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Is the antiproteinuric effect of ACE inhibition mediated by interference in the renin-angiotensin system?

RON T. GANSEVOORT, DICK DE ZEEUW, and PAUL E. DE JONG

Department of Medicine, Division of Nephrology, State University Hospital, Groningen, The Netherlands

Is the antiproteinuric effect of ACE inhibition mediated by interference in the renin-angiotensin system? Angiotensin converting enzyme (ACE) inhibition causes specific renal effects, such as a rise in effective renal plasma flow, a fall in filtration fraction and a lowering of proteinuria. The mechanism of these renal effects is still debated. Recent animal studies suggest that non-angiotensin (Ang) II related actions of ACE inhibition, such as bradykinin accumulation, may have a role. We therefore investigated the effects of specific intervention in the renin-angiotensin system with the Ang II receptor antagonist losartan, and compared these effects to those obtained with ACE inhibition, as this comparison might resolve the question whether or not the effects of ACE inhibition are Ang II related. The effects of losartan and enalapril were studied in eleven patients with non-diabetic proteinuria and hypertension. The protocol consisted of seven periods, each lasting one month, in which patients received once daily placebo, 50 mg losartan, 100 mg losartan, placebo, 10 mg enalapril, 20 mg enalapril, and placebo, respectively. At the end of each study period proteinuria, blood pressure, and renal function were determined. On both doses of losartan and enalapril proteinuria and blood pressure fell, whereas ERPF increased and GFR remained stable. The fall in urinary protein excretion was similar for both drugs: 46.3% (28.3% to 63.1%) on 100 mg losartan versus 51.6% (37.0% to 69.2%) on 20 mg enalapril (expressed as Wilcoxon-based estimated median with 95% CI). The same held true for the fall in blood pressure [15.1% (12.7% to 20.2%) vs. 17.3% (15.4% to 22.0%)], the rise in ERPF [13.3% (4.2% to 23.4%) vs. 13.1% (4.1% to 27.0%)] and the fall in FF [15.1% (5.7% to 20.6%) vs. 14.6% (4.7% to 22.7%)]. In conclusion, the Ang II receptor antagonist losartan induces changes in blood pressure, renal hemodynamics, and proteinuria similar to those induced by ACE inhibition. These data support the idea that the antiproteinuric effect of ACE inhibition, as well as the renal hemodynamic effects, are primarily mediated by interference in the renin-angiotensin system.

Angiotensin I converting enzyme (ACE) inhibitors show, in addition to their antihypertensive capacity, unique renal effects, such as a rise in effective renal plasma flow, a lowering in filtration fraction and a decrease in urinary protein excretion [1]. Because of these qualities, ACE inhibitors have drawn much attention as possible renoprotective agents and as treatment modality for patients with the nephrotic syndrome [1, 2]. The mechanism of these renal effects is not yet fully understood. Since in experimental renal disease infusion of angiotensin II (Ang II) was proven to induce opposite effects, that is, renal vasoconstriction and an increase in proteinuria [3–6], it was assumed that the renal effects of ACE inhibitors are the result of inhibition of Ang II formation.

However, angiotensin I converting enzyme is identical to kininase II, the enzyme that catabolyzes the vasodilator hormone bradykinin [7]. Therefore, ACE inhibition is also hypothesized to result in accumulation of bradykinin. Indeed, a number of recent animal studies suggest that bradykinin-related effects of ACE inhibition contribute to the renal effects [8–15]. Moreover, we recently showed in patients that Ang II infusion did not reverse the antiproteinuric effect of long-term ACE inhibition, despite offsetting the systemic and renal hemodynamic effects induced by this treatment [16]. These findings confirm the role of Ang II in the renal hemodynamic effects of ACE inhibition; however, the role of Ang II in the antiproteinuric effect is clearly questioned.

Recently, an orally active nonpeptide Ang II antagonist has been developed. This novel drug (losartan), and its active carboxylic acid metabolite effectively block the Ang II receptor (type 1). Both lack agonistic activity and appear to antagonize all known physiological functions of the renin-angiotensin system [17, 18]. Interestingly, losartan has no effect on the kinin-kallikrein system [19]. The objectives of the present study were first to investigate the hemodynamic and antiproteinuric effects of specific Ang II receptor antagonism in hypertensive proteinuric patients, and second, to compare the responses obtained with losartan to those obtained during ACE inhibition, as this comparison might resolve the question whether or not the effects of ACE inhibition are Ang II related.

Methods

Patients and protocol

Patients were selected from the cohort that consecutively attended our renal outpatient department from the starting day of the study. Entry criteria for this study were: non-diabetic chronic renal disease, with mild to moderate hypertension (90 mm Hg $\leq$ DBP $\leq$ 115 mm Hg), normal or mildly impaired stable renal function (creatinine clearance $\geq$ 60 ml/min), and stable proteinuria exceeding 2.0 grams per day. Patients with diabetes, edema, or renovascular hypertension were not allowed to participate. Before enrollment all antihypertensive medications, including diuretics, were withdrawn for at least four weeks. All subjects gave their informed consent for participation in this protocol, which was approved by the local Medical Ethical Committee.

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Table 1. The effects of Ang II receptor antagonism and ACE inhibition in eleven patients

			Ang II A	
		Placebo	50 mg	100 mg
MAP *mm Hg*		114.6 [108.9–120.0]	99.7 [94.6–104.0][a]	95.8 [90.0–102.0][a]
GFR *ml/min*		70.1 [59.5–82.3]	70.1 [60.3–81.2]	67.0 [60.6–75.5]
ERPF *ml/min*		321 [289–349]	366 [326–407][a]	363 [334–397][a]
FF %		23.4 [19.0–24.5]	20.1 [17.6–21.6]	19.0 [16.9–20.4][a]
U_{prot} *g/day*		4.48 [3.33–8.84]	3.00 [2.11–6.85][a]	2.43 [1.33–6.70][a]
ACE activity *U/liter*	07:45	27.0 [22.0–35.8]	29.1 [24.5–34.0]	28.5 [21.3–36.0]
	12:00	22.3 [21.0–29.5][b]	22.8 [19.9–25.0][b]	23.0 [20.5–31.9][b]
PRA *nmol/liter/hr*	07:45	1.7 [0.8–3.3]	4.9 [2.4–8.3][a]	6.4 [3.8–8.6][a]
	12:00	1.4 [0.8–3.2]	5.8 [2.7–9.1][a]	9.9 [3.4–16.1][ab]
Ang II *pmol/liter*	07:45	10.3 [6.2–13.0]	21.8 [13.8–28.5][a]	25.9 [17.9–33.0][a]
(N = 10)	12:00	7.4 [5.1–12.4]	29.5 [15.7–42.9][ab]	39.3 [21.0–53.2][a]
U_{Na} *mmol/day*		127 [84–181]	118 [103–143]	116 [88–154]
U_{urea} *mmol/day*		346 [298–414]	351 [315–390]	363 [327–408]

The study was performed on an ambulatory basis, and was designed in single-blind, longitudinal order. During the study patients adhered to a diet containing 1 g protein per kg body weight and 100 mmol sodium per day. Patients were followed during seven periods, each of four weeks, in which they received once daily placebo, losartan (50 mg), losartan (100 mg), placebo, enalapril (10 mg), enalapril (20 mg) and again placebo, respectively. Blood pressure and urinary protein excretion (24 hr urine) were measured once weekly during the first four periods, and at the end of the last three periods. Blood pressure was recorded after patients had taken study medication at home. At the end of all study period patients collected three consecutive 24-hour urines (of which the mean value is used for data evaluation), whereafter patients were admitted for an in-hospital renal function assessment. On these renal function study days patients took medication at 8:00 a.m., and blood pressure was measured at 12:00 a.m. Both before (07:45 a.m.) and after drug administration (12:00 a.m.) blood was drawn for the determination of PRA, ACE activity and Ang II concentration. Medication compliance was assessed by tablet counts. Patients who forgot to take study medication more than once a week were to be excluded from analysis.

Clinical and laboratory procedures

Serum and urinary electrolytes, urea and creatinine were determined with an automated multi-analyzer (SMA-C, Technicon®), while urinary protein was determined in each urine sample with the pyrogallol red-molybdate method [20]. The intra-assay coefficient of variation of this method is less than 3.3%, while the inter-assay coefficient of variation is less than 3.0%. During every visit, after at least 15 minutes of supine rest, blood pressure was measured five consecutive times using a Hawksley random-zero-sphygmomanometer. The first and fifth Korotkoff sounds were used as indications for systolic and diastolic blood pressure. The mean of three intermediate readings was recorded. MAP was calculated as the sum of one-third of the systolic and two-thirds of the diastolic blood pressure. GFR and ERPF were measured according to a previously described method using a constant infusion of ^{125}I-iothalamate and ^{131}I-hippuran, respectively [21]. The intra-patient day-to-day coefficient of variation of this method is 2.2% for GFR and 5.0% for ERPF. Both parameters were corrected for standard body surface area (1.73 m^2). Filtration fraction was calculated as the ratio of GFR and ERPF. Serum ACE activity was measured using a HPLC-assisted assay [22]. Plasma for the determination of PRA and Ang II levels was drawn after 30 minutes of supine rest in prechilled tubes. Blood was immediately centrifuged at 4°C, and plasma was stored at −20°C until analysis. PRA was assessed by the quantitation of generated Ang I as measured by radioimmunoassay (Rianen® Ang I RIA kit) [23]. Blood for determination of Ang II levels was collected in tubes containing 1,10-phenantroline, EDTA and captopril to prevent *in vitro* generation or degradation of Ang II. Ang II was determined by radioimmunoassay [24]. Cross-reactivity of the Ang II antibody with Ang I is less than 0.1%, and 100% with Ang III. The detection range of this assay is 2.5 to 200 fmol/ml with an intra-assay coefficient of variation of 8%.

Data analysis

Data are expressed as a Wilcoxon-based estimated median, with 95% confidence intervals (95% CI) [25], unless otherwise indicated. Parameters are expressed as absolute value or as percentage change from the placebo baseline value. To test for differences between the various placebo periods a Friedmann two-way, non-parametric ANOVA was used using baseline placebo data as reference [26], followed by Duncan's correction for multiple comparisons [27]. The same procedure was used to test differences between baseline and active treatment. The median difference in response on the Ang II antagonist and the ACE inhibitor, both expressed as percentage change from baseline, was estimated with a Wilcoxon-based method for paired observations, including their 95% CI [25]. For post-hoc analysis, the individual responses during treatment with the Ang II antagonist were expressed as percentage of the response obtained during ACE inhibition. The type II error (β-error) was calculated with a Wilcoxon-based method for paired observations. Changes in hormonal parameters pre-dose versus post-dose were tested using a Wilcoxon matched-pairs test. Statistical significance was assumed at a 5% level (two-sided).

Results

Eleven patients completed the protocol. None of these had to be excluded from analysis because of insufficient medication compliance. Two were of female gender. All patients were Caucasian and their median age was 42.0 years (range 20 to 61 years). All had mild to moderate hypertension [SBP 151.0 mm

Table 1. *Continued*

Placebo	ACEi 10 mg	ACEi 20 mg	Placebo
110.2 [104.1–115.7][a]	96.5 [91.4–103.3][a]	92.9 [88.1–98.0][a]	107.3 [98.5–115.7]
71.5 [62.2–81.2]	70.4 [62.8–76.8]	65.2 [58.9–77.0]	70.6 [62.7–78.5]
324 [284–347]	363 [333–396][a]	360 [329–387][a]	315 [281–341]
23.1 [19.7–24.4]	19.4 [17.2–21.5][a]	18.8 [16.8–20.7][a]	23.3 [20.2–24.9]
4.28 [2.43–7.44]	2.49 [1.48–6.70][a]	2.14 [1.23–5.45][a]	4.23 [2.11–7.06]
28.0 [22.3–34.0]	15.3 [9.3–20.8][a]	14.3 [7.5–23.8][a]	29.5 [23.0–36.1]
24.5 [20.0–29.3][b]	4.0 [2.5–5.5][ab]	3.0 [2.5–4.0][ab]	26.5 [21.5–31.8]
2.3 [1.4–3.7]	6.4 [3.4–9.7][a]	10.4 [6.6–14.1][a]	1.9 [1.2–2.7]
2.2 [1.4–3.4][a]	9.7 [4.0–17.1][ab]	13.1 [6.6–22.0][a]	1.7 [1.1–2.4]
8.0 [5.8–9.1]	8.6 [5.7–11.1]	7.7 [5.2–10.7]	7.5 [4.2–10.5]
7.8 [5.5–9.5]	4.4 [2.7–6.7][ab]	5.0 [2.9–7.1][ab]	7.5 [4.7–10.3]
143 [107–179]	118 [92–150]	126 [87–157]	131 [95–174]
354 [299–410]	386 [336–446]	382 [347–414]	354 [326–402]

Measurements were performed at the end of each one-month lasting study period. Parameters are expressed as median and 95% confidence interval. Abbreviations are: MAP, mean arterial pressure; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; Uprot, proteinuria; PRA, plasma renin activity; Ang II, angiotensin II; U_{Na}, urinary sodium excretion; U_{urea}, urinary urea excretion.

[a] $P < 0.05$ vs. baseline, two-way ANOVA (Friedman)

[b] $P < 0.05$ vs. value obtained at 07:45 same study period, paired Wilcoxon

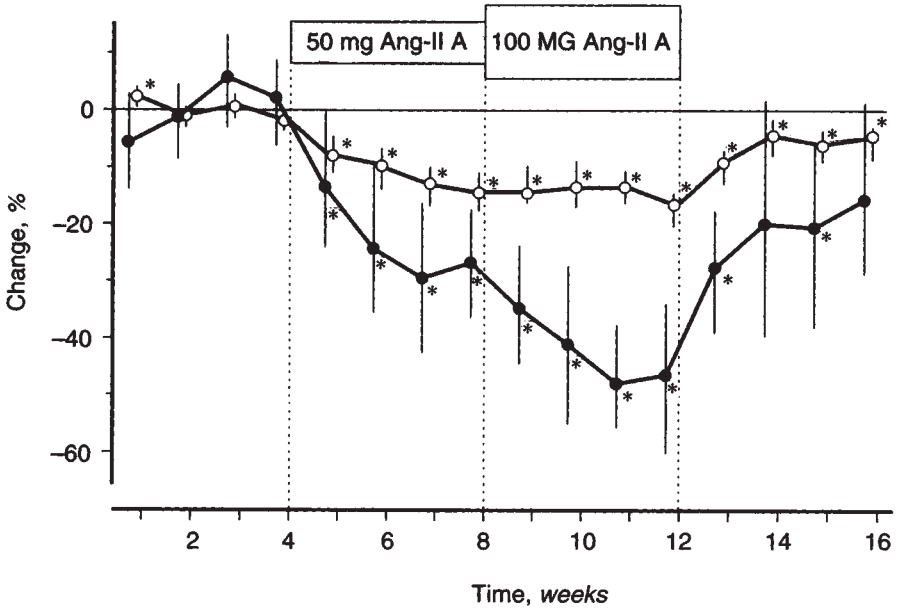

Fig. 1. *The course of the effect (median with 95% CI) of Ang II antagonism (50 and 100 mg losartan oid, respectively) on blood pressure (○) and urinary protein excretion (●) in eleven patients with non-diabetic renal disease.* Parameters are expressed as percentage change from the mean of the four values obtained at the end of the placebo baseline period. *$P < 0.05$ vs. the mean of baseline values (two-way ANOVA, Friedman)

Hg (range 139 to 180 mm Hg), DBP 97.3 mm Hg (range 90 to 111 mm Hg)]. Renal function ranged from normal to mildly impaired (creatinine clearance 94.5 ml/min (range 60 ml/min to 114 ml/min). Patients showed nephrotic range proteinuria with a median of 4.19 g/day (range 2.2 to 14.4 g/day). Histological diagnosis was glomerulosclerosis [5], membranous glomerulopathy [4], IgA nephropathy [1], and thin basement membrane disease [1]. Most of these patients showed no signs or symptoms of a concomitant disease. One patient suffered from hypothyroidism (necessitating levothyroxine treatment), another suffered from asthma (necessitating treatment with salbutamol and ipratropium-bromide), while two had familiar hyperlipidemia (no treatment). Their medication was not changed during the protocol. None of the other patients used concomitant medication.

The time course of the effects of the Ang II antagonist on blood pressure and proteinuria is shown in Figure 1. The antihypertensive effect of the 50 mg dose showed a slow onset, reaching a maximal effect approximately three weeks after start of treatment. On the higher dose almost no additional effect on blood pressure was observed. During the 50 mg dose a progressive decrease in proteinuria was also observed, stabilizing approximately three weeks after the start of treatment. When increasing the dose to 100 mg, a further fall in proteinuria was seen, again reaching its maximal effect after three weeks.

Table 1 and Figure 2 show the effects on blood pressure, renal

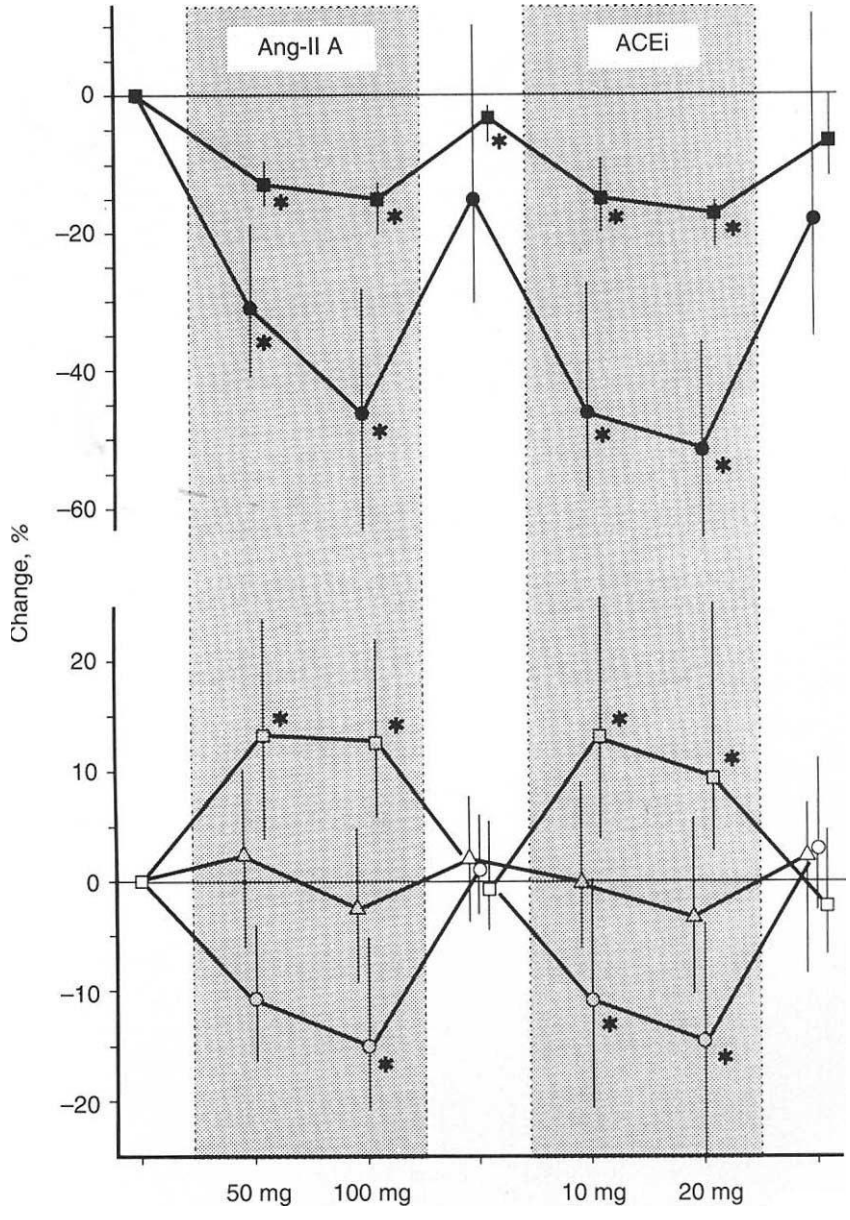

Fig. 2. *The effects (median with 95% CI) of Ang II receptor antagonism (50 and 100 mg oid) and ACE inhibition (10 and 20 mg oid) in eleven patients with proteinuria due to non-diabetic renal disease.* Shaded areas represent study periods in which active treatment (Ang II A, angiotensin II antagonist; ACEi, ACE inhibition) was given, while non-shaded areas represent study periods in which placebo was given. Changes in blood pressure (■) and urinary protein excretion (●) are depicted in the upper part, changes in glomerular filtration rate (△), effective renal plasma flow (□), and filtration fraction (○) are depicted in the lower panel. Parameters are expressed as percentage change from baseline. *$P < 0.05$ vs. baseline (two-way ANOVA, Friedman)

hemodynamics and proteinuria during the two doses of both the Ang II antagonist and the ACE inhibitor. Measurements were performed at the end of all study periods. Blood pressure fell by 12.8% (9.6% to 16.0%) during the 50 mg dose of losartan, and by 15.1% (12.7% to 20.2%) on the 100 mg dose (expressed as Wilcoxon-based estimated median with 95% CI). During the subsequent wash-out period blood pressure returned towards baseline. ACE inhibition induced an antihypertensive effect of 14.9% (9.1% to 20.1%) during the 10 mg dose of enalapril, and 17.3% (15.4% to 22.0%) during the 20 mg dose. The difference in antihypertensive response obtained during the high dose of the ACE inhibitor when compared to that on the high dose of the Ang II antagonist was 2.3% (−2.2% to 6.6%; $P = 0.25$). Glomerular filtration rate remained stable during all study periods. Effective renal plasma flow, however, rose maximally 13.3% (4.2% to 23.4%) during Ang II antagonism, and 13.1% (4.1% to 27.0%) during ACE inhibition [difference 1.2% (−7.1% to 8.4%), $P = 0.79$]. As a result a fall in filtration fraction was observed for both drugs, maximally 15.1% (5.7% to 20.6%) during the Ang II antagonist and 14.6% (4.7% to 22.7%) during the ACE inhibitor [difference 0.5% (−5.8% to 5.8%; $P = 0.86$). When measured at the end of both study periods, proteinuria fell by 31.0% (18.6% to 41.1%) and 46.3% (28.3% to 63.1%) on the two doses of the Ang II antagonist, respectively. These responses were comparable with the decrease in proteinuria of 46.9% (27.4% to 57.9%) and 51.6% (37.0% to 69.2%) during the two doses of the ACE inhibitor (Fig. 1). The antiproteinuric responses during the high doses of both drugs did not differ significantly [11.7% (−5.5% to 24.5%), $P = 0.14$].

Post-hoc analysis of the β-error revealed that the probability that the Ang II antagonist induced an effect that was 70% (or less) of the effect obtained during ACE inhibition was 0.04 for

blood pressure, 0.01 for ERPF, 0.07 for FF and 0.16 for proteinuria.

Plasma renin activity rose during Ang II antagonism both pre- and post-dose, indicating that Ang II activity was inhibited throughout the day (Table 1). During ACE inhibition a similar PRA change was observed. This indicates that both drugs induced an equivalent blockade of Ang II activity. Serum ACE activity was stable during Ang II receptor antagonism, and decreased during ACE inhibition, as expected. During Ang II antagonism the plasma concentration of Ang II increased, while during ACE inhibition a decrease in the effector hormone of the renin-angiotensin system was observed post-dose.

Patients, in general complied well with the instituted dietary restrictions, reflected by the stable median urinary sodium and urea excretion. Both the ACE inhibitor and the novel Ang II antagonist were well tolerated, as patients did not complain about side-effects while on either drug.

Discussion

In the present study we found the Ang II antagonist losartan to induce a fall in blood pressure, a rise in effective renal plasma flow, a lowering of the filtration fraction, and particularly to induce a decrease in urinary protein excretion. Interestingly, these changes were both qualitatively and quantitatively similar to those observed with the ACE inhibitor enalapril.

Our study is the first to investigate the effects of an Ang II receptor antagonist in patients with renal disease. The antihypertensive effect compares well to that found in patients with essential hypertension [28]. Data on renal hemodynamics and proteinuria have not been published previously in any patient category. Our finding of a stable GFR with a concomitant increase in ERPF is compatible with renal vasodilation. The reduction in proteinuria may be the result of a fall in intraglomerular pressure and/or changes in glomerular basement membrane characteristics. This systemic and renal profile closely resembles that of ACE inhibitors, which are known for their blood pressure lowering efficacy, renal vasodilation and antiproteinuric effect. This could well suggest that both drugs have a similar mode of action. Before drawing this conclusion one, however, has to consider the possible limitations of our study: drug choice, dose choice and the longitudinal design.

With regard to the choice of enalapril, two recent meta-analyses did not provide clues that one ACE inhibitor would be superior to another as far as hemodynamic or antiproteinuric effects are concerned [29, 30]. It is therefore unlikely that we would have obtained different results in this comparison trial using another ACE inhibitor. The choice of losartan was dictated, as only one Ang II antagonist is available for clinical purposes. However, losartan appears to be very specific in blocking only the type 1 Ang II receptor, without interfering in other hormonal systems [19]. These premises allow us, within the objective of our study, to draw conclusions on the role of the angiotensin system in the antiproteinuric effect of ACE inhibitors.

When comparing the efficacy of two drugs, this should be done with a dose of either drug that induces the maximal effect. As far as this is concerned, a comparison appears justified since both drugs were at near maximal dose. Indeed, blood pressure, renal hemodynamic parameters, and to a lesser extent also proteinuria, showed only a modest response to doubling of the dose of both the Ang II antagonist and ACE inhibition. Moreover, the effects of both drugs closely mimic the maximal effects described in literature [1, 28, 31].

The study should, of course, ideally have been performed according to a randomized, placebo-controlled, crossover design. Limited clinical experience, however, forced us to perform this phase II study as a single-blind longitudinal one. This design might have caused carry-over effects, though a distinct impact of this bias on the conclusion drawn seems rather unlikely. First, both active treatment modalities were separated by a four-week placebo period. Moreover, the effects of the ACE inhibitor used for comparison were obtained 12 weeks after withdrawal of the Ang II antagonist. It is hard to imagine that the former drug will have interfered with the latter. This consideration is strengthened by the observation of a near complete recovery of the principal parameters in the placebo wash-out and recovery periods. Second, even when carryover effects, or the course of the underlying disease are in play, this can only have worked to the disadvantage of the conclusion drawn. The ACE inhibitor-induced effects are related to the first placebo period (pre-losartan). Since proteinuria and blood pressure did not reach baseline completely in the intermediate placebo period, this may only have exaggerated the effects of the ACE inhibitor. When the response on both drugs is expressed as change compared to the respective preceding placebo periods, most parameters change even less during ACE inhibition than during Ang II antagonism. Thus, we may conclude that Ang II antagonism induced similar effects as ACE inhibition on the studied parameters.

What are the consequences of our observation that the Ang II antagonist and the ACE inhibitor induce similar effects? First of all, it indicates that the Ang II antagonist may be of similar clinical value as ACE inhibitors in treating patients with hypertension or the nephrotic syndrome. Both drugs appear to lower blood pressure adequately, while inducing a renal vasodilation and a decrease of urinary protein excretion. This profile has been hypothesized to be protective against progression of renal function decline.

Second, the strikingly similar response to Ang II antagonism and ACE inhibition has interesting renal physiological implications, as it suggests that the ACE inhibitor-induced effects are indeed caused by inhibition of Ang II activity, and not so much by interference in the kinin-kallikrein system as has been suggested so often by various experimental animal studies [8–15]. Although species differences seem a logical explanation for these conflicting results, differences in design of animal studies also have to be considered, since several other animal studies found Ang II antagonism and ACE inhibition to be equally effective in renal protection [32–35]. The present findings may seem contradictory to the above-mentioned observation of Heeg et al [16], who found that acute angiotensin II infusion could not offset the antiproteinuric effect of chronic ACE inhibition, though restoring the systemic and renal hemodynamic effects to control values. While that study questions a role of angiotensin II in the antiproteinuric effect of ACE inhibition, the present study indicates that blockade of angiotensin II activity is pivotal. A possible explanation for this discrepancy may be that infusion of Ang II is not the correct way of substituting the endogenous Ang II that has been

diminished by ACE inhibition, since the exogenous administered hormone may not reach all "Ang II deprived" effector sites. Alternatively, an explanation can be found in the different time scales in both studies. Ang II infusion was only continued for hours, whereas ACE inhibition and Ang II antagonist therapy lasted for weeks. Intriguingly, we recently demonstrated that the antiproteinuric effect of ACE inhibition has a slow onset, reaching its maximal effect only several weeks after the start of treatment [36]. Thus, the fact that Ang II infusion does not offset the antiproteinuric effect of ACE inhibition may be due to too short an infusion time. In this context it is important to note that the antiproteinuric effect of losartan showed a similar slow onset, reaching its maximum only three weeks after start of treatment. This observation demonstrates that the effects of Ang II antagonism should be assessed only several weeks after start of treatment, both in clinical practice and in future studies.

In conclusion, in patients with renal disease, the Ang II receptor antagonist losartan reduces blood pressure, filtration fraction, and proteinuria. These effects were similar to those obtained with the ACE inhibitor enalapril. The present data support the idea that the beneficial renal effects specific for ACE inhibitors are primarily mediated by interference in the renin-angiotensin system. Whether Ang II antagonists, by means of their greater specificity, will prove to be drugs of choice for the future has yet to be disclosed in large comparative trials.

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Reprint requests to D. de Zeeuw, M.D., Department of Medicine, State University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

References

1. DE ZEEUW D, HEEG JE, DE JONG PE: The antiproteinuric effect of angiotensin converting enzyme inhibitors in human renal disease, in *Intemational Yearbook of Nephrology*, edited by ANDREUCCI VE, FINE LG, London, Springer Verlag, 1992, pp. 95–113
2. TOLINS JP, RAIJ L: Angiotensin converting enzyme inhibitors and progression of chronic renal failure. *Kidney Int* 38(Suppl 30):S118–S122, 1990
3. EISENBACH GM, VAN LIEW JB, BOYLAN JW: Effect of angiotensin on the filtration of protein in the rat kidney: A micropuncture study. *Kidney Int* 8:80–87, 1975
4. BOHRER MP, DEEN WM, ROBERTSON CR, BRENNER BM: Mechanism of angiotensin II induced proteinuria in the rat. *Am J Physiol* 233:F13–F21, 1977
5. OLIVETTI G, KITHIER K, GIACOMELLI F, WIENER J: Characterization of glomerular permeability and proteinuria in acute hypertension in the rat. *Kidney Int* 25:599–607, 1984
6. YOSHIOKA T, RENNKE HG, SALANT DJ, DEEN WM, ICHIKAWA I: Role for abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: A study in early passive Heymann nephritis. *Circ Res* 61:531–538, 1987
7. DORER FE, KAHN JR, LENTZ KE, LEVINE M, SKEGGS LT: Hydrolysis of bradykinin by angiotensin-converting enzyme. *Circ Res* 34:824–827, 1974
8. HUTCHISON FN, MARTIN VI: Effects of modulation of renal kallikrein-kinin system in the nephrotic syndrome. *Am J Physiol* 258:F1237–F1244, 1990
9. FENOY FJ, SCICLI G, CARRETERO O, ROMAN RJ: Effect of an angiotensin II and a kinin receptor antagonist on the renal hemodynamic response to captopril. *Hypertension* 17:1038–1044, 1991
10. HAJJ-ALI AF, ZIMMERMAN BG: Kinin contribution to renal vasodilator effect of captopril in rabbit. *Hypertension* 17:504–509, 1991
11. MATTSON DL, ROMAN RJ: Role of kinins and angiotensin II in the renal hemodynamic response to captopril. *Am J Physiol* 260(29): F670–F679, 1991
12. HUTCHISON FN, WEBSTER SK: Effect of ANG II receptor antagonist on albuminuria and renal function in passive Heymann nephritis. *Am J Physiol* 263:F311–F318, 1992
13. VENIANT M, CLOZEL JP, HESS P, FISCHLI W: Effects of renin-angiotensin system blockade in guinea pigs. *Hypertension* 19:255–262, 1992
14. BOHEN E, YEUN J, YUAN C, CHEN S, MOORE J, PAMNANI MB: Chronic effects of Dup 753 and enalapril in 25% reduced renal mass rats with streptozocin induced diabetes and hypertension. (abstract) *J Am Soc Nephrol* 3:755, 1992
15. KON V, FOGO A, ICHIKAWA I: Bradykinin causes selective efferent arteriolar dilatation during angiotensin I converting enzyme inhibition. *Kidney Int* 44:545–550, 1993
16. HEEG JE, DE JONG PE, VAN DER HEM GK, DE ZEEUW D: Angiotensin II does not acutely reverse the reduction of proteinuria by long-term ACE inhibition. *Kidney Int* 40:734–741, 1991
17. WEXLER RR, CARINI DJ, DUNCIA JV, JOHNSON AL, WELLS GJ, CHIU AT, WONG PC, TIMMERMANS PBMWM: Rationale for the chemical development of Angiotensin II receptor antagonists. *Am J Hypertens* 5:209S–220S, 1992
18. TIMMERMANS PBMWM, BENFIELD P, CHIU AT, HERBLIN WF, WONG PC, SMITH RD: Angiotensin II receptors and functional correlates. *Am J Hypertens* 5:221S–235S, 1992
19. RHALEB NE, ROUISSI N, NANTEL F, D'ORLEANS-JUSTE P, REGOLI D: Dup 753 is a specific antagonist for the angiotensin receptor. *Hypertension* 17:480–484, 1991
20. WATANABE N, KAMEI S, OHKUBO A, YAMANAKE M, OHSAWA S, MAKINO K, TOKUDA K: Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. *Clin Chem* 32/8:1551–1554, 1986
21. DONKER AJM, VAN DER HEM GK, SLUITER WJ, BEEKHUIS H: A radioisotope method for simultaneously determination of the glomerular filtration rate and the effective renal plasma flow. *Neth J Med* 20:97–103, 1977
22. KWARTS E, BEUKENVELD G, GAZEDAM J: Evaluation of a simple colorimetric assay for serum angiotensin-converting enzyme: Comparison with a new ion-pair liquid chromatography-assisted assay. *Ann Clin Biochem* 19:227–232, 1982
23. COHEN EL, GRIM CE, CONN JW, BLOUGH WM JR, GUYER RB, KEM DC, LUCAS CP: Accurate and rapid measurement of plasma renin activity by radioimmunoassay. Results in normal and hypertensive people. *J Lab Clin Med* 77:1025–1038, 1971
24. SUZUKI H, FERRARIO CM, SPETH RC, BROSHNIKAN KB, SMEBY RR, DE SILVA P: Alterations in plasma and cerebrospinal fluid norepinephrine and angiotensin II during the development of renal hypertension in conscious dogs. *Hypertension* 5(Suppl 1):139–148, 1983
25. CAMPBELL MJ, GARDNER MJ: Calculating confidence intervals for some non-parametric analyses, in *Statistics with Confidence*, edited by GARDNER MJ, ALTMAN DG, London, British Medical Journal, 1989
26. ALTMAN DG: Non-parametric two way analysis of variance, in *Practical Statistics for Medical Research*, London, Chapman and Hall, 1991, pp. 334–335
27. DUNCAN DB: Multiple range and multiple F-tests. *Biometrics* 11:1–42, 1955
28. NELSON E, ARCURI K, IKEDA L, SNAVELY D, SWEET C: Efficacy and safety of losartan in patients with essential hypertension. (abstract) *Am J Hypertens* 5:20A, 1992

29. WEIDMANN P, BOEHLEN LM, DE COURTEN: Effects of different antihypertensive drugs on human diabetic proteinuria. *Nephrol Dial Transplant* 8:582–584, 1993
30. KASISKE BL, KALIL RSN, MA JZ, LIAO M, KEANE WF: Effect of antihypertensive therapy on the kidney in patients with diabetes: A meta-regression analysis. *Ann Intern Med* 118:129–138, 1993
31. MUNAFO A, CHRISTEN Y, NUSSBERGER J, SHUM LY, BORLAND RM, LEE RJ, WAEBER B, BIOLLAZ J, BRUNNER HR: Drug concentration response relationships in normal volunteers after oral administration of losartan, an angiotensin II receptor antagonist. *Clin Pharmacol Ther* 51:513–521, 1992
32. LAFAYETTE RA, MAYER G, PARK SK, MEYER TW: Angiotensin II receptor blockade limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 90:766–771, 1992
33. IMAMURA A, MACKENZIE HS, HUTCHISON FN, PLOTH DW: Albuminuria and the effects of chronic renin angiotensin system inhibition in 2-kidney, 1 clip hypertensive rats. (abstract) *J Am Soc Nephrol* 3:521, 1992
34. DIVISH B, POLLOCK DM, POLAKOWSKI J, OPGENORTH TJ: Effect of AII receptor blockade in rats with reduced renal mass. (abstract) *J Am Soc Nephrol* 3:737, 1992
35. SALADINI D, JOVER B, RIBSTEIN J, MIMRAN A: Renal compensation to uninephrectomy and the renin-angiotensin system. (abstract) *J Am Soc Nephrol* 3:478, 1992
36. GANSEVOORT RT, DE ZEEUW D, DE JONG PE: Dissociation between the course of the hemodynamic and antiproteinuric effects of angiotensin I converting enzyme inhibition. *Kidney Int* 44:579–584, 1993