

## Original Article

# Differing Anti-Proteinuric Action of Candesartan and Losartan in Chronic Renal Disease

Hiroto MATSUDA<sup>\*,\*\*</sup>, Koichi HAYASHI<sup>\*\*</sup>, Koichiro HOMMA<sup>\*\*</sup>, Kyoko YOSHIOKA<sup>\*\*</sup>,  
Takeshi KANDA<sup>\*\*</sup>, Ichiro TAKAMATSU<sup>\*\*</sup>, Satoru TATEMATSU<sup>\*\*</sup>,  
Shu WAKINO<sup>\*\*</sup>, and Takao SARUTA<sup>\*\*</sup>

It has becoming clear that angiotensin receptor blockers (ARBs) show varying levels of angiotensin II type 1 (AT1) receptor blocking activity. Although the duration of activity and the efficacy on blood pressure of ARB are reported to vary, depending on the agents used, it has not been examined whether the effects on proteinuria and urinary nitrite/nitrate (NO<sub>x</sub>) excretion differ in hypertensive patients with chronic renal disease. In the present study, patients with hypertension (>140 and/or 90 mmHg) and chronic renal disease (proteinuria >0.5 g/day; serum creatinine <265 μmol/l or creatinine clearance >30 ml/min/1.72 m<sup>2</sup>) were randomly assigned to perindopril- (*n* = 15), trandolapril- (*n* = 15), candesartan- (*n* = 17), and losartan-treated groups (*n* = 15), and were followed up for 96 weeks. All agents decreased blood pressure to the same level, and none of them had any effect on creatinine clearance. Candesartan, perindopril, and trandolapril reduced proteinuria markedly (from 3.0 ± 0.6 to 1.8 ± 0.5 g/day, 2.7 ± 0.5 to 1.6 ± 0.4 g/day, and 2.7 ± 0.5 to 1.7 ± 0.4 g/day, respectively) at 12 weeks, and the beneficial effect persisted throughout the study. The effect of losartan, however, diminished over the study period. Whereas perindopril, trandolapril, and candesartan markedly increased urinary NO<sub>x</sub> excretion (from 257 ± 23 to 1,011 ± 150 μmol/day, 265 ± 70 to 986 ± 130 μmol/day, and 260 ± 62 to 967 ± 67 μmol/day at 12 weeks, respectively), a relatively blunted increase was observed with losartan (from 309 ± 42 to 596 ± 64 μmol/day). In conclusion, renal action of ARB varies, with relatively less proteinuria-sparing, as well as NO<sub>x</sub>-enhancing, effects observed with candesartan showing the greatest reduction of proteinuria and greatest enhancement of NO<sub>x</sub>. Furthermore, renal nitric oxide may contribute to the renal protective action of these agents when administered to patients with chronic renal disease.

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**Key Words:** candesartan, losartan, angiotensin receptor blockers, nitric oxide, chronic renal disease

## Introduction

Since the recognition of the intrarenal renin-angiotensin system as a determinant of the progression of renal injury (1), there has been a growing body of evidence that angiotensin (ANG) converting enzyme inhibitors (ACE-Is) exert beneficial actions on both diabetic and non-diabetic renal disease (2–4). Furthermore, a new tool for the blockade of ANG II activity, the ANG receptor blocker (ARB), has become

available for clinical use, and has recently been demonstrated to be effective in retarding the progression of renal injury in type II diabetic renal disease (5, 6). With the further development of ARBs, it has become clear that these agents show differential levels of activity on the vascular tissue. Thus, in a study examining the pharmacological properties of the ARB, losartan was shown to bind to ANG II type 1 (AT1) receptors in a surmountable fashion, whereas candesartan showed stronger binding to AT1 receptors (7) and manifested insurmountable affinity (7, 8). These differential

From the <sup>\*</sup> Ashikaga Red Cross Hospital, Ashikaga, Japan, and <sup>\*\*</sup> Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan. Address for Reprints: Koichi Hayashi, M.D., Department of Internal Medicine, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan. E-mail: khayashi@sc.itc.keio.ac.jp  
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activities of ARBs *in vitro* suggest that the agents will show similarly heterogeneous actions *in vivo*. Indeed, in a report by Hansson, candesartan was shown to be more potent than other ARBs in reducing systemic blood pressure (9). Nevertheless, the effects of losartan and candesartan on proteinuria and the progression of non-diabetic renal disease remain undetermined.

Several lines of evidence have demonstrated that the pharmacological action of ARB can be attributed in part to the bradykinin-nitric oxide (NO) pathway (10–12). In cardiac muscles, the AT1 blockade by losartan is reported to stimulate bradykinin production (10), which is thought to cause NO production (11). Furthermore, Siragy *et al.* (12) demonstrated that during the pretreatment with losartan, the administration of ANG II elicited an increase in intrarenal cyclic guanosine monophosphate (cGMP) contents. These findings suggest that NO makes a substantial contribution to the action of ARBs, and that intrarenal NO might potentiate the renal vasodilator action of ARBs, just as demonstrated with ACE-Is (13, 14). Nevertheless, although such a contribution of NO to the action of ARBs appears possible, it has not been determined whether ARBs alter the NO production in patients with renal disease.

In the present study, we evaluated the effect of candesartan and losartan on the development of proteinuria for a period of 96 weeks. Furthermore, whether the effect of these agents on urinary excretion of NO metabolites differs was examined.

## Methods

The inclusion criteria for entry into the study were hypertension ( $> 140$  and/or  $90$  mmHg) and proteinuria ( $> 0.5$  g/day). Serum creatinine level  $< 265$   $\mu\text{mol/l}$  or creatinine clearance  $> 30$  ml/min/1.72 m<sup>2</sup> was confirmed before entry into this protocol. The underlying renal diseases were proliferative glomerulonephritis ( $n = 58$ ), membranous nephropathy ( $n = 2$ ), or focal segmental glomerulosclerosis ( $n = 2$ ). Patients with diabetic nephropathy, polycystic kidney disease, or chronic pyelonephritis were excluded from this study. The patients had been educated on dietary therapy, including the importance of low protein (0.8 g/kg/day) and low sodium intake (7 g/day) for at least 3 months before entry into this study. Adherence to the dietary restrictions was evaluated by measuring daily urinary sodium and urea nitrogen excretion. The study was approved by the institutional ethical committee, and informed consent was obtained from all patients.

At entry, the patients were randomly assigned to ACE-I-treated and ARB-treated groups. After the 4-week observational period, either perindopril (2 mg/day), trandolapril (0.5 mg/day), losartan (25 mg/day), or candesartan cilexetil (4 mg/day) was administered once a day, and the doses were titrated to achieve systemic blood pressure of less than 135/85 mmHg. Of 62 patients, 14 patients had received antiplatelet therapy (dipyridamole or dilazep dihydrochloride), and these drugs were continued throughout the protocol.

## Laboratory Procedures

On every visit, systolic (SBP) and diastolic blood pressure (DBP) were measured twice after the 5-min sedentary position in the morning (during 9:00 to 12:00 AM). Twenty-four hour creatinine clearance was used for evaluation of renal function and was evaluated during the control period and treatment periods (12, 24, 48, 72, and 96 weeks). Laboratory examinations, including assays of blood chemistry (serum creatinine and potassium) and daily urinary protein excretion, were made at 12, 24, 48, 72, and 96 weeks. Urinary protein was measured at a central laboratory (Laboratory Division, Ashikaga Red Cross Hospital, Ashikaga, Japan) using a standard method (the pyrogallol red molybdate method; Micro TP-Test, Wako, Tokyo, Japan), and daily urinary protein excretion was determined by collecting 24-h urine samples.

Daily excretions of urinary metabolites of NO (*i.e.*, nitrate and nitrite) were assessed by Griess reaction (14, 15), and the sum of these values (NO<sub>x</sub>) was used as a marker of urinary excretion of NO. The intra-assay coefficient of variation for NO<sub>x</sub> was less than 6%.

## Data Analysis

Results are expressed as the mean  $\pm$  SEM. Statistical analysis was performed with two-way analysis of variance with repeated-measures, followed by Fisher's post hoc test. Values of  $p < 0.05$  were considered to indicate statistical significance.

## Results

### Effects of ACE-I and ARB on Systemic and Renal Function

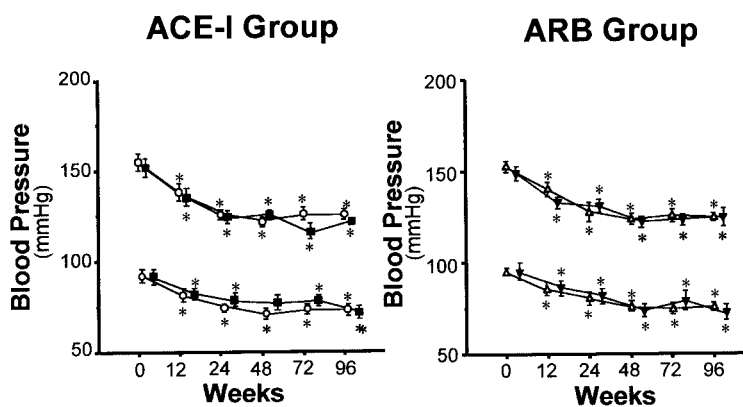
The baseline profiles in each group are shown in Table 1. There was no difference in age, SBP, DBP, serum creatinine, creatinine clearance, serum potassium, or urinary protein excretion among these groups.

Figure 1 illustrates the changes in SBP and DBP in patients treated with an ACE-I (left) or an ARB (right). ACE-Is caused marked reductions in SBP (perindopril, from  $155 \pm 3$  to  $136 \pm 3$  mmHg,  $p < 0.01$ ,  $n = 15$ ; trandolapril, from  $154 \pm 6$  to  $132 \pm 4$  mmHg,  $p < 0.01$ ,  $n = 15$ ) and DBP (perindopril, from  $91 \pm 4$  to  $78 \pm 5$  mmHg,  $p < 0.01$ ; trandolapril, from  $90 \pm 3$  to  $79 \pm 3$  mmHg,  $p < 0.01$ ) at 12 weeks. In the patients treated with an ARB, baseline blood pressures did not differ between the candesartan- ( $152 \pm 2/93 \pm 2$  mmHg,  $n = 17$ ) and losartan-treated groups ( $150 \pm 4/93 \pm 3$  mmHg,  $n = 15$ ). Twelve-week treatment with an ARB also caused significant reductions in SBP (candesartan,  $138 \pm 3$  mmHg,  $p < 0.05$ ; losartan,  $134 \pm 3$  mmHg,  $p < 0.05$ ) and DBP (candesartan,  $79 \pm 3$  mmHg,  $p < 0.05$ ; losartan,  $83 \pm 3$  mmHg,  $p < 0.05$ ), and these reductions were sustained throughout the study.

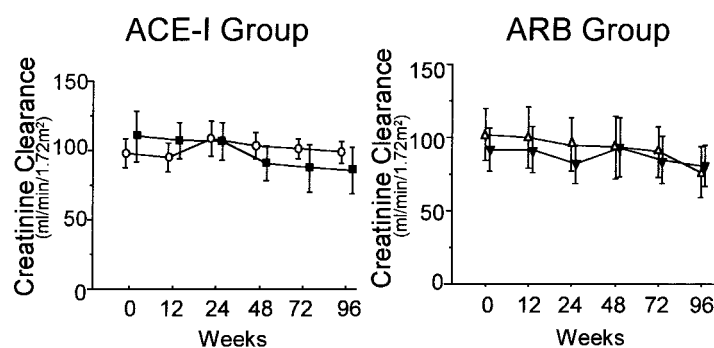
**Table 1. Baseline Characteristics of Patients with Proteinuria**

	ACE-I		ARB	
	Perindopril	Trandolapril	Candesartan	Losartan
N (male/female)	15 (8/7)	15 (9/6)	17 (9/8)	15 (7/8)
Age (y/o)	51 ± 4	50 ± 5	58 ± 5	51 ± 3
Systolic blood pressure (mmHg)	155 ± 3	154 ± 6	152 ± 2	150 ± 4
Diastolic blood pressure (mmHg)	91 ± 4	90 ± 3	93 ± 2	93 ± 3
Serum creatinine (μmol/l)	79.6 ± 8.0	79.6 ± 8.7	88.5 ± 8.2	97.3 ± 17.1
Creatinine clearance (ml/min/1.72 m <sup>2</sup> )	98 ± 10	115 ± 18	102 ± 18	89 ± 15
Serum potassium (mEq/l)	4.1 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.0 ± 0.2
Urinary protein excretion (g/day)	2.7 ± 0.5	2.7 ± 0.5	3.0 ± 0.6	2.5 ± 0.4

ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers.



**Fig. 1.** Changes in systemic blood pressure by angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) in patients with proteinuria. Circles, perindopril; squares, trandolapril; triangles, candesartan; inverted triangles, losartan. Results are the means ± SEM. \*  $p < 0.05$  vs. 0 week.

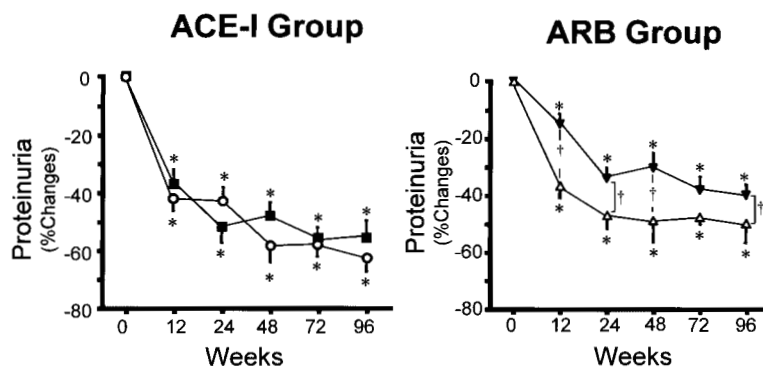


**Fig. 2.** Changes in creatinine clearance by angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) in patients with proteinuria. Circles, perindopril; squares, trandolapril; triangles, candesartan; inverted triangles, losartan. Results are the means ± SEM.

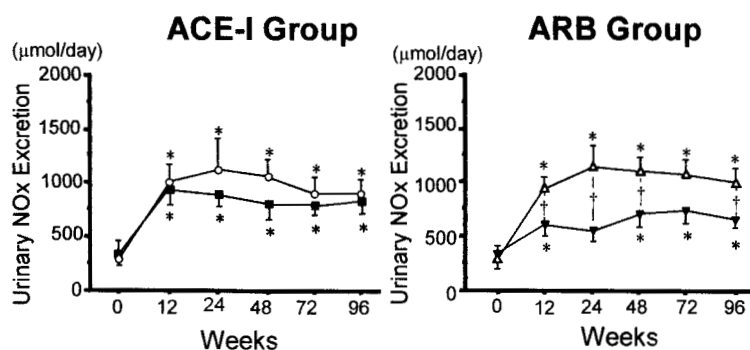
There were no significant differences in blood pressure or in the changes in blood pressure between these treatment groups at any period. The final doses of the agents used were 6.6 mg/day, 1.8 mg/day, 7.8 mg/day, and 81 mg/day for perindopril, trandolapril, candesartan, and losartan, respectively.

The changes in creatinine clearance are shown in Fig. 2. Neither ACE-I nor ARB had any effect on creatinine clearance throughout the study protocol. Serum potassium levels were also unaltered throughout the protocol in all groups (data not shown).

The effects of ACE-Is and ARBs on urinary protein excre-



**Fig. 3.** Effects of angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) on urinary protein excretion in patients with proteinuria. Circles, perindopril; squares, trandolapril; triangles, candesartan; inverted triangles, losartan. Results are the means  $\pm$  SEM. \*  $p < 0.05$  vs. 0 week. †  $p < 0.05$ .



**Fig. 4.** Effects of angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) on urinary nitrite/nitrate ( $\text{NO}_x$ ) excretion in patients with proteinuria. Circles, perindopril; squares, trandolapril; triangles, candesartan; inverted triangles, losartan. Results are the means  $\pm$  SEM. \*  $p < 0.05$  vs. 0 week. †  $p < 0.05$ .

tion are summarized in Fig. 3. At 12 weeks, both perindopril and trandolapril elicited  $42 \pm 6\%$  (from  $2.7 \pm 0.5$  to  $1.6 \pm 0.4$  g/day,  $p < 0.05$ ,  $n = 15$ ) and  $37 \pm 6\%$  (from  $2.7 \pm 0.5$  to  $1.7 \pm 0.4$  g/day,  $p < 0.05$ ,  $n = 15$ ) reductions in proteinuria, and the decreases in proteinuria reached  $60 \pm 7\%$  ( $1.0 \pm 0.4$  g/day) and  $53 \pm 7\%$  ( $1.3 \pm 0.4$  g/day) at 96 weeks, respectively. Candesartan also produced a marked (*i.e.*,  $38 \pm 4\%$ ) decrease in proteinuria (from  $3.0 \pm 0.6$  to  $1.8 \pm 0.5$  g/day,  $n = 17$ ) at 12 weeks, and exerted a sustained anti-proteinuric action throughout the study. In contrast, the ability of losartan to decrease proteinuria was diminished compared with that of candesartan. Thus, proteinuria was reduced by  $12 \pm 3\%$  at 12 weeks (from  $2.6 \pm 0.4$  to  $2.3 \pm 0.4$  g/day,  $p < 0.05$ ,  $n = 15$ ), and the diminished action was observed throughout the protocol ( $p < 0.05$ ). At 96 weeks, losartan caused a  $36 \pm 4\%$  decrease in proteinuria, a value significantly less than that by candesartan ( $49 \pm 5\%$  decrement,  $p < 0.05$ ).

#### Link between NO and Urinary Protein Excretion in Patients with Moderate Proteinuria

We further examined the effects of ACE-Is and ARBs on

urinary  $\text{NO}_x$  excretion in patients with proteinuria (Fig. 4). In both the perindopril and trandolapril groups, urinary  $\text{NO}_x$  excretion was markedly increased at 12 weeks (perindopril, from  $257 \pm 23$  to  $1,011 \pm 150$   $\mu\text{mol/day}$ ,  $p < 0.01$ ,  $n = 15$ ; trandolapril, from  $265 \pm 70$  to  $986 \pm 130$   $\mu\text{mol/day}$ ,  $p < 0.01$ ,  $n = 15$ ), and the augmented excretion persisted throughout the study. Candesartan elicited increases in urinary  $\text{NO}_x$  excretion comparable to those observed with the ACE-Is. Thus, at 12 weeks, urinary  $\text{NO}_x$  excretion increased markedly from  $260 \pm 62$  to  $967 \pm 67$   $\mu\text{mol/day}$  (*i.e.*, a  $272 \pm 31\%$  increment,  $p < 0.01$ ,  $n = 17$ ), and remained elevated throughout the study. In contrast, losartan caused  $93 \pm 34\%$  increments (from  $309 \pm 42$  to  $596 \pm 64$   $\mu\text{mol/day}$ ,  $p < 0.05$ ,  $n = 15$ ) at 12 weeks ( $p < 0.05$  vs. candesartan). At 96 weeks, the urinary  $\text{NO}_x$  excretion ( $617 \pm 60$   $\mu\text{mol/day}$ ) was less than that observed with candesartan ( $1,002 \pm 89$   $\mu\text{mol/day}$ ,  $p < 0.05$ ).

#### Discussion

Despite the recognition that chronic renal injury relentlessly progresses to end-stage renal disease requiring dialysis therapy, specific treatment modalities that consistently retard the

progression of non-diabetic renal disease have only recently become available. Since the discovery of the renal protective action of benazepril (4), however, there has been a growing body of evidence that ACE-Is exhibit renal protective action in non-diabetic renal disease (16, 17). Similarly, it has recently been demonstrated that the progression of renal injury in diabetic nephropathy is prevented by the inhibition of ANG II activity by ARBs (5, 6, 18). Although these observations clearly indicate the renal protective action of ARBs, there exists some heterogeneity in their antagonistic activity on AT1 receptors (7, 9, 19, 20). Furthermore, it has been demonstrated ARBs, like ACE-Is, enhance the production of NO *via* AT1 receptor blockade (10, 21). Thus, it remains to be clarified whether all of the ARBs have similarly potent antiproteinuric activity in chronic renal disease. Furthermore, it has not been examined whether NO production differs among ARBs.

In the present study, we have demonstrated that in patients with proteinuria, all of the ACE-I and ARB studied reduce blood pressure by a similar magnitude (Fig. 1). Similarly, creatinine clearance was unaltered by 96-week treatment with any of the ACE-Is or ARBs (Fig. 2). However, these agents showed varying effects on proteinuria (Fig. 3). Both ACE-I and candesartan decreased proteinuria at 12 weeks, and elicited a sustained anti-proteinuric action thereafter. In contrast, losartan induced much smaller decreases in proteinuria. These observations indicate heterogeneity in the renal action of these agents, and further suggest that the divergent temporal profiles of proteinuria are attributable to the renal action of these agents *per se*. However, a caveat is in order here, since in the present study the blood pressure was measured at the clinic after 2–5 h of administration of the antihypertensive agents. It has been demonstrated that candesartan possesses a longer blocking activity on AT1 receptors than losartan (7, 9, 20, 22). Thus, although the clinic blood pressure was nearly identical, the trough level of blood pressure may have differed between these treatment groups (22). Apparently, the heterogeneity in the activity of these agents may affect the proteinuria-sparing effect. Of interest, the decreased proteinuria returned to the pre-treatment level at the end of the protocol in 3 patients of the trandolapril-treated group, whereas no such escape was observed in the other groups. Whether this phenomenon is associated with aldosterone escape (23) requires additional evaluation.

Whereas it has been established that the action of ACE-Is involves multiple mechanisms, including ANG inhibition and bradykinin accumulation, the effect of ARBs on renal NO production remains undetermined. In the present study, we demonstrated that ACE-Is caused prominent increases in urinary NO<sub>x</sub> excretion at 12 weeks, and the increased levels persisted until the end of the study (Fig. 4). These effects were expected due to the NO synthase stimulation by the ACE-I-mediated bradykinin accumulation. In contrast, candesartan and losartan elicited distinct actions on the urinary NO<sub>x</sub> excretion. Candesartan enhanced the urinary NO<sub>x</sub> excre-

tion to nearly the same level as ACE-Is, whereas the ability of losartan to increase the NO<sub>x</sub> excretion was diminished, particularly in the earlier periods. Although the mechanism responsible for this differential action on urinary NO<sub>x</sub> excretion was not readily apparent, ARBs have been reported to stimulate bradykinin production in the systemic vasculature (24, 25), cardiac muscles (10), and renal parenchyma (11, 12). Since ARBs preferentially antagonize AT1 receptors, leaving ANG II type 2 (AT2) receptors relatively unblocked, and chronic ARB administration is expected to elevate plasma ANG II levels (26), the bradykinin-induced NO may play a substantial role in mediating the increase in urinary NO<sub>x</sub> excretion during treatment with an ARB. In addition, the fact that candesartan exhibits more beneficial effects than losartan may be related, at least in part, to the fact that the former drug shows greater selectivity for the blocking of AT1 receptors over the blocking of AT2 receptors, which could affect the NO production (7). Alternatively, it has recently been demonstrated that the AT1 receptor blockade *per se* elicits NO production (27).

The role of NO in mediating the antiproteinuric action of ARB merits comment. The present study demonstrated an inverse relationship between the changes in urinary NO<sub>x</sub> excretion and those of proteinuria (Figs. 3 and 4). Although there is still controversy regarding the origin of urinary NO<sub>x</sub>, urinary NO<sub>x</sub> reflects, at least in part, the renal production (28, 29). Since renal NO is assumed to protect against the progression of renal injury (30, 31), the enhanced renal NO, in addition to ANG blockade, teleologically would contribute to the reduction in proteinuria. Furthermore, bradykinin, which stimulates NO production (12, 14), elicits predominant dilation of the efferent arterioles (13, 32), and therefore would reduce glomerular capillary pressure. Alternatively, the elevation in urinary NO<sub>x</sub> may simply be a consequence of the renal protection conferred by long-term treatment. Clearly, further studies will be needed to clarify the mechanism of the heterogeneity in the renal protective action of ACE-I and ARB. Whether candesartan confers better renal protection than losartan remains to be determined.

In conclusion, the present study showed that blockade of ANG activity by ACE-Is (perindopril and trandolapril) and ARBs (candesartan and losartan) decreased blood pressure and urinary protein excretion in patients with proteinuria. The ability of these agents to reduce proteinuria differs, however, with losartan showing the lesser ability to decrease proteinuria compared with candesartan and the ACE-Is. Furthermore, the changes in proteinuria paralleled those of urinary NO<sub>x</sub> excretion. The heterogeneity in the ability to reduce proteinuria and enhance NO<sub>x</sub> excretion among these agents may represent agent-specific characteristics, and could be related to differences in the drugs' ability to block ANG activity.

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