*Kidney International, Vol. 54 (1998), pp. 2037–2044*



# Nephroprotection by long-term ACE inhibition with ramipril in spontaneously hypertensive stroke prone rats

**WOLFGANG LINZ, REINHARD H.A. BECKER, BERNWARD A. SCHÖLKENS, GABRIELE WIEMER, MANFRED KEIL, and KARL HEINZ LANGER**

*DG Cardiovascular Research and Department of Pathology, Hoechst Marion Roussel, Frankfurt am Main, Germany*

**Nephroprotection by long-term ACE inhibition with ramipril in spontaneously hypertensive stroke prone rats.**

*Background*. The effect of life-long treatment with the ACE inhibitor ramipril on hypertension-induced histological changes in the kidney was tested in stroke-prone spontaneously hypertensive rats (SHR-SP).

*Methods*. One-month-old pre-hypertensive SHR-SP were randomized into three groups of 45 animals each, and exposed via drinking water for their lifetime to a dose of: 1 $mg \cdot kg^{-1} \cdot d^{-1}$ ramipril (antihypertensive dose, HRA); 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$ slight dose of ramipril (non-antihypertensive dose, LRA); or placebo. Histological and biochemical assessments were conducted after 15 months in ten rats each, when about 80% of the placebo group had died.

*Results*. Kidneys from placebo treated SHR-SP showed pronounced arterial wall hypertrophy and sclerosis, arterial fibrinoid necrosis, glomerulopathy and tubular interstitial injury that were, in concert with normalized blood pressure, completely prevented by HRA treatment. LRA treatment did not affect any blood pressure increase, and also attenuated the development of arterial wall hypertrophy, sclerosis and arterial fibrinoid necrosis, though to a minor extent only, but did not change glomerular and tubulointerstitial degeneration. These effects of ramipril were associated with a dose-dependent inhibition of plasma and renal tissue ACE activities as well as lower serum concentrations of creatinine, but there were no changes in serum potassium.

*Conclusions*. Life-long HRA-induced ACE inhibition protects against hypertension-induced renal damages in SHR-SP. This is associated with a doubeling of the lifespan in these animals [1].

The incidence of hypertension-related disease states such as chronic renal failure is increased in high risk patients with long-lasting hypertension [2]. In a three year follow-up study in patients with hypertension and chronic non-diabetic nephropathy and proteinuria (Ramipril Efficacy In Nephropathy - REIN Study) deterioration of renal function under angiotensin converting enzyme (ACE) inhibition with ramipril almost ceased, and hence, kidney survival increased as compared to blood pressure controlled patients without ramipril [3]. Analogous to human hypertensives, spontaneously hypertensive rats (SHR) develop kidney damage that resembles nephropathy seen in patients [4]. In such rats the onset of kidney damage was delayed when treated for 12 weeks or 1.5 years with an antihypertensive dose of perindopril or enalapril, respectively, starting at the age of 15 weeks [4, 5]. Also, histopathological changes start to reverse in the kidneys of 73-week-old SHR after three weeks of treatment with an antihypertensive dose of an ACE inhibitor [6].

It was recently shown that early onset life-long treatment with ramipril in an antihypertensive dose doubled the lifespan, and a non-antihypertensive dose prolonged the lifespan from 15 to 18 months of stroke-prone spontaneously hypertensive rats (SHR-SP). This observation correlated with preservation of cardiac function, metabolism and size as well as endothelial function [1]. The present report investigates the nephroprotection associated with this treatment in animals developing malignant hypertension.

## METHODS

### Animals

Male SHR-SP were purchased from Møllegrad Denmark at the age of one month. They were housed three per cage under standardized conditions of temperature, humidity and light. The rats had free access to standard diet (Altromin® Maintenance Diet 1320, sodium content 0.2%) and drinking water *ad libitum*. All experiments were performed in accordance with the German animal protection law.

### Study design

As recently outlined, 135 animals were randomized into three groups [1]. Each group ($N = 45$) was treated via drinking water with a high antihypertensive dose of ramipril (HRA; 1 $mg \cdot kg^{-1} \cdot d^{-1}$) [7], a low non-antihypertensive

**Key words:** angiotensin converting enzyme inhibition, SHR-SP, hypertension, sclerosis, hypertrophy.

Received for publication January 23, 1998
and in revised form June 3, 1998
Accepted for publication July 14, 1998

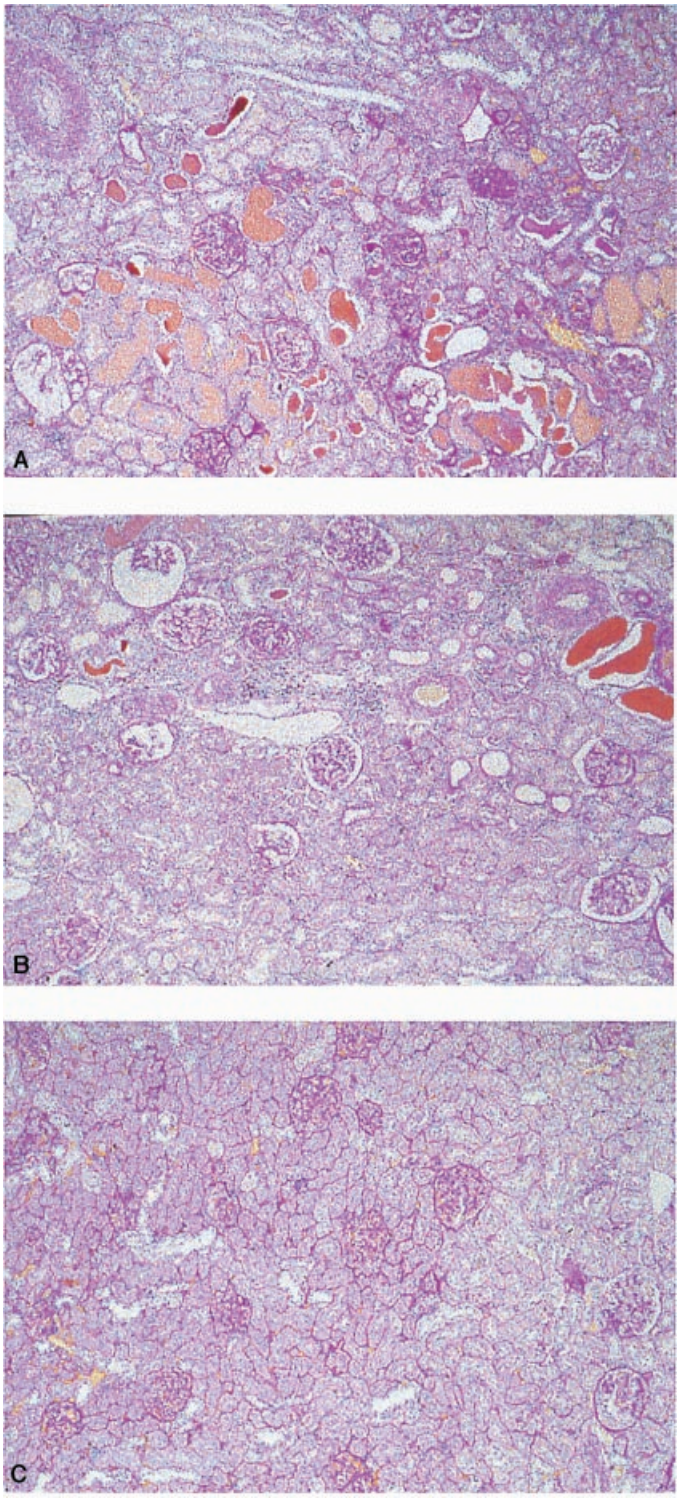


**Fig. 1. Overview of the kidney cortex (*A*) with arterial, glomerular and tubulointerstitial injuries in placebo-treated SHR-SP, (*B*) minor changes after LRA treatment (non-antihypertensive dose of ramipril, 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$), and (*C*) no changes after HRA treatment (antihypertensive dose of ramipril, 1 $mg \cdot kg^{-1} \cdot d^{-1}$).** PEARSE staining, magnification a - c ×52.

**Table 1.** Plasma and kidney ACE activities, serum creatinine and potassium concentrations in SHR-SP

| | Plasma $nmol \cdot min^{-1} \cdot ml^{-1}$ | Kidney $nmol \cdot mg\ protein^{-1} \cdot hr^{-1}$ | Creatinine | Potassium |
|---|---|---|---|---|
| | | | *mmol/liter* | |
| Placebo | 166 ± 17[b,c] | 126 ± 21[b,c] | 63 ± 11[b] | 3.3 ± 0.4 |
| HRA | 11 ± 5[a,c] | 10 ± 2[a,c] | 46 ± 4[a] | 3.4 ± 0.2 |
| LRA | 68 ± 13[a,b] | 25 ± 2[a,b] | 53 ± 4 | 3.5 ± 0.3 |

Data are given as mean ± 95% CI. Abbreviations are: HRA: antihypertensive high dose of ramipril (1 $mg \cdot kg^{-1} \cdot d^{-1}$); LRA, non-antihypertensive low dose of ramipril (10 $\mu g \cdot kg^{-1} \cdot d^{-1}$).
[a] vs. Placebo, [b] vs. HRA, [c] vs. LRA, $P < 0.05$ by one way ANOVA

dose of ramipril (LRA; 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$) [7] known to completely inhibit renal tissue ACE activity [8], or placebo. Treatment commenced immediately after randomization and was adjusted to the actual consumption of drinking water. Histological and biochemical data as well as kidney and adrenal gland weights of 10 animals per group were assessed after 15 months, when about 80% of the placebo group had died.

The rats were anesthetized with hexobarbitone, 80 mg/kg i.p., for direct recording of mean arterial blood pressure in the left carotid artery. Thereafter, blood samples, kidneys, adrenals as well as other organs were removed for biochemical and morphological analyses. ACE activities in plasma and kidney were radioenzymatically measured using $^3$H-Gly-Gly as substrate (Hycor, ACE-activity test). Serum potassium concentrations were measured by flame photometry. Serum concentrations of creatinine were measured by the creatinine PAP method, (Boehringer Mannheim).

Kidneys, adrenal glands, pancreas, mesenterium, testis and epididymidis were fixed in 10% neutral buffered formalin and embedded in paraffin for light microscopic examination. Two to 3 $\mu$m thick sections were stained with hematoxylin and eosin, or with perjodic acid Schiff-orange G according to PEARSE for detection of fibrin. Histological examinations and associated scoring were performed in a blinded and separate fashion by two investigators, and eventually by consensus. Alterations of vessel walls, glomeruli, and interstitial sites were gradually classified for hypertrophy, necrosis, and fibrosis from 0 = no alteration as observed in juvenile rats, to 5 = massive destruction, with 1 = minimal, 2 = low, 3 = moderate, and 4 = severe as intermediate scores.

### Statistical analysis

The data are given as mean ± 95% CI (confidence interval limits; Table 1) or median with 95% CI (Table 2). For exploratory purposes, one way ANOVA followed by multiple pairwise comparisons according to Tukey were employed on blood pressure and clinical chemistry data, and MANOVA with Holm adjusted $P$ values on organ weights, while the Kruskal-Wallis tests of cumulative scores were used for morphometric assessments, and rank ordered

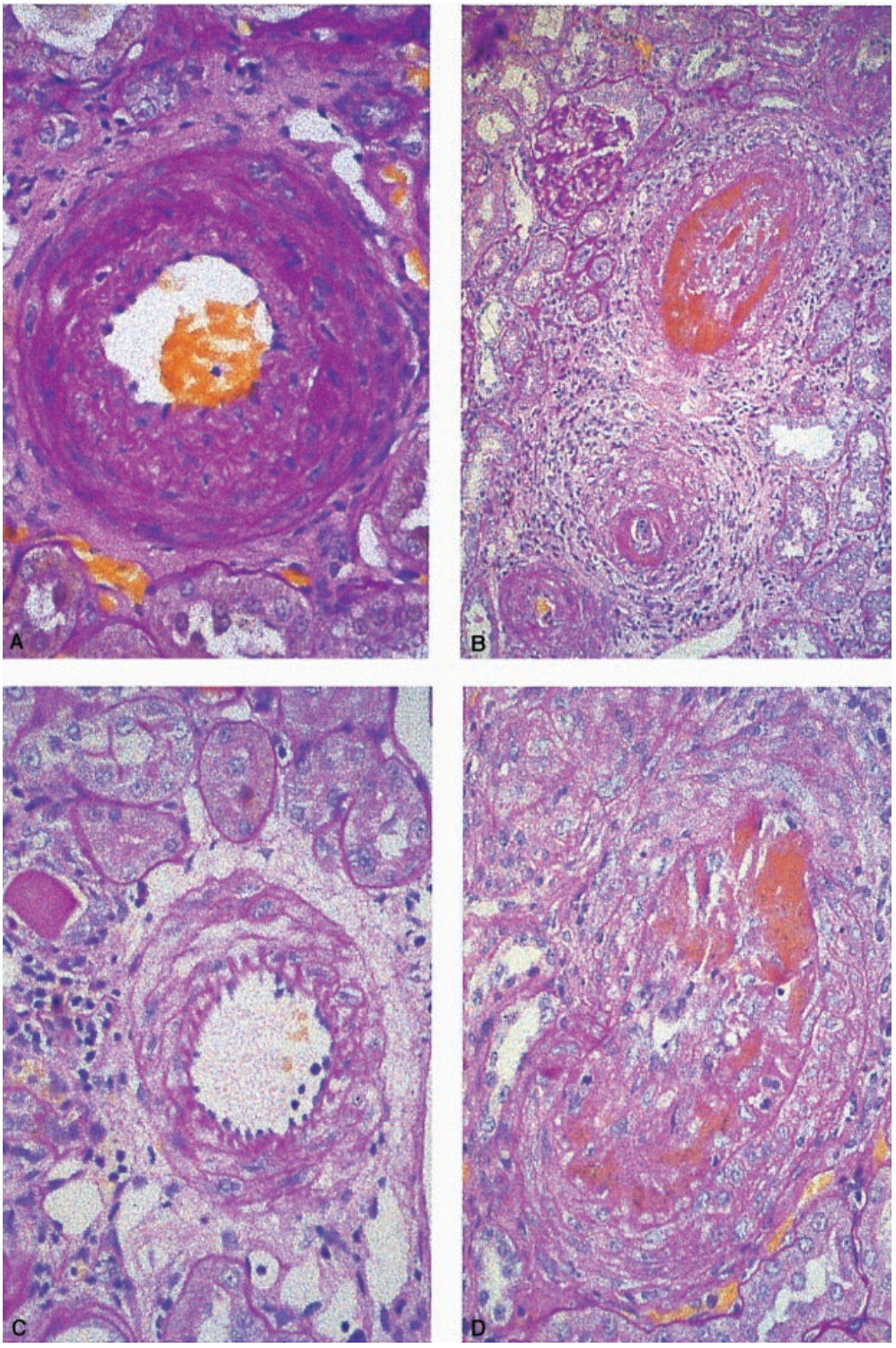


**Fig. 2. Arteriae intralobulares after placebo (*A and B*) and after LRA treatment (see Fig. 1) (*C and D*).** (A) media hypertrophy and sclerosis and (B) fibrinoid necrosis and periarteritis (like panarteritis nodosa); (C) minimal hypertrophy and no intimal sclerosis, and (D) fibrinoid necrosis, but not combined with periarteritis. PEARSE staining, magnification A, C, D ×330, B ×130.

analyses according to Spearman for correlations. Null-hypotheses were rejected at $P < 0.05$.

## RESULTS

Body weights increased from 72 ±8 g at the age of one month to 366 ± 17 g at the age of 15 months in placebo treated SHR-SP. Ramipril treatments did not affect the increases in body weights. As reported, systolic blood pressure was 98 ± 8 mm Hg in young (1 month old) placebo treated SHR-SP as measured by tail plethysmography, increased significantly over the following three months, and climaxed after 12 months at 233 ± 14 mm Hg.

**Table 2.** Median scores of histological lesions in the kidneys observed in placebo and ramipril-treated SHR-SP at 15 months, and life expectancy

| | Placebo | | HRA | | LRA | |
|---|---|---|---|---|---|---|
| | C.I. (range) | *N* affected | C.I. (range) | *N* affected | C.I. (range) | *N* affected |
| Hypertrophy of arterial wall | 4.0 (3–4)[b,c] | 10/10 | 0.0 (0–0)[a,c] | 0/10 | 3.0 (2–3)[a,b] | 10/10 |
| Arterial fibrinoid necrosis | 2.5 (0–4)[b] | 6/10 | 0.0 (0–0)[a,c] | 0/10 | 1.0 (0–1)[b] | 7/10 |
| Glomerulopathy | 4.0 (3–4)[b] | 10/10 | 1.0 (1–2)[a,b] | 10/10 | 3.0 (3–4)[b] | 10/10 |
| Tubulointerstitial injuries | 4.0 (3–4)[b] | 10/10 | 1.0 (1–1)[a,c] | 10/10 | 4.0 (3–4)[b] | 10/10 |
| Total score[d] | 13.0 (9–16)[b] | | 2.0 (2–3)[a,c] | | 10.5 (9–13)[b] | |
| Arterial fibrinoid necrosis in other organs | 3.0 (3–4) | 10/10 | 0.0 (0–0) | 0/10 | 1.0 (0–1) | 10/10 |
| Life expectancy *days* | 423 (408–430)[b,c] | | 744 (535–767)[a,c] | | 456 (427–487)[a,b] | |

Data are given as median with 95% CI (range), and number of affected organs
[a] vs. Placebo, [b] vs. HRA, [c] vs. LRA, $P < 0.05$ by ANOVA on Ranks, [d] Median of individual sums

At 15 months, mean arterial blood pressures measured in the carotid artery were 150 ± mm Hg after placebo, 109 ± 11 mm Hg after HRA and 156 ± 12 mm Hg after LRA [1]. Kidney weights did not differ between the groups (right kidneys, 471 ± 16 g/100 g body wt; left kidneys, 476 ± 19 mg/100 g body wt). Weights of adrenal glands were dose-dependent less with either treatment independent of site: placebo 107 ± 14; HRA 76 ± 6; LRA 91 ± 10 $\mu$g/100 g body wt).

Reductions in plasma and kidney ACE activities by ramipril were dose-dependent (Table 1). Other components of the renin-angiotensin system such as plasma renin activities were increased only by HRA treatment, whereas plasma angiotensin II (Ang II) and plasma aldosterone were significantly reduced by both doses of ramipril [1].

Serum creatinine after HRA, was lower as compared to placebo treated animals; however, serum potassium was not changed by either dose of ramipril (Table 1).

Morphological evaluation of kidneys from placebo treated SHR-SP showed arterial wall hypertrophy, intimal sclerosis, arterial fibrinoid necrosis as well as glomerular and tubulointerstitial injuries (Table 2, and Figs. 1A, 2 A, B, and 3 A, B).

Middle to small sized arteries (A. arcuatae, interlobares and afferentia) presented with marked hypertrophy of the media combined with an enlargement of the intima (fibroelastosis) resulting in intense luminal stenosis (Fig. 2A), while large interlobar arteries were not affected. These alterations were accompanied by arterial fibrinoid wall necrosis with inflammatory reactions in intima and adventitia (Fig. 2B). The extent of fibrinoid affections varied from discrete intimal depositions to circumferential imbibition with destruction or edemateous swelling of the media containing erythrocytes or their debris as siderin accumulating macrophages in the adventitia. However, there were no signs of infarcteous loss of cortical parenchyma. In some cases the adventitia of these arteries, apart from granulocytes, contained considerable amounts of macrophages (Fig. 2B). Similar changes were found in arteries of other organs such as the adrenal gland, pancreas, mesenterium, testis and epididymidis (Table 2).

Glomerular changes were found in two features: (*1*) mostly juxtamedullary enlarged glomerula with ectatic capillary loops, often with reduction of capillary and mesangial cells and sometimes with fibrin thrombosis (Fig. 3A); and (*2*) sclerotic changes ranging from segmental to total capillary obliteration (Fig. 3B). Consequently, the Bowman's capsular space contained often albumin or fibrin (Figs. 1A, and 3 A, B).

There was a massive presence of protein casts in the tubules (Fig. 1A), in the Henles loops and in the collecting ducts as a consequence of an increased glomerular permeability or destruction. Proximal tubules often contained an accumulation of protein droplets. Tubular atrophies were mainly localized close to sclerotic glomerula, and the peritubular interstium showed round cell infiltration or fibrosis corresponding to the degree of atrophy (Fig. 1A).

HRA treatment completely prevented arterial wall hypertrophy and arterial necrotic lesions. On glomerular as well as the tubulointerstitial changes HRA treatment was less effective. However, these changes in HRA treated SHR-SP appeared to be even less as compared to age matched normotensive control rats (Langer, personal observation; Table 2, Figs. 1C and 4).

Treatment with LRA attenuated arterial wall hypertrophy in the kidneys (Table 4, Figs. 1B and 2 C, D) and arterial necrotic lesions in testes (not further quantified), but did not protect against glomerular and tubulointerstial damages (Figs. 1B and 3 C, D). Moreover, the degree of necrosis in kidneys of the LRA group was significantly less in those affected as compared to placebo. The scatterplot matrix revealed that the histological changes correlated to blood pressure and plasma ACE activity, whereas kidney ACE activity did not (Fig. 5). This correlation is based on the changes under HRA, as attested by nonsignificant Spearman rank correlation coefficients when the HRA data are excluded ($r = -0.19$ histomorphological score vs. blood

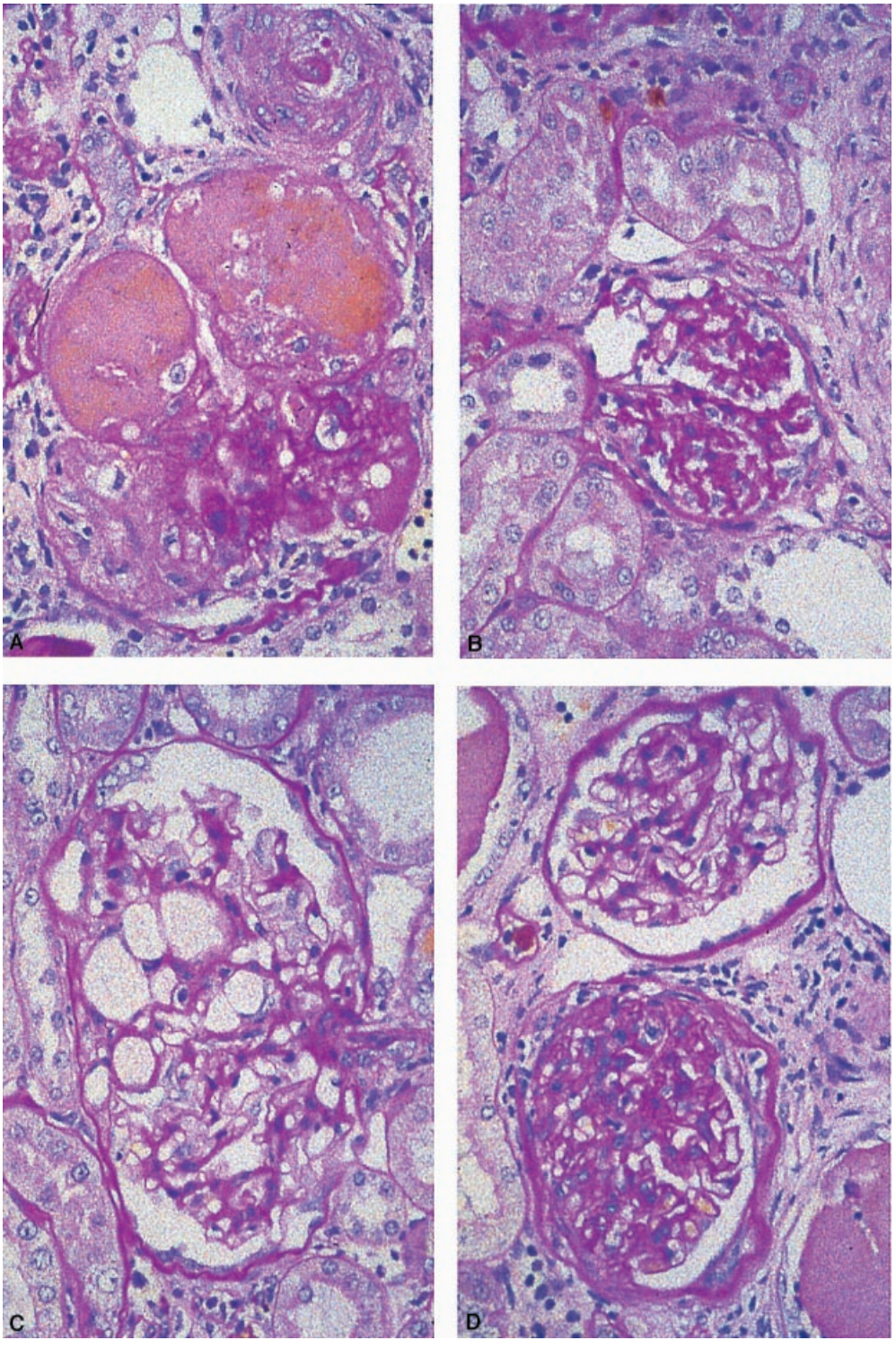


**Fig. 3. Glomeruli after placebo (*A and B*) and LRA treatment (see Fig. 1) (*C and D*).** (A) fibrinoid necrosis in microcystic capillary loops, (B) sclerosis with capillary obliteration; (C) enlarged glomerulum (like that in A) with widened capillary loops juxtamedullary typical for hypertension, (D) capillary sclerosis with capsular adherence besides a normal glomerulum. PEARSE staining, magnification A - C ×330, D ×130.

pressure, −0.41 histomorphological score vs. ACE, −0.17 histomorphological score vs. kidney ACE).

## DISCUSSION

Early onset life-long administration of the ACE inhibitor ramipril to SHR-SP in an antihypertensive dose was able to prevent hypertension-induced complications of the kidney seen in placebo treated age-matched animals, while a dose that did not affect the rise in blood pressure was only partly effective. This nephroprotection, beyond the reported cardioprotective and vasculoprotective effects, is associated with a dose-dependent lifespan extension [1].

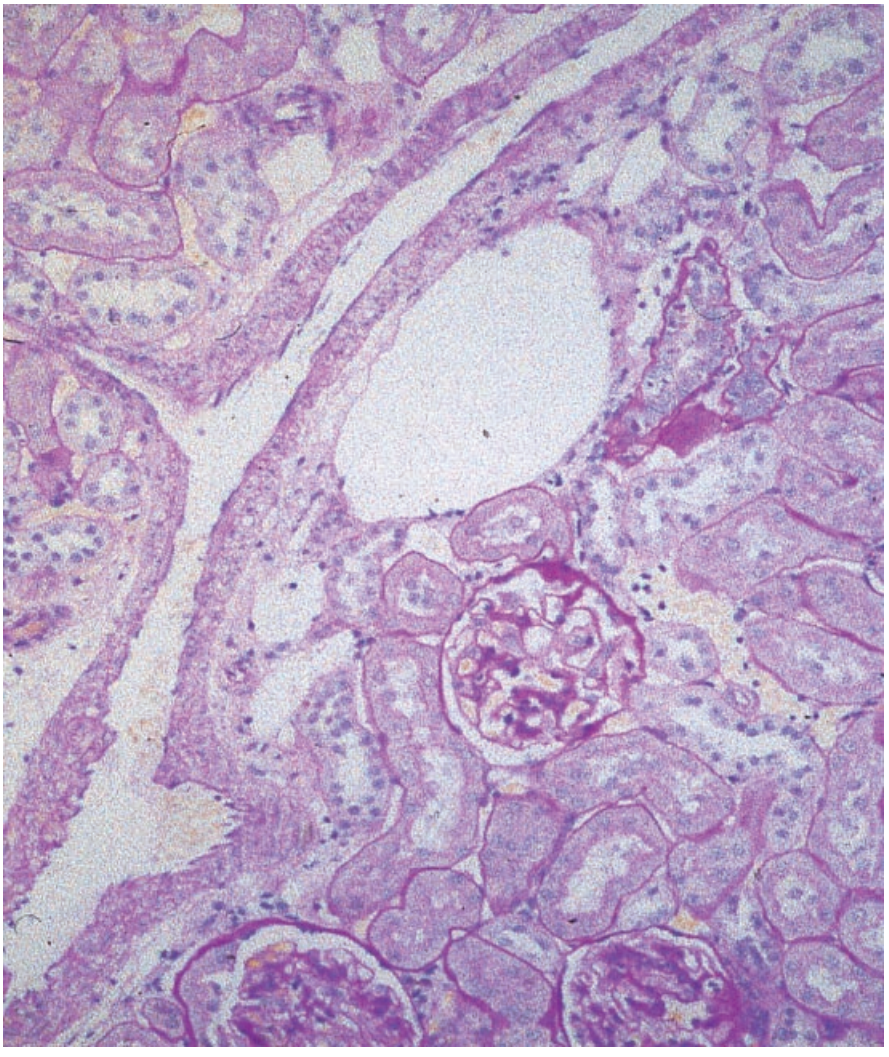

**Fig. 4. After HRA treatment (see Fig. 1), arteria arcuata (cross section below) and intralobularis, glomerula and tubulointerstitial area are without any change.** PEARSE staining, magnification ×130.

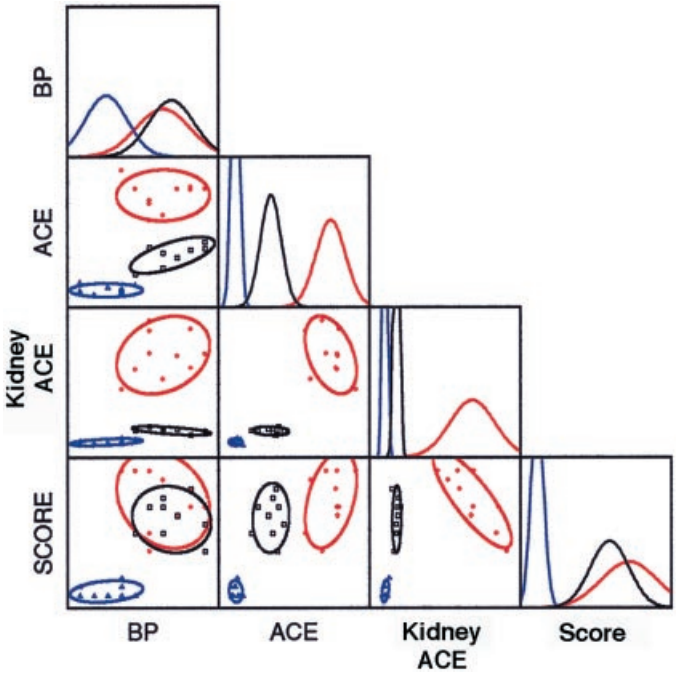


**Fig. 5. Scatterplot correlation matrix with normal curve density display of blood pressure (BP), plasma angiotensin converting enzyme (ACE), kidney ACE activity, and histomorphological score (Score).** Symbols are: red (●) placebo; blue (▲) HRA; and black (□) LRA (see Fig. 1 for definitions).

The beneficial effects of ACE inhibition with ramipril on kidney morphology correlated with decreased plasma Ang II and aldosterone concentrations, and lower weights of the adrenal glands. They reflect the suppression of renin-angiotensin system components in plasma and tissue, which is most likely involved in the protection of the kidney. It has been convincingly demonstrated in rats that chronic exposure to Ang II causes an adverse increase in glomerular capillary pressure that is associated with albuminuria and glomerular sclerosis [9–11].

Also, locally increased kinins seem to be involved in the prevention of renal injuries [12] by contributing to increased renal blood flow and diuresis seen in animals with low to normal renin levels under ACE inhibition [13]. Furthermore, impaired NO synthesis and release described in rats [14] and also in aged hypertensive humans with attentuated renal vascular dilation [15] may be prevented by ACE inhibition [16].

The role of the recently described heptapeptide Ang 1-7, formed by the neutral endopeptidase 24.11 from Ang I, is subject of a scientific dispute, since it also acts through specific receptors presumably involved in the control of hydroelectrolyte balance [17–19].

The serum potassium concentrations did not differ between placebo and ramipril treated animals in this study, which is not in line with the hypothesis that a reduced propensity for arrhythmias due to a preserved potassium concentration may have contributed to the lifespan extension in these SHR-SP. An increased sensitivity to epinephrine inducible arrhythmias was observed in potassium-deficient rats [20], and moreover, less cardiac arrhythmias in patients with congestive heart failure treated with enalapril were associated with higher serum potassium concentrations [21].

The serum creatinine concentration was significantly lower by long-term ACE inhibition as compared to the placebo treatment. This is in context with results from a double-blind controlled trial in patients with renal insufficiency caused by various disorders, where benazepril halved the overall risk of progressive renal insufficiency, defined as doubling of baseline serum creatinine as a surrogate parameter [22].

The histomorphological changes in the kidneys of placebo-treated SHR-SP (Figs. 1A, 2 A, B, and 3 A, B) were mainly the result of chronic pressure overload. The fibrinoid necroses found in arteries and glomerula correspond to the decompensating hypertension-induced nephrosclerosis in humans. Therefore, the "early" death of placebo treated animals was associated with strong renal injuries above cardiac failure [1]. The considerable amount of macrophages and granulocytes (Fig. 2B) also found in some cases in the adventitia of arteries in rats [23] corresponds to the human panarteriitis nodosa.

No renal hypertensive degeneration was seen in HRA treated animals. The marginal changes in glomerular and tubulointerstitial areas can be viewed as the beginning of an age-related nephropathy also seen in age-matched normotensive control rats (Langer, personal observation).

The effect of ACE inhibition seems to be partly independent of its antihypertensive action since LRA treatment without effect on the development of high blood pressure was also nephroprotective, although to a minor extent, as only arterial hypertrophy, fibrinoid necrosis and sclerosis were opposed (Figs. 1C and 2 C, D). This favors an additional organoprotective effect in the kidney by inhibition of a locally activated renin-angiotensin system [24, 25], and possibly by an improved endothelial function through preserved NO availability [16]. However, the present study clearly recommends that blood pressure must be controlled to fully render ACE inhibition with both nephroprotective and live saving doses of ramipril.

A similar study in spontaneously hypertensive rats with enalapril in an antihypertensive dose did not demonstrate the same degree of morphological protection [4]. Furthermore, the morphological changes in placebo treated animals were somewhat less dramatic [4]. The difference to the present study may be due to the more severe hypertension developing in SHR-SP and the earlier commencement of treatment with ramipril. Moreover, this ACE inhibitor is endowed with higher affinity to the enzyme as compared to enalapril, which consequently may result in the more efficacious protection [26].

The present results show that the beneficial effects of life-long antihypertensive ACE inhibitor treatment with ramipril on renal morphology accompany and hence may be causally linked to the doubeling of lifespan extension of genetically hypertensive rats. Although these effects were in part seen with the non-antihypertensive dose of ramipril, the majority of the nephroprotectic effects was associated with the prevention of the development of high blood pressure. These data are in line with the advantageous effects of ramipril in the REIN study in which the decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy were safely reduced [3].

## ACKNOWLEDGMENTS

We kindly thank Peter Pleines for coordination of the study, as well as Heinz Otto Stern and Wolfgang Jung for technical assistance, and Reiner Uhl for support on statistics.

*Reprint requests to Dr. Wolfgang Linz, Hoechst Marion Roussel, DG Cardiovascular Research (H821), D-65926 Frankfurt/Main, Germany.*
*E-mail: wolfgang.linzhmrag.com*

## REFERENCES

1. Linz W, Jessen T, Becker RHA, Schölkens BA, Wiemer G: Long-term angiotensin converting enzyme inhibition doubles lifespan of hypertensive rats. *Circulation* 96:3164–3172, 1997
2. National High Blood Pressure Education Program Coordinating Committee: The sixth report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Intern Med* 157:2413–2446, 1997

3. The GISEN Group: Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy - REIN Study. *Lancet* 349:1857–1863, 1997
4. Feld LG, Cachero S, Liew JBV, Zamlauski-Tucker M, Noble B: Enalapril and renal injury in spontaneously hypertensive rats. *Hypertension* 16:544–554, 1990
5. Lee RMKW, Delaney KH, Lu M: Perindopril treatment prolonged the lifespan of spontaneously hypertensive rats. *J Hypertens* 13:471–476, 1995
6. Komatsu K, Frohlich ED, Ono H, Ono Y, Numabe A, Willis GW: Glomerular dynamics and morphology of aged spontaneously hypertensive rats. Effects of ACE inhibition. *Hypertension* 25:207–213, 1995
7. Becker RHA, Baldes L, Fürst U, Schulze KJ: Sustained diurnal blood pressure reduction in SHR with ramipril assessed by telemetric monitoring. *Clin Exp Hypertens* 19:1233–1246, 1997
8. Becker RHA, Baldes L, Treudler M, Metzger G, Maier R: Long-term fall in blood pressure, plasma- and tissue converting enzyme inhibition. Two rat studies with ramipril. (abstract) *Eur J Pharmacol* 83:1309, 1990
9. Schunkert H, Tang S-S, Litwin SE, Diamant D, Riegger G, Dzau VJ, Ingelfinger JR: Regulation of intrarenal and circulating renin-angiotensin systems in severe heart failure in the rat. *Cardiovasc Res* 27:731–735, 1993
10. Zoja C, Donadelli R, Corna D, Testa D, Facchinetti D, Maffi R, Luzzana E, Colosio V, Bertani T, Remuzzi G: The renoprotective properties of angiotensin-converting enzyme inhibitors in a chronic model of membranous nephropathy are solely due to the inhibition of angiotensin II: Evidence based on comparative studies with a receptor antagonist. *Am J Kidney Dis* 29:254–264, 1997
11. Rocha R, Chander PN, Khanna K, Zuckerman A, Stier CT: Mineralcorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. *Hypertension* 31:451–458, 1998
12. Naitoh M, Suzuki H, Arakawa K, Matsumoto A, Ichihara A, Matsuda H, Kubota E, Murakami M, Nakamoto H, Saruta T: Role of kinin and renal ANG II blockade in acute effects of ACE inhibitors in low-renin hypertension. *Am J Physiol* 272(Heart Cir Physiol 41):H679–H687, 1997
13. 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 (Dallas)* 17:1038–1044, 1991
14. Schnackenberg C, Patel AR, Kirchner KA, Granger JP: Nitric oxide, the kidney and hypertension. *Clin Exp Pharmacol Physiol* 24:600–606, 1997
15. Higashi Y, Oshima T, Ozono R, Matsuura H, Kajiyama G: Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension* 30:252–258, 1997
16. Wiemer G, Linz W, Hatrik S, Schölkens BA, Malinski T: ACE inhibition alters NO and $O_2^-$ release in normotensive and hypertensive rats. *Hypertension* 30:934–941, 1997
17. Santos RAS, Simoes e Silva AC, Magaldi AJ, Khosla MC, Cesar KR, Passaglio KT, Baracho NCV: Evidence for a physiological role of angiotensin-(1-7) in the control of hydroelectrolyte balance. *Hypertension* 27:875–884, 1996
18. Botelho LM, Block CH, Khosla MC, Santos RAS: Plasma angiotensin-(1-7) levels is increased by water deprivation, salt load and hemorrhage. *Peptides* 15:723–729, 1994
19. Heyne N, Beer W, Muhlbauer B, Osswald H: Renal response to angiotensin-(1-7) in anesthetized rats. *Kidney Int* 47:975–976, 1995
20. Miletich DJ, Minshall RD, Albrecht RF: The influence of chronic hypokalemia on myocardial adrenergic receptor densities: Enhanced sensitivity to epinephrine-induced arrhythmias. *Anesth Analg* 84:734–739, 1997
21. Gurlek A, Erol C, Basesme E: Antiarrhythmic effect of converting enzyme inhibitors in congestive heart failure. *Int J Cardiol* 43:315–318, 1994
22. Maschio G, Alberti D, Janin G, Locatelli F, Mann JFE, Motolese M, Ponticelli C, Ritz E, Zucchelli P: ACE Inhibition In Progressive Renal Insufficiency study group: Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. *N Engl J Med* 334:939–945, 1996
23. Suzuki T, Oboshi S, Sato R: Periarteritis nodosa in spontaneously hypertensive rats-incidence and distribution. *Acta Pathol Jpn* 29:697–703, 1979
24. Richer C, Fornes P, Vacher E, Bruneval P, Giudicelli J-F: Trandolapril's protective effects in stroke-prone spontaneously hypertensive rats persist long after treatment withdrawal. *Am J Cardiol* 73:26C–35C, 1994
25. Münter K, Hergenröder S, Jochims K, Kirchengast M: Individual and combined effects of verapamil or trandolapril on attenuating hypertensive glomerulopathic changes in the stroke-prone rat. *J Am Soc Nephrol* 7:681–686, 1996
26. Bünning P: Inhibition of angiotensin converting enzyme by 2-[N-[(S)-1Carboxy-3-phenylpropyl]-L-alanyl]-(1S, 3S, 5S)-2azabicyclo [3.3.0]octane-3-carboxylic Acid (HOE 498 Diacid). *Arzneim Forsch/Drug Res* 34:1406–1410, 1984