impaired vascular function in the kidneys, heart and other systemic organs. Elevated ADMA levels are associated not only with endothelial dysfunction [8, 13, 14] but also with increased oxidative stress [15, 16], thereby linking vascular function and redox mechanisms in CKD and CVD. In addition, serum ADMA levels have been reported to be markers of endothelial dysfunction and/or atherosclerosis [17] and to be associated not only with renal function but also with proteinuria in CKD [8, 18]. The results of our study demonstrated that serum ADMA levels were significantly correlated with the intrarenal vascular lesions even in normotensive patients with CKD. With regard to the clinical data, serum ADMA levels were correlated with renal function and proteinuria. Taking

these results into account, the increase of serum ADMA levels is believed to be the first step in the process of the progression of CKD. Therefore, these findings suggested that serum ADMA levels reflect the severity of CKD and could be used as a surrogate marker for the progression of CKD.

Many reports have shown the efficacy of RAS-Is in renal diseases. Angiotensin-converting enzyme inhibitor and ARB have been shown to reduce urinary protein excretion and slow the progression of renal dysfunction [2, 3]. However, only few studies have demonstrated the efficacy of RAS-Is in normotensive patients with CKD in clinical settings. Makino et al. [19] reported that ARB reduces the transition to overt nephropathy in normotensive diabetic patients, suggesting that ARB has favorable BP-independent effects on CKD. Web et al. [20, 21] demonstrated that RAS-Is effectively reduced proteinuria in normotensive children with CKD.

Angiotensin II, which acts on angiotensin II type 1 receptors, increases oxidative stress, in part by activating nicotinamide adenine dinucleotide phosphate oxidase and ADMA, which in turn activates the local renin-angiotensin system [15, 22, 23]. Therefore, RAS-Is can lower oxidative stress, which has been suggested to be associated with a reduction in serum ADMA levels independently of lowering the BP. In patients with CKD, RAS-Is has been reported to reduce ADMA, proteinuria levels and oxidative stress [24, 25]. However, no study has actually demonstrated that RAS-Is can reduce ADMA levels in normotensive patients with CKD.

The main limitation of our study was the small number of study patients. However, our patients were carefully and appropriately treated and we closely observed them prospectively and longitudinally. Although only few prospective and longitudinal studies have elucidated the detailed mechanisms of the progression of CKD on this topic in the early stages of CKD, we performed both a biochemical analysis and histological evaluations. Therefore, we consider the results of the present study to be valuable for understanding the mechanisms and effects of RAS-Is on the progression of CKD. Another limitation was that the present study included a group of patients with heterogeneous renal etiologies. However, all study patients were normotensive and relatively young. Other patient characteristics were very similar. In the near future, we plan to conduct a randomized controlled trial with a large number of study patients to clarify these details.

Our data suggest that RAS-Is prevent the progression of chronic glomerulonephritis by reducing serum ADMA levels and oxidative stress in normotensive patients with CKD. In addition, the findings suggest that ADMA may be associated with intrarenal lesions and can be used as a useful surrogate marker for the effects of treatment in the early stages of CKD.

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Disclosure Statement

None of the authors have any conflict of interest to report.

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