Dimethylarginine Dimethylaminohydrolase Prevents Progression of Renal Dysfunction by Inhibiting Loss of Peritubular Capillaries and Tubulointerstitial Fibrosis in a Rat Model of Chronic Kidney Disease

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Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, is mainly degraded by dimethylarginine dimethylaminohydrolase (DDAH). It was recently reported that reduced DDAH expression could contribute to ADMA accumulation and subsequent elevation of BP in an experimental model of chronic kidney disease (CKD). ADMA is a strong predictor of the progression of CKD as well. However, a role for the ADMA-DDAH in the pathogenesis of CKD remains to be elucidated. This study investigated the effects of DDAH-elicited ADMA lowering on renal function and pathology in a rat remnant kidney model. Four weeks after five-sixths subtotal nephrectomy (Nx), the rats were given tail-vein injections of recombinant adenovirus vector encoding DDAH-I (Adv-DDAH) or control vector expressing bacterial β-galactosidase (Adv-LZ) or orally administered 20 mg/kg per d hydralazine (Hyz), which served as a BP control model. In comparison with Adv-LZ or Hyz administration, Adv-DDAH decreased plasma levels of ADMA and inhibited the deterioration of renal dysfunction. Plasma levels of ADMA were associated with decreased number of peritubular capillaries, increased tubulointerstitial fibrosis, and proteinuria levels in Nx rats. These changes were progressed in Adv-LZ– or Hyz-treated Nx rats, which were ameliorated by DDAH overexpression. In addition, semiquantitative reverse transcriptase–PCR and immunohistochemistry for TGF-β revealed that Adv-DDAH inhibited upregulation of TGF-β expression in Nx rats. These data suggest that ADMA may be involved in peritubular capillary loss and tubulointerstitial fibrosis, thereby contributing to the progression of CKD. Substitution of DDAH protein or enhancement of its activity may become a novel therapeutic strategy for the treatment of CKD.

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Nitric oxide (NO) is synthesized by stereospecific oxidation of terminal guanidine nitrogen of L-arginine by the action of the NO synthase (NOS). The synthesis of NO can be blocked by inhibition of the NOS active site with guanidino-substituted analogues of L-arginine, such as asymmetric dimethylarginine (ADMA) (1,2). We, along with others, have demonstrated that elevated plasma ADMA is associated with cardiovascular risk factors such as hypertension (3,4), diabetes (4,5), and chronic kidney disease (CKD) (6,7), thereby being one of the useful biomarkers for atherosclerosis and future cardiovascular events (5,7–9). ADMA is mainly metabolized by an enzyme dimethylarginine dimethylaminohydrolase (DDAH) (1,2). We recently found that reduced DDAH expression could contribute to ADMA accumulation and subsequent elevation of BP in an experimental model of CKD (10). Furthermore, plasma level of ADMA is known to be a strong predictor of the progression of renal dysfunction in patients with CKD (11,12). According to the recent comprehensive review on the potential role of ADMA in renal disease progression (13), there may be two major possible mechanisms by which ADMA could contribute to the progression of CKD; one is a BP-dependent effect of ADMA described previously (4,10), and the other is a BP-independent, direct effect of ADMA on renal microvasculature. As to the latter, Kang *et al.* (14) demonstrated that administration of an inhibitor of NOS accelerated renal injury and impaired angiogenic response and peritubular capillary formation in the remnant kidney model, whose harmful effects were greater than expected from the increase in BP levels, thus suggesting that an important role of NO in maintaining renal microvasculature (15,16). Therefore, it is plausible that DDAH could protect against renal damage by suppressing the inhibitory effect of ADMA on NO generation. In this study, we investigated the effects of DDAH-elicited ADMA lowering on renal function and tubulointerstitial changes in a rat remnant kidney model. To address the issue of whether DDAH could protect against renal injury in a BP-independent manner, we compared the renoprotective effects of DDAH with those of hydralazine (Hyz), an antihypertensive drug with equihypotensive property.

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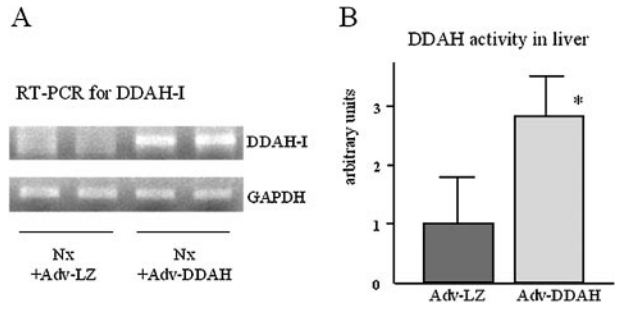

Figure 1. Dimethylarginine dimethylaminohydrolase (DDAH) expression levels in liver. (A) Representative results of reverse transcription–PCR (RT-PCR) bands of DDAH-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. (B) DDAH enzymatic activity of the liver 3 d after infection. *$P < 0.05$ *versus* adenovirus vector–expressing bacterial β-galactosidase (Adv-LZ)-treated diabetic rats.

Materials and Methods

Recombinant Adenoviruses

The plasmid, including the entire coding region of DDAH-I cDNA, was cloned as described previously (17). To produce adenovirus vector encoding human DDAH-I protein under the control of the cytomegalovirus promoter (Adv-DDAH), the entire coding region of DDAH was inserted into an E1, E3-deleted, human adenovirus serotype 5 mutant, dl7001, with homologous recombination in 293 cells (American Tissue Culture Collection, Bethesda, MD) as described previously (17).

Experimental Protocol

After male Sprague-Dawley rats (200 to 250 g) underwent baseline measurement of BP and renal function, five-sixths subtotal nephrectomy (Nx; right nephrectomy with surgical resection of the lower and upper thirds of left kidney) was performed as described previously (10). Four weeks after Nx, BP was measured using a tail-cuff sphygmomanometer using an automated system with a photoelectric sensor (BP-98A; Softron, Tokyo, Japan), and blood and urine samples were collected. Then, the rats were randomly divided into four groups: Rats that were immediately killed ($n = 11$); rats that were treated with tail-vein injection of 1.5×10^{10} plaque-forming units of Adv-DDAH ($n = 15$) or control adenovirus vector–expressing bacterial β-galactosidase (Adv-LZ; $n = 15$); and rats that were orally administered 20 mg/kg per d Hyz, which served as a BP control model ($n = 6$). Fourteen days after the treatment, BP was measured and the rats were transferred to metabolic cages for 2 d for urinalysis. Then, the rats were killed. All experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the ethical committee of our institution.

Chemical Analysis

Plasma levels of L-arginine, ADMA, and symmetric dimethylarginine (SDMA), an inert isomer of ADMA, were measured by HPLC as described previously (10,17). Serum level of creatinine (cr) was determined with commercial kits (Denka Seiken, Co., Tokyo, Japan). Creatinine clearance (Ccr, ml/min) was determined as the following formula: [urine creatinine (mg/dl)] × [urine volume (ml/d)]/[plasma creatinine (mg/dl)]/[1440 (min)].

Immunohistochemistry

The kidneys were removed and fixed in 4% paraformaldehyde. Then the kidneys were embedded in paraffin wax for sectioning. Three-micrometer paraffin sections were incubated with monoclonal JG-12 antibody raised against aminopeptidase P of capillary endothelial cells (Bender MedSystems, San Bruno, CA) or polyclonal antibody raised against TGF-β (Santa Cruz Biotechnology, Santa Cruz, CA). After exposure to peroxidase-labeled secondary anti-mouse antibody, the sections were incubated with 3,3′-diaminobenzidine solution. The intensity of JG-12 or TGF-β staining was analyzed by an image analysis software (Optimas version 6.57; Media Cybernetics, Silver Spring, MD).

Renal Histology Analysis

Three-micrometer paraffin sections were stained with Azan to evaluate interstitial fibrosis. The intensity of the positive for Azan was also quantified by computer image analysis (Optimas version 6.57).

Primers

Primer sequences that were used in semiquantitative reverse transcription–PCR (RT-PCR) were 5′-AGACAGCCGCATCTTCTTGT-3′ and 5′-CCACAGTCTTCTGAGTGGCA-3′ for glyceraldehyde-3-

Table 1. L-arginine and dimethylarginine[a]

Parameter	L-Arginine (μM)	ADMA (μM)	SDMA (μM)	L-Arginine/ADMA
Adv-LZ				
before	80.8 (29.9 to 126.1)	0.70 (0.59 to 0.87)	0.82 (0.66 to 1.06)	104.1 (45.3 to 185.4)
after	64.6 (25.6 to 149.4)	0.73 (0.56 to 0.98)	0.84 (0.62 to 1.56)	86.3 (31.6 to 266.8)
Adv-DDAH				
before	64.2 (33.5 to 128.4)	0.73 (0.45 to 0.98)	0.80 (0.74 to 1.02)	88.9 (42.9 to 173.5)
after	62.6 (13.6 to 153.8)	0.52 (0.40 to 0.74)[b,c]	0.84 (0.70 to 1.05)	118.0 (24.3 to 334.4)[b]
Hyz				
before	69.4 (49.9 to 113.2)	0.71 (0.54 to 0.95)	0.84 (0.6 to 1.41)	81.6 (58.0 to 182.6)
after	71.1 (25.6 to 149.4)	0.74 (0.63 to 0.99)	0.82 (0.69 to 1.60)	83.8 (50.1 to 158.0)

[a]Data are median (range). Adv-LZ, nephrectomized (Nx) rats that were treated with adenovirus encoding β-galactosidase; Adv-DDAH, Nx rats that were treated with adenovirus encoding dimethylarginine dimethylaminohydrolase; Hyz, Nx rats that were treated with hydralazine.

[b]$P < 0.05$ *versus* rats before treatment.

[c]$P < 0.05$ *versus* Adv-LZ.

Table 2. Baseline clinical variables[a]

Variable	*n*	cr	Ccr	UP	SBP
Adv-LZ	15	0.94 (0.71 to 1.46)	0.91 (0.66 to 1.20)	6.0 (2.0 to 24.9)	132 (110 to 142)
Adv-DDAH	15	0.93 (0.56 to 1.41)	1.03 (0.51 to 1.28)	4.7 (1.7 to 16.7)	122 (91 to 155)
Hyz	6	0.98 (0.72 to 1.31)	0.92 (0.63 to 1.47)	5.5 (2.5 to 8.1)	128 (121 to 135)

[a]Data are median (range). Ccr, creatinine clearance (ml/min); UP, urinary protein excretion (g/g creatinine); SBP, systolic blood pressure (mmHg).

phosphate dehydrogenase, 5′-CGTGGCCGTGGTGTGCGAGGA-3′ and 5′-CAGTTCAGACATGCTCACGGGG-3′ for detecting DDAH-I, and 5′-AGACATTCGGGAAGCAGTGCCAG-3′ and 5′-CATGAGGAGCAGGAAGGGTCGG-3′ for TGF-β.

Semiquantitative RT-PCR

Poly(A)$^+$RNA were isolated from kidney cortex and analyzed by RT-PCR as described previously (9,17). The amounts of poly(A)$^+$RNA templates (30 ng) and cycle numbers (23 cycles for DDAH-I gene, 28 cycles for TGF-β gene, and 22 cycles for glyceraldehyde-3-phosphate dehydrogenase gene) for amplification were chosen in quantitative ranges, where reactions proceeded linearly, which had been determined by plotting signal intensities as functions of the template amounts and cycle numbers (18).

DDAH Activity

Total DDAH activity was measured as described previously (17). Briefly, homogenized liver tissue was incubated with 4 μmol/L ADMA and 0.1 mol/L sodium phosphate buffer (pH 6.5) in a total volume of 0.5 ml for 6 h at 37°C. The reaction was stopped by the addition of equal volume of 10% TCA, and the supernatant was boiled with diacetyl monoxime (0.8% [wt/vol] in 5% acetic acid) and antipyrine (0.5% [wt/vol] in 50% sulfuric acid). The amounts of L-citrulline formed were determined with the spectrophotometric analysis at 466 nm.

Statistical Analyses

All data were expressed as median and range or mean ± SE. Experimental groups were compared by ANOVA and, when appropriate, with Scheffe test for multiple comparisons. Linear regression analysis was performed between plasma dimethylarginine and Ccr, peritubular capillary density, the intensity of Azan staining, or proteinuria levels. A level of $P < 0.05$ was accepted as statistically significant.

Results

DDAH Gene Transfer

Determination of infection efficiencies by *in situ* X-Gal staining of the liver revealed that at least 80% of cells were positive

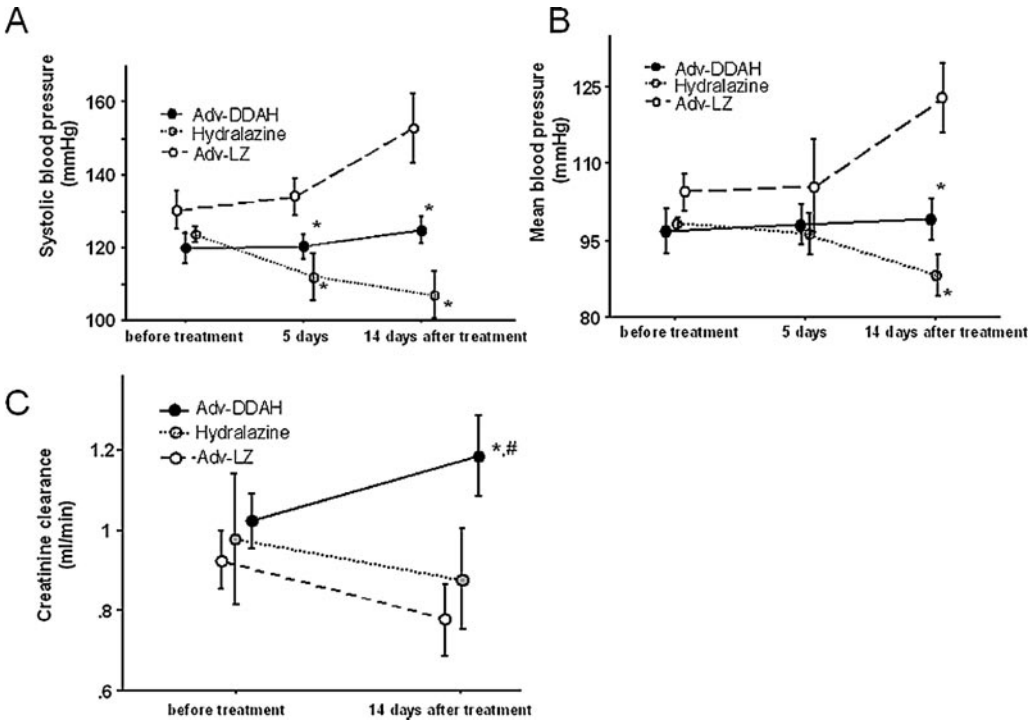

Figure 2. Effects of DDAH overexpression on BP and renal function in rats that underwent five-sixths subtotal nephrectomy (Nx). Four weeks after the Nx, the rats were treated with Adv-DDAH, Adv-LZ, or hydralazine (Hyz). Then, systolic BP (SBP; A), mean BP (B), and creatinine clearance (Ccr; C) were measured at the indicated days after treatment. Data are means ± SEM. *$P < 0.05$ *versus* Adv-LZ; #$P < 0.05$ *versus* Hyz-treated rats.

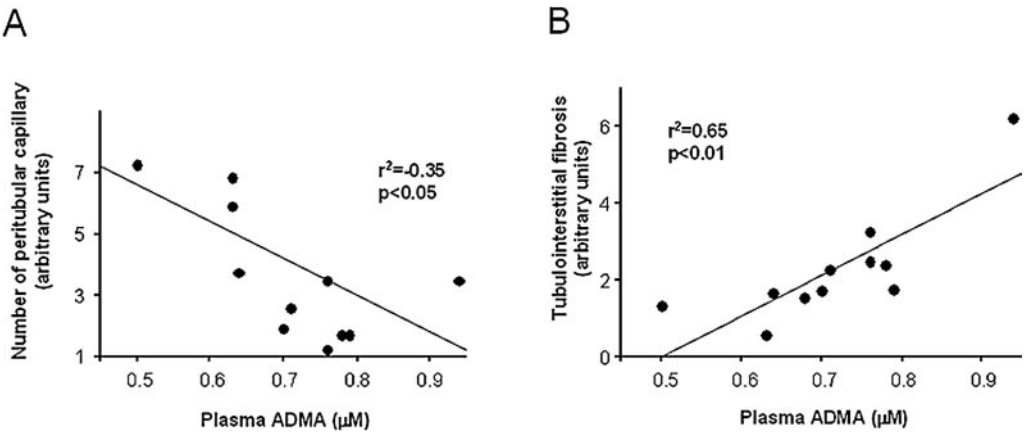

Figure 3. Correlations between plasma levels of asymmetric dimethylarginine (ADMA) and number of peritubular capillaries (A) and extent of tubulointerstitial fibrosis (B). Four weeks after Nx, 11 rats were killed. Then immunohistochemical analysis was performed. Peritubular capillary endothelial cells were stained with JG-12 antibody directed against aminopeptidase P. Tubulointerstitial fibrosis was evaluated by Azan staining.

for β-galactosidase 14 d after the infection with Adv-LZ (data not shown). DDAH infection was found actually to increase its mRNA level and enzymatic activity in the liver by approximately 2.5-fold compared with that of Adv-LZ–infected rats (Figure 1). Adv-DDAH but not Adv-LZ or Hyz significantly decreased plasma levels of ADMA (Table 1). Although each treatment did not affect plasma levels of L-arginine or SDMA, L-arginine/ADMA ratio, which was considered to be a good marker for NO production capacity (1,19), was significantly increased by the treatment with DDAH overexpression and tended to be higher than that in Adv-LZ–treated rats.

Data of Clinical Variables

As shown in Table 2, baseline renal function (cr and Ccr), urinary excretion of protein (UP), and BP were similar among the three groups. As shown in Figure 2, A and B, systolic BP (SBP) as well as mean BP were progressively elevated in Adv-LZ–transfected Nx rats (SBP levels: before infection 130.4 ± 7.7 mmHg, 14 d after infection 153.9 ± 15.9 mmHg; mean BP: levels before infection 104.6 ± 3.5 mmHg, 14 d after infection 122.8 ± 8.7 mmHg), whereas that of Adv-DDAH–transfected Nx rats remained unchanged (SBP levels: before infection 119 ± 4.3 mmHg, 14 d after infection 122 ± 3.8 mmHg; mean BP levels: before infection 96.8 ± 4.4 mmHg, 14 d after infection 99.2 ± 4.0 mmHg). Hyz treatment also prevented the elevation of BP levels in Nx rats (SBP levels: before treatment 126.9 ± 2.2 mmHg, 14 d after treatment 105.8 ± 10.3 mmHg; mean BP levels: before treatment 98.2 ± 1.4 mmHg, 14 d after treatment 88.3 ± 4.1 mmHg).

Dimethylarginine and Renal Function

A simple correlation analysis revealed that baseline Ccr value was inversely correlated to plasma levels of SDMA ($r^2 = -0.56$, $P < 0.01$) and ADMA ($r^2 = -0.20$, $P < 0.05$) in our model. These findings suggest that SDMA is also a good marker of renal function in rats as described previously (20–22). Furthermore, compared with Adv-LZ (Ccr value: before infection 0.93 ± 0.07 ml/min, 14 d after infection 0.77 ± 0.09 ml/min) or Hyz treatment (Ccr value: before treatment 0.98 ± 0.16 ml/min, 14 d after treatment 0.87 ± 0.12 ml/min), Adv-DDAH prevented the decrease of Ccr (Ccr value: before infection 1.04 ± 0.09 ml/min, 14 d after infection 1.20 ± 0.14 ml/min) in Nx rats (Figure 2C).

Relationship between Plasma ADMA Levels and Tubulointerstitial Changes

NO inhibition progresses renal damage in a remnant kidney model by impairing angiogenesis and subsequently causing fibrosis in tubulointerstitial regions (14,23,24). Therefore, we investigated the relationship between plasma ADMA levels and tubulointerstitial changes in Nx rats. As shown in Figure 3, plasma levels of ADMA were associated with decreased number of peritubular capillaries (Figure 3A) and increased tubulointerstitial fibrosis in Nx rats (Figure 3B).

Effects of DDAH Overexpression on Tubulointerstitial Changes

We next investigated the ADMA-lowering effects of Adv-DDAH on tubulointerstitial changes in Nx rats. As shown in Figures 4 and 5, progressive loss of peritubular capillaries and increase in tubulointerstitial fibrosis were observed in Adv-LZ– or Hyz-treated Nx rats, which were ameliorated by the treatment with Adv-DDAH.

Effects of DDAH Overexpression on Renal TGF-β Gene Expression

Because TGF-β is recognized as a major causative factor for the pathogenesis of tubulointerstitial fibrosis in CKD (25,26), we next examined the effect of DDAH overexpression on TGF-β expression in Nx rats. Semiquantitative RT-PCR analysis revealed that TGF-β gene expression was increased in Adv-LZ– or Hyz-treated Nx rats, which was significantly reduced by Adv-DDAH (Figure 6A). Furthermore, immunohistochemistry for TGF-β revealed that tubular protein expression levels of

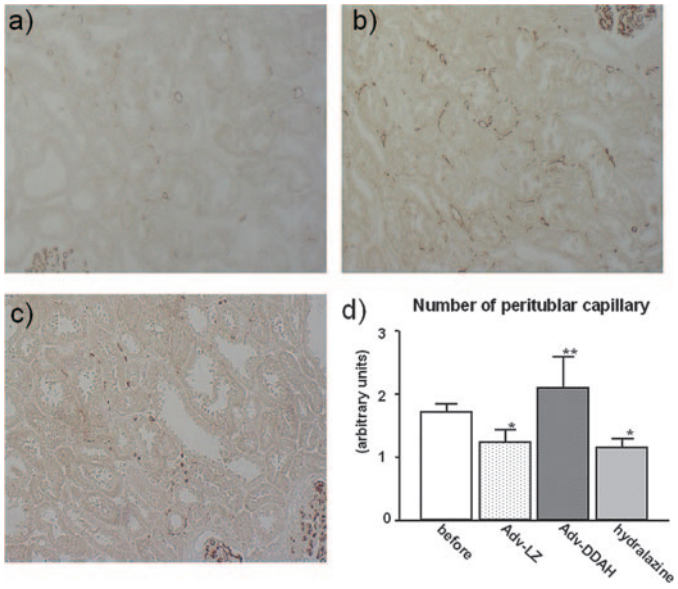

Figure 4. Effects of DDAH overexpression on progressive loss of peritubular capillaries. Representative microphotographs are shown. (A) Adv-LZ–treated rats. (B) Adv-DDAH–treated rats. (C) Hyz-treated rats. (D) Quantitative analysis of staining of peritubular capillaries. *$P < 0.05$ *versus* rats before treatment; **$P < 0.01$ *versus* Adv-LZ or Hyz. Magnification, ×200.

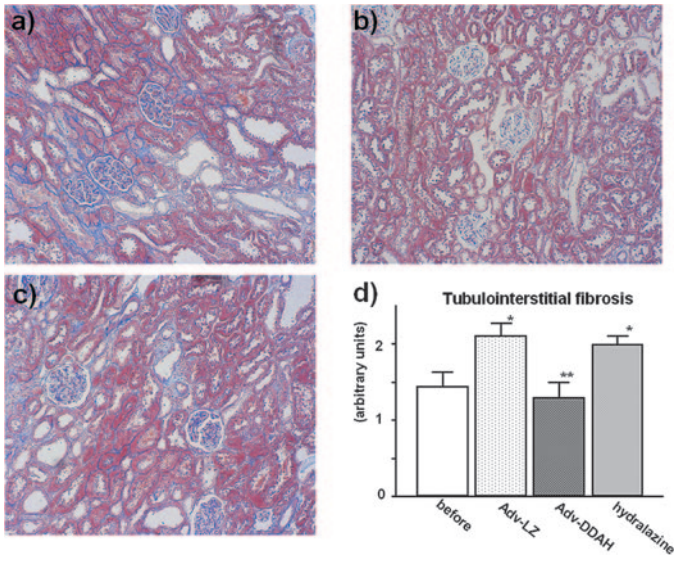

Figure 5. Effects of DDAH overexpression on tubulointerstitial fibrosis. Representative microphotographs are shown. (A) Adv-LZ–treated rats. (B) Adv-DDAH–treated rats. (C) Hyz-treated rats. (D) Quantitative analysis of the extent of tubulointerstitial fibrosis. *$P < 0.05$ *versus* rats before treatment; **$P < 0.01$ *versus* Adv-LZ or Hyz. Magnification, ×12.5.

TGF-β were also increased in Nx rats, which was blocked by DDAH overexpression but not Hyz (Figure 6B).

Effects of DDAH Overexpression on Proteinuria Levels

Recently, Caglar *et al.* (27) showed that ADMA correlates with proteinuria in patients with CKD stage 1. Therefore, we next investigated whether this is also the case in our experimental model. As shown in Figure 7A, plasma levels of ADMA were positively correlated to urinary protein excretion levels. Furthermore, DDAH overexpression but not Adv-LZ or Hyz administration prevented the increase in urinary protein excretion levels (Figure 7B). These observations suggest that ADMA may play a role in the progression of proteinuria in our CKD model.

Discussion

The salient findings of this study were (*1*) overexpression of DDAH-I, a rate-limiting enzyme for the degradation of ADMA, significantly decreased plasma levels of ADMA and subsequently blocked the progression of renal dysfunction in the remnant kidney model (Nx rats); (*2*) plasma levels of ADMA were associated with decreased number of peritubular capillaries and increased tubulointerstitial fibrosis in Nx rats; (*3*) DDAH overexpression prevented the progressive loss of peritubular capillaries and increase in interstitial fibrosis; (*4*) DDAH overexpression suppressed TGF-β gene and protein expression in an experimental model of CKD; (*5*) plasma levels of ADMA were positively correlated with proteinuria; and (*6*) DDAH overexpression significantly prevented the increase in urinary protein excretion levels. Although Hyz treatment blocked the elevation of BP, it did not decrease plasma ADMA level or improve tubulointerstitial changes, renal dysfunction, or urinary protein excretion levels. Therefore, these observations suggest that DDAH could block the progression of renal damage in CKD by reducing plasma ADMA but not BP levels.

The remnant kidney model is widely considered to be the classic model of CKD. Previous studies have reported that the blockade of NO production in this model is associated with increased systemic and glomerular pressure and loss of peritubular capillaries, thereby resulting in a more rapid course of renal scarring (14). Because ADMA is an endogenous NOS inhibitor and its plasma level is elevated in patients with CKD (6,13,28,29), our study suggests that DDAH overexpression may have a protective role against the progression of renal damage in the remnant kidney model by suppressing the inhibitory effect of ADMA on NO generation. These findings that plasma levels of ADMA were associated with tubulointerstitial changes in Nx rats and that Adv-DDAH but not Adv-LZ or Hyz decreased plasma ADMA levels and ameliorated tubulointerstitial changes, renal dysfunction, and proteinuria further support the pathologic role for the ADMA-DDAH system in the progression of CKD. Although a couple of articles suggested that SDMA also could be implicated in NOS inhibition indirectly by interfering in L-arginine transport (21,30), it is unlikely that DDAH overexpression exerted renoprotective and antihypertensive effects in our CKD model *via* modulation of SDMA levels because only ADMA but not SDMA is metabolized by DDAH. We confirmed here that DDAH overexpression did not affect plasma SDMA levels in our model (Table 1).

In our CKD model, DDAH overexpression significantly reduced both systolic and mean BP levels. It was in contrast with the previous findings that DDAH I transgenic mice exhibited no difference in mean BP levels, only in SBP levels (31). Al-

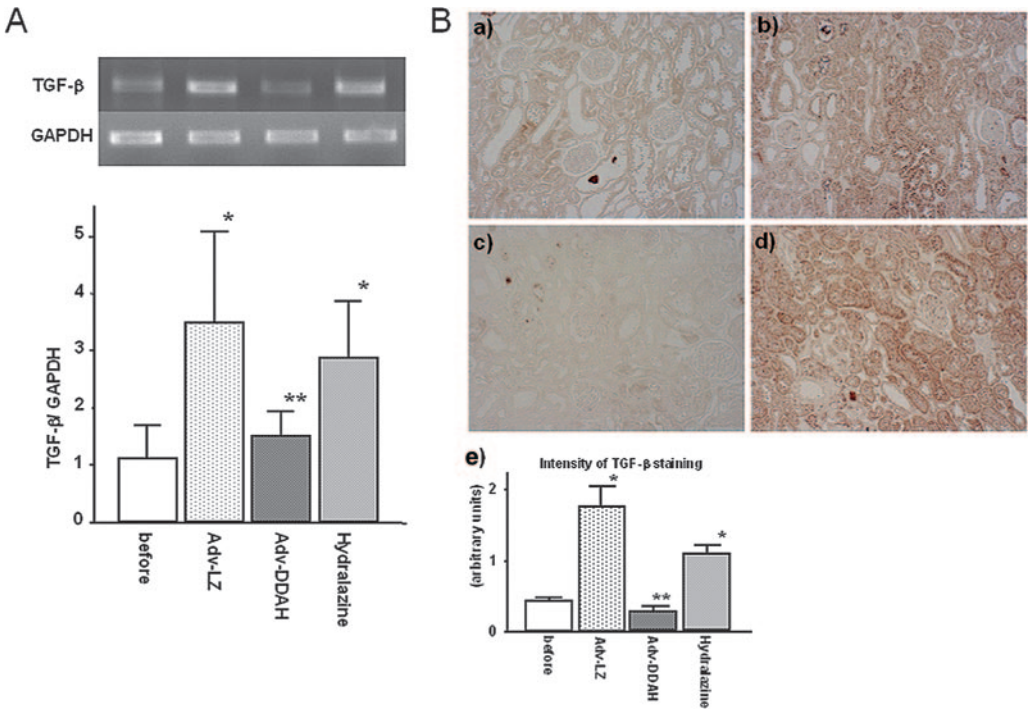

Figure 6. Effect of DDAH overexpression on TGF-β expression. (A, top) Representative results of RT-PCR bands of TGF-β gene. (A, bottom) Quantitative representation of TGF- β gene induction. Data were normalized by the intensity of GAPDH mRNA (n = 5, respectively). (B) Representative microphotographs of TGF-β immunostaining: before treatment (a), Adv-LZ–treated rats (b), Adv-DDAH–treated rats (c), and Hyz-treated rats (d). (e) Quantitative analysis of TGF-β immunostaining. *$P < 0.05$ *versus* rats before treatment; **$P < 0.01$ *versus* Adv-LZ or Hyz. Magnification, ×200.

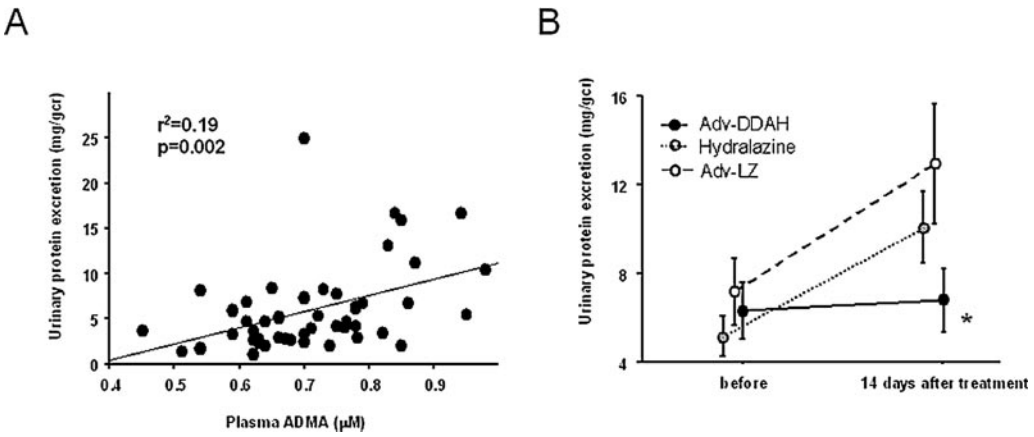

Figure 7. (A) Correlation between plasma levels of ADMA and urinary protein excretion levels in Sprague-Dawley rats 4 wk after Nx (n = 47). (B) Effects of DDAH overexpression on urinary protein excretion levels. Data are means ± SEM. *$P < 0.05$ *versus* Adv-LZ or Hyz.

though we do not know the exact reason for the discrepant results between ours and theirs, the differences of animal species and models and the different method of DDAH overexpression could partly explain the discrepancy.

It has been recognized that proximal tubular cell atrophy and tubulointerstitial fibrosis are more important than glomerulopathy in terms of renal prognosis in CKD (32,33). Peritubular capillary loss may be involved in chronic ischemia and hypoxia in tubulointerstitium, which could stimulate the renal scarring process (32,33). Indeed, a close relationship between progressive interstitial fibrosis and the loss of peritubular capillary was shown in patients with CKD and animal models of CKD (14,34). Because NO inhibits apoptosis of endothelial cells (35) and increases their proliferation and migration (36,37), DDAH could block the progression of renal damage in Nx rats by maintaining the number of peritubular capillaries. In support of this, DDAH transfection into cultured endothelial cells enhances vascular endo-

thelial growth factor mRNA expression and stimulates tube formation of these cells (38). Transfection of DDAH into a glioma tumor cell line promotes tumor angiogenesis and growth *in vivo* (39). Moreover, in a murine model of hindlimb ischemia, enhanced neovascularization and limb perfusion were observed in DDAH transgenic mice, which were associated with reduced plasma levels of ADMA (40). These observations suggest that the ADMA-DDAH axis may regulate angiogenic responses in various diseases.

It has been reported that TGF-β gene is upregulated under hypoxic conditions (41). Therefore, the ADMA-elicited peritubular capillary loss could cause tubulointerstitial ischemia and subsequently enhance TGF-β expression in Nx rats. Because several pieces of evidence have implicated TGF-β as a major causative agent in the pathogenesis of tubulointerstitial fibrosis in CKD (25,26), DDAH may ameliorate tubulointerstitial fibrosis in the remnant kidney model by suppressing tubular ischemia and TGF-β overexpression. Recently, blockade of NO synthesis or NO deficiency was reported to promote cardiac or renal fibrosis, respectively, *via* induction of TGF-β (24,42), thus suggesting the pathologic role for the ADMA-elicited NO reduction in fibrosis in various devastating disorders.

In our CKD model, ADMA levels were correlated to urinary protein excretion levels, and DDAH overexpression but not Hyz prevented the increase in proteinuria. These findings were consistent with the previous report of Caglar *et al.* (27) that showed that ADMA levels were correlated with proteinuria in patients with CKD stage 1. Impaired NO production is the characteristic feature of endothelial dysfunction (1). There is a growing body of evidence that endothelial dysfunction is linked to proteinuria (43–45). Furthermore, ADMA levels are related to endothelial dysfunction in an animal model and patients with CKD (46,47). These observations suggest that the elevation of ADMA levels may exacerbate proteinuria in our CKD model by suppressing the generation of NO.

Conclusion

These data suggest that ADMA may be involved in peritubular capillary loss and tubulointerstitial fibrosis, thereby contributing to the progression of CKD. Although these results may be one of many mechanisms of how ADMA causes deterioration of renal function, substitution of DDAH protein or enhancement of its activity may become a novel therapeutic strategy for the treatment of CKD.

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Disclosures

None.

References

1. Cooke JP: Dose ADMA cause endothelial dysfunction? *Arterioscler Thromb Vasc Biol* 20: 2032–2037, 2000
2. Ueda S, Yamagishi S, Kaneyuki U, Okuda S: Asymmetric dimethylarginine (ADMA) and cardiovascular disease. *Vasc Dis Prev* 3: 291–295, 2006
3. Matsuoka H, Itoh S, Kimoto M, Kohno K, Tamai O, Wada Y, Yasukawa H, Iwami G, Okuda S, Imaizumi T: Asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in experimental hypertension. *Hypertension* 29: 242–247, 1997
4. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T: Endogenous nitric oxide synthase inhibitor: A novel marker of atherosclerosis. *Circulation* 99: 1141–1146, 1999
5. Tarnow L, Hovind P, Teerlink T, Stehouwer CD, Parving HH: Elevated plasma asymmetric dimethylarginine as a marker of cardiovascular morbidity in early diabetic nephropathy in type 1 diabetes. *Diabetes Care* 27: 765–769, 2004
6. Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339: 572–575, 1992
7. Zoccali C, Bode-Boger SM, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frolich J, Boger R: Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. *Lancet* 358: 2113–2117, 2001
8. Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso S, Laaksonen R: Risk of acute coronary event and serum concentration of asymmetrical dimethylarginine. *Lancet* 358: 2127–2128, 2001
9. Schnabel R, Blankenberg S, Lubos E, Lackner KJ, Rupprecht HJ, Espinola-Klein C, Jachmann N, Post F, Peetz D, Bickel C, Cambien F, Tiret L, Munzel T: Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: Results from the AtheroGene Study. *Circ Res* 97: e53–e59, 2005
10. Matsuguma K, Ueda S, Yamagishi S, Matsumoto Y, Kaneyuki Utako, Shibata R, Fujimura T, Matsuoka H, Kimoto M, Kato S, Imaizumi T, Okuda S: Molecular mechanism for elevation of asymmetric dimethylarginine (ADMA) and its role for hypertension in chronic kidney disease. *J Am Soc Nephrol* 17: 2176–2183, 2006
11. Fliser D, Kronenberg F, Kielstein JT, Morath C, Bode-Boger SM, Haller H, Ritz E: Asymmetric dimethylarginine and progression of chronic kidney disease: The mild to moderate kidney disease study. *J Am Soc Nephrol* 16: 2456–2461, 2005
12. Ravani P, Tripepu G, Mallberti F, Testa S, Mallamaci F, Zoccali C: Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: A competing risks modeling approach. *J Am Soc Nephrol* 16: 2449–2455, 2005
13. Zoccali C, Kielstein JT: Asymmetric dimethylarginine: A new player in the pathogenesis of renal disease? *Curr Opin Nephrol Hypertens* 15: 314–320, 2006

14. Kang DH, Nakagawa T, Feng L, Johnson RJ: Nitric oxide modulates vascular disease in the remnant kidney model. *Am J Pathol* 161: 239–248, 2002
15. Erdely A, Wagner L, Muller V, Szabo A, Baylis C: Protection of Wistar Furth rats from chronic renal disease is associated with maintained renal nitric oxide synthesis. *J Am Soc Nephrol* 14: 2526–2533, 2003
16. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, Kearney M, Chen D, Symes JF, Fishman MC, Huang PL, Isner JM: Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest* 101: 2567–2578, 1998
17. Ueda S, Kato S, Matsuoka H, Kimoto M, Okuda S, Morimatsu M, Imaizumi T: Regulation of cytokine-induced nitric oxide synthesis by asymmetric dimethylarginine: Role of dimethylarginine dimethylaminohydrolase. *Circ Res* 92: 226–233, 2003
18. Yamagishi S, Nakamura K, Ueda S, Kato S, Imaizumi T: Pigment epithelium-derived factor (PEDF) blocks angiotensin II signaling in endothelial cells via suppression of NADPH oxidase: A novel anti-oxidative mechanism of PEDF. *Cell Tissue Res* 320: 437–445, 2005
19. Boger RH, Bode-Boger SM, Thiele W, Junker W, Alexander K, Frolich JC: Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation* 95: 2068–2074, 1997
20. Kielstein JT, Salpeter SR, Bode-Boeger SM, Cooke JP, Fliser D: Symmetric dimethylarginine (SDMA) as endogenous marker of renal function: A meta-analysis. *Nephrol Dial Transplant* 21: 2446–2451, 2006
21. Bode-Boger SM, Scalera F, Kielstein JT, Martens-Lobenhoffer J, Breithardt G, Fobker M, Reinecke H: Symmetrical dimethylarginine: A new combined parameter for renal function and extent of coronary artery disease. *J Am Soc Nephrol* 17: 1128–1134, 2006
22. Carello KA, Whitesall SE, Lloyd MC, Billecke SS, D'Alecy LG: Asymmetrical dimethylarginine plasma clearance persists after acute total nephrectomy in rats. *Am J Physiol Heart Circ Physiol* 290: H209–H216, 2006
23. Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ: Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J Am Soc Nephrol* 12: 1448–1457, 2001
24. Boffa JJ, Lu Y, Placier S, Stefanski A, Dussaule JC, Chatziantoniou C: Regression of renal vascular and glomerular fibrosis: Role of angiotensin II receptor antagonism and matrix metalloproteinases. *J Am Soc Nephrol* 14: 1132–1144, 2003
25. Tamaki K, Okuda S, Ando T, Iwamoto T, Nakayama M, Fujishima M: TGF-beta 1 in glomerulosclerosis and interstitial fibrosis of Adriamycin nephropathy. *Kidney Int* 45: 525–536, 1994
26. Okuda S, Languino LR, Ruoslahti E, Border WA: Elevated expression of transforming growth factor-beta and proteoglycan production in experimental glomerulonephritis. Possible role in expansion of the mesangial extracellular matrix. *J Clin Invest* 86: 453–462, 1990
27. Caglar K, Yilmaz MI, Sonmez A, Cakir E, Kaya A, Acikel C, Eyileten T, Yenicesu M, Oguz Y, Bilgi C, Oktenli C, Vural A, Zoccali C: ADMA, proteinuria, and insulin resistance in non-diabetic stage I chronic kidney disease. *Kidney Int* 70: 781–787, 2006
28. Kielstein JT, Boger RH, Bode-Boger SM, Frolich JC, Haller H, Ritz E, Fliser D: Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. *J Am Soc Nephrol* 13: 170–176, 2002
29. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, Boger R; CREED Investigators: Asymmetric dimethylarginine, c-reactive protein, and carotid intima-media thickness in end-stage renal disease. *J Am Soc Nephrol* 13: 490–496, 2002
30. Closs EI, Basha FZ, Habermeier A, Forstermann U: Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric Oxide* 1: 65–73, 1997
31. Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuehlinger MC, Wang BY, Tsao PS, Kimoto M, Vallance P, Patterson AJ, Cooke JP: Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: Genetic and physiological evidence. *Circulation* 108: 3042–3047, 2003
32. Fine LG, Orphanides C, Norman JT: Progressive renal disease: The chronic hypoxia hypothesis. *Kidney Int* 65[Suppl]: S74–S78, 1998
33. Nangaku M: Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. *J Am Soc Nephrol* 17: 17–25, 2006
34. Choi YJ, Chakraborty S, Nguyen V, Nguyen C, Kim BK, Shim SI, Suki WN, Truong LD: Peritubular capillary loss is associated with chronic tubulointerstitial injury in human kidney: Altered expression of vascular endothelial growth factor. *Hum Pathol* 31: 1491–1497, 2000
35. Suschek CV, Schnorr O, Hemmrich K, Aust O, Klotz LO, Sies H, Kolb-Bachofen V: Critical role of L-arginine in endothelial cell survival during oxidative stress. *Circulation* 107: 2607–2614, 2003
36. Ziche M, Parenti A, Ledda F, Dell'Era P, Granger HJ, Maggi CA, Presta M: Nitric oxide promotes proliferation and plasminogen activator production by coronary venular endothelium through endogenous bFGF. *Circ Res* 80: 845–852, 1997
37. Murohara T, Witzenbichler B, Spyridopoulos I, Asahara T, Ding B, Sullivan A, Losordo DW, Isner JM: Role of endothelial nitric oxide synthase in endothelial cell migration. *Arterioscler Thromb Vasc Biol* 19: 1156–1161, 1999
38. Smith CL, Birdsey GM, Anthony S, Arrigoni FI, Leiper JM, Vallance P: Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype. *Biochem Biophys Res Commun* 308: 984–989, 2003
39. Kostourou V, Robinson SP, Cartwright JE, Whitley GS: Dimethylarginine dimethylaminohydrolase I enhances tumour growth and angiogenesis. *Br J Cancer* 87: 673–680, 2002
40. Jacobi J, Sydow K, von Degenfeld G, Zhang Y, Dayoub H, Wang B, Patterson AJ, Kimoto M, Blau HM, Cooke JP: Overexpression of dimethylarginine dimethylaminohydrolase reduces tissue asymmetric dimethylarginine levels and enhances angiogenesis. *Circulation* 111: 1431–1438, 2005
41. Zhang B, Liang X, Shi W, Ye Z, He C, Hu X, Liu S: Role of impaired peritubular capillary and hypoxia in progressive

interstitial fibrosis after 56 subtotal nephrectomy of rats. *Nephrology (Carlton)* 10: 351–357, 2005
42. Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, Yamamoto H, Tamaki K, Shimokawa H, Takeshita A: Early induction of transforming growth factor-beta via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 32: 273–279, 1998
43. Stehouwer CD, Smulders YM: Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. *J Am Soc Nephrol* 17: 2106–2111, 2006
44. Arcos MI, Fujihara CK, Sesso A, de Almeida Prado EB, de Almeida Prado MJ, de Nucci G, Zatz R: Mechanisms of albuminuria in the chronic nitric oxide inhibition model. *Am J Physiol Renal Physiol* 279: F1060–F1066, 2000
45. Sharma M, McCarthy ET, Savin VJ, Lianos EA: Nitric oxide preserves the glomerular protein permeability barrier by antagonizing superoxide. *Kidney Int* 68: 2735–2744, 2005
46. Okubo K, Hayashi K, Wakino S, Matsuda H, Kubota E, Honda M, Tokuyama H, Yamamoto T, Kajiya F, Saruta T: Role of asymmetrical dimethylarginine in renal microvascular endothelial dysfunction in chronic renal failure with hypertension. *Hypertens Res* 28: 181–189, 2005
47. Cross JM, Donald A, Vallance PJ, Deanfield JE, Woolfson RG, MacAllister RJ: Dialysis improves endothelial function in humans. *Nephrol Dial Transplant* 16: 1823–1829, 2001

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