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Temporary losartan or captopril in young SHR induces malignant hypertension despite initial normotension

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Temporary losartan or captopril in young SHR induces malignant hypertension despite initial normotension.

Background. Exposure of normotensive rats to angiotensin-converting enzyme (ACE) inhibitors in early life causes hypertrophy of intrarenal arteries. Similar defects have been found in knockout mice lacking angiotensinogen, ACE, or angiotensin II type 1 (AT_1) receptors. On the other hand, transient inhibition of the renin-angiotensin system from 2 weeks of age in spontaneously hypertensive rats (SHR), either with ACE inhibitors or with AT_1 receptor antagonists partially prevents the increase in blood pressure. However, permanent treatment of SHR from conception onwards with ACE inhibitors completely prevents hypertension. Although these studies demonstrated protection from hypertension-induced changes in the heart and large arteries, renal arteries were not studied and follow-up did not extend beyond 6 months of age. We postulated that while brief exposure to ACE inhibitors or AT_1 receptor antagonists in young SHR would temporarily decrease blood pressure, it would also be associated with development of intrarenal arterial malformation, and ultimately have deleterious effects.

Methods. Direct effects on intrarenal arterial morphology of an ACE inhibitor (captopril, 100 mg/kg/day) and an AT_1 receptor antagonist (losartan, 50 mg/kg/day), administered from the last week of gestation until 8 weeks of age were examined in SHR. After stopping treatment at 8 weeks, we continued to monitor blood pressure until spontaneous death.

Results. Systolic blood pressure at 8 weeks was normalized by captopril and losartan (SHR control 187 ± 8 mm Hg; captopril 118 ± 5 mm Hg; and losartan 120 ± 9 mm Hg). However, by 30 weeks, blood pressure had increased to control SHR levels. At 4 weeks, the media of renal arteries and arterioles was hypertrophied. Marked smooth muscle cell hyperplasia of cortical arteries resulted in significantly increased wall thickness by 8 weeks, despite similar external diameter. Arterial wall structure was disrupted, with fragmentation of elastic fibers and irregular distribution of collagen type I fibers. After stopping treatment, the rats gradually began to show poor health and all had died by 1 year of age, while all 1-year-old control SHR females were in good health. The cause of morbidity and mortality in the rats treated in early life was clearly malignant hypertension. Severe hypertrophy of renal arterioles was found, as well as cerebral hemorrhage.

Conclusion. Despite initial normalization of blood pressure interference with the renin-angiotensin system during a crucial stage of development in SHR can initiate marked smooth muscle cell hyperplasia and disruption of the wall structure of the intrarenal arteries. Subsequent progression of this intrarenal process after cessation of treatment suggests an independent process that eventually results in malignant hypertension and early death.

Key words: neonatal toxicity, ACE inhibitor, AT1 antagonist, intrarenal arteries.

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Multiple studies have reported that transient inhibition of the renin-angiotensin system from 2 weeks of age in spontaneously hypertensive rats (SHR), either with angiotensin-converting enzyme (ACE) inhibitors or with angiotensin II type 1 (AT_1) receptor antagonists, blunts the increase in blood pressure for up to 21 weeks after cessation of treatment [1]. However, the change in blood pressure in comparison to age-matched untreated SHR averaged 14% ± 6%, ranging from no change to a decrease of 25%, and even this effect usually waned over time.

Permanent treatment of SHR from conception onwards with ACE inhibitors completely prevents hypertension [2, 3]. On the other hand, exposure of normotensive rats to ACE inhibitors from birth to 2 or 3 weeks of age causes hypertrophy of intrarenal arteries [4, 5]. Similar defects have been found in knockout mice lacking angiotensinogen, ACE, or AT_1 receptors [6–9]. Recently, Inokuchi et al [10] studied the morphology of the intrarenal arteries of AT_1a receptor null mice in more detail and found hyperplasia of the vascular smooth muscle cells, loss of circular mechanical integrity, and increased extracellular matrix [10]. Exposure of very young SHR to ACE inhibitor or an AT_1 antagonist could well result in similar detrimental effects on morphology of the intrarenal arteries if the beneficial hemodynamic effect is offset by these morphologic abnormalities. In the long-term, such effects on the

intrarenal arteries could be detrimental with respect to kidney damage, blood pressure, and survival. Although the studies where SHR were exposed to ACE inhibition from conception onwards demonstrated protection from hypertension-induced changes in the heart and large arteries, the renal vasculature was not studied and follow-up did not extend beyond 6 months of age [2, 3].

We postulated that while brief exposure to ACE inhibitors or AT_1 receptor antagonists in young SHR would temporarily decrease blood pressure, it would also be associated with the development of intrarenal arterial malformation, and ultimately have deleterious long-term effects and decreased survival. Because treatment from 2 to at least 6 weeks of age appears to be the minimum required to blunt the increase in blood pressure in SHR [1], treatment was continued in and shortly beyond this period. Therefore, we examined the direct effects in SHR of ACE inhibitors and AT_1 receptor antagonists administered from the last week of gestation until 8 weeks of age on intrarenal arterial morphology. After stopping treatment at 8 weeks, we continued to monitor blood pressure until spontaneous death.

METHODS

Animals

Pregnant SHR (Harlan-Olac, Blackthorn, Bichester, Oxon, UK) were kept at an ambient temperature of 22°C, humidity 60%, exposed to a 12-hour light/dark cycle. Rats were fed a standard natural diet (RMH-TH, Hope Farms, Woerden, The Netherlands). Sentinel animals housed in the same conditions were monitored regularly for infection with nematodes and pathogenic bacteria and antibodies to rodent viral pathogens (ICLAS, Nijmegen, The Netherlands).

During the last 7 days of pregnancy and throughout lactation pregnant rats received either tap water (control), or the ACE inhibitor captopril (1000 mg/L) or the AT_1 receptor antagonist losartan (500 mg/L) dissolved in drinking water. This realized a dose of captopril of 100 mg/kg/day and a dose of losartan of 50 mg/kg/day. Captopril was obtained from Sigma Chemical Co. (St. Louis, MO, USA), and R.D. Smith, Ph.D. (DuPont-Merck Pharmaceuticals) kindly provided losartan. All litters were carried to full gestation; the total number of pups (male and female) varied from 5 to 15 per litter. Pups were sacrificed at weaning (4 weeks), or were continued on treatment until sacrifice at 8 weeks. In the pups that continued to receive treatment, the dose was maintained; the drug concentration in drinking water was adjusted according to changes in water intake of the rapidly growing pups. Female offspring not sacrificed at 8 weeks (eight controls, six captopril, and five losartan) were no longer treated and followed until moribund. Pups were weighed weekly beginning with 4 weeks of age. Systolic blood pressure was measured from 8 weeks of age by the tail-cuff method (IITC, San Diego, CA, USA). At 4 and 8 weeks and at regular intervals when off-treatment rats were placed in metabolic cages for 24 hours for urine collection. At 4 and 8 weeks, some rats were anesthetized (pentobarbital sodium, 60 mg/kg intraperitoneally) and killed either by exsanguination or by perfusion fixation with 1.5% glutaraldehyde in 0.1 mol/L phosphate buffer at pH 7.4 at 200 mm Hg for morphometry and ultrastructural evaluation. Kidneys and heart were removed and weighed. Plasma and urinary creatinine, and urinary sodium, potassium, osmolality, and protein were determined by standard techniques.

Renal and cerebral morphology

High-resolution T2-weighted spin-echo images of the brain (61 slices of 0.5 mm; repetition time 3 seconds; echo time 17.5 mseconds; field of view 2 × 2 cm; matrix 128 × 128) were collected on a 4.7 Tesla horizontal bore nuclear magnetic resonance (NMR) spectrometer (Varian, Palo Alto, CA, USA). The kidneys and the brain were immersion-fixed in phosphate-buffered saline (PBS) formaldehyde (4%, pH 7.35) and embedded in paraffin. Sections stained with hematoxylin-eosin (H&E) and periodic-acid Schiff (PAS) were examined by light microscopy. Ultrastructural evaluation was performed in losartan-treated and control male SHR. Small blocks of renal tissue (2 × 3 mm) were postfixed in OSO_4 (1% for 2 hours) and subsequently dehydrated and embedded into Epon by standard procedures. Semithin sections (1 μm thick) were cut on an ultramicrotome using a diamond knife, stained as described [11], and examined by light microscopy. In sections of three to four blocks of each animal, all profiles of intracortical arteries were photographed (six to eight per animal), arcuate arteries at the corticalmedullary border as well as afferent arterioles were excluded. In all perpendicularly or obliquely sectioned profiles of intracortical arteries the outer and inner diameters were measured twice, and the mean wall thickness was calculated. In obliquely sectioned profiles, the shortest outer and corresponding inner diameters were measured [10]. Analysis on the electron microscopic level was performed in a qualitative way. From areas of interest selected in the semithin sections, ultrathin section were cut and studied with a Philips 301 electron microscope.

Calculations and statistical analyses

Values are expressed as mean ± SEM. Data were compared with unpaired *t* test and two-way analysis of variance (ANOVA) for repeated measurements where appropriate. Linear regression analysis was employed to examine the correlation between wall thickness and outer arterial diameter.

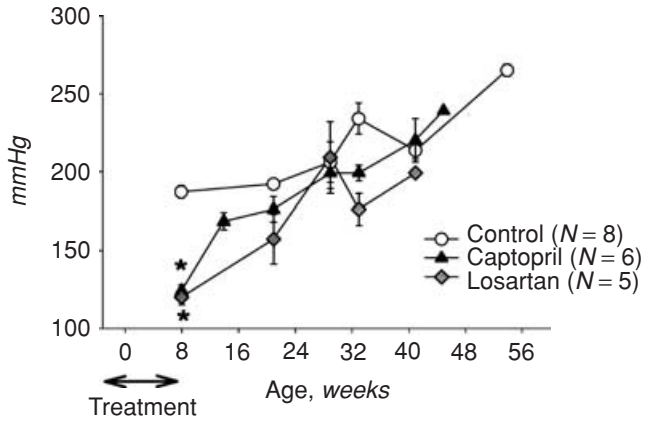

Fig. 1. Systolic blood pressure in female control spontaneously hypertensive rats (SHR) and SHR treated for 2 weeks in utero and up to 8 weeks of age.

RESULTS

Functional studies

At 8 weeks of age, systolic blood pressure was reduced from 187 ± 8 mm Hg in control SHR to 118 ± 5 mm Hg in captopril-treated SHR and 120 ± 9 mm Hg in losartan-treated SHR (Fig. 1). Changes in systolic blood pressure at 8 weeks of age correlated with heart weight (Table 1). However, by 30 weeks, systolic blood pressure was at control SHR level (Fig. 1). Treatment with either captopril or losartan tended to increase daily water intake and diuresis and reduce urinary osmolality, the difference being significant by 8 weeks of age (Table 1). There was no significant effect of captopril on plasma creatinine, but losartan caused a slight increase. No effect was observed on sodium and potassium handling (not shown), and none of the 8-week-old rats developed proteinuria (Table 1). However, after stopping treatment, mild proteinuria developed with a maximum varying from 20 to 56 mg/day vs. 5 to 7 mg/day in 42-week-old control SHR ($P < 0.05$).

Morphologic studies

Losartan treatment during pregnancy and lactation reduced kidney weight (Table 2). At 4 weeks of age in the losartan-treated SHR, hypertrophy of the media of renal arteries and arterioles was observed (Fig. 2). This was more pronounced at 8 weeks. Marked smooth muscle cell hyperplasia of cortical arteries was observed, particularly at 8 weeks (Fig. 3A vs. B). This resulted in a significantly increased wall thickness by 8 weeks (Table 2), despite similar external diameter (Fig. 3C). At the ultrastructural level, pronounced disruption of the preglomerular arterial wall structure was observed at 8 weeks. Note that this was in the presence of a systolic blood pressure of about 120 mm Hg (Fig. 1). At variance with control SHR, fragmentation of the elastic layers was observed in the losartan-treated SHR, and the media of interlobular arteries contained abundant type I collagen with characteristic periodic banding, distributed in an irregular pattern (Fig. 4A vs. B to D).

Table 1. Body and heart weight and renal water handling in control and treated young male spontaneously hypertensive rats (SHR)

	Control SHR	Captopril-treated SHR	Losartan-treated SHR
Age 4 weeks			
Number	9	5	7
Body weight *g*	37 ± 1	41 ± 2	41 ± 3
Heart weight *% body weight*	0.53 ± 0.02	0.47 ± 0.02	0.50 ± 0.01
Diuresis *mL/kg/day*	93 ± 5	125 ± 7[a]	91 ± 8
Plasma creatinine *μmol/L*	54 ± 1	49 ± 3	59 ± 7
Proteinuria *mg/day*	2.3 ± 0.5	2.4 ± 0.9	1.4 ± 0.2
Urine osmolality *mOsm/kg*	1298 ± 148	1316 ± 71	1517 ± 68
Age 8 weeks			
Number	8	6	5
Body weight *g*	157 ± 5	159 ± 2	147 ± 11
Heart weight *% body weight*	0.41 ± 0.01	0.36 ± 0.01[a]	0.36 ± 0.01[a]
Diuresis *mL/kg/day*	65 ± 11	103 ± 9[a]	113 ± 14[a]
Plasma creatinine *μmol/L*	52 ± 4	60 ± 2	66 ± 4[a]
Proteinuria *mg/day*	4.8 ± 0.7	4.3 ± 1.4	2.5 ± 0.2
Urine osmolality *mOsm/kg*	1478 ± 133	996 ± 100[a]	1070 ± 201[a]

[a] $P < 0.05$ vs. age-matched controls.

Table 2. Morphometric analysis of proximal interlobular arteries in control and losartan-treated young male spontaneously hypertensive rats (SHR)

	Control SHR	Losartan-treated SHR
Age 4 weeks		
Number	4	4
Left kidney weight *% body weight*	0.68 ± 0.03	0.56 ± 0.02[a]
Outer diameter *μm*	34.2 ± 2.0	41.5 ± 7.0
Wall thickness *μm*	4.81 ± 0.32	5.29 ± 0.90
Age 8 weeks		
Number	4	4
Left kidney weight *% body weight*	0.46 ± 0.01[b]	0.39 ± 0.03[a,b]
Outer diameter *μm*	47.5 ± 6.3	58.6 ± 3.2
Wall thickness *μm*	4.88 ± 0.38	8.96 ± 1.16[a,b]

[a] $P < 0.05$ vs. age-matched controls.
[b] $P < 0.05$ vs. 4 weeks of age.

Survival

After stopping treatment, the rats gradually began to show signs of poor health: ruffled hair, weight loss, and 100% mortality by 1 year of age. All 1-year-old control SHR females were still alive and in good health (Fig. 5). The cause of morbidity and mortality in the perinatally treated rats was clearly malignant hypertension. Very

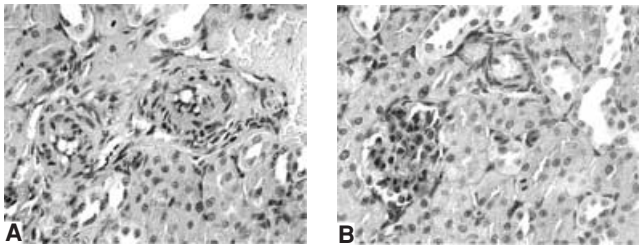

Fig. 2. At 4 weeks of age, hypertrophy of the media of renal arteries and arterioles was observed in losartan-treated spontaneously hypertensive rats (SHR) (*A*) as compared to control SHR (*B*).

severe hypertrophy of renal arteries and arterioles, and occasional fibrinoid necrosis of glomeruli was found (Fig. 6). Postmortem analysis of rats of 30 weeks and older of the cerebrum with magnetic resonance imaging (MRI) revealed hypointense areas in the basal ganglia in the four rats that could be studied (Fig. 7A). This is caused by the T2 shortening effect of iron. The presence of hemorrhage at these locations was confirmed by histology (Fig. 7B).

DISCUSSION

Exposure of young SHR to ACE inhibitors or AT_1 antagonists indeed results in a temporary decrease in blood pressure. However, already at 8 weeks of age, the decreased blood pressure is accompanied by severe disruption of the normal vascular architecture of intrarenal arteries and arterioles. Ultrastructural changes at 8 weeks included media hypertrophy due to smooth muscle cell hyperplasia, fragmentation of elastic fibers, and irregular distribution of type I collagen fibers. Moreover, these intrarenal defects became progressively more severe. Initially in the presence of normal blood pressure, but later, after exposure to these agents was stopped, in the presence of high blood pressure, ultimately the rats died showing symptoms, both renal and extrarenal, of malignant hypertension between 8 and 12 months of age. Apparently, interference with the renin-angiotensin system during a crucial stage of development in SHR can initiate this disturbance. Subsequently this intrarenal process progresses despite cessation of antihypertensive treatment.

Transient exposure to ACE inhibitors or AT_1 antagonists from 2 to 20 weeks of age in SHR has repeatedly been reported to blunt the increase in blood pressure for months after cessation of treatment [1, 12–14]. Renal arterial resistance was also reported to be decreased 15 weeks after treatment for 2 to 10 weeks of age [12]. In other studies permanent treatment of SHR from conception onwards with ACE inhibitors or AT_1 antagonists completely prevented hypertension and the accompanying left ventricular hypertrophy [2, 13, 15, 16]. However, the kidney was not investigated in any of these studies and the rats were sacrificed at 28 to 30 weeks of age. None of these studies included a long-term follow-up to evaluate target organ damage or survival.

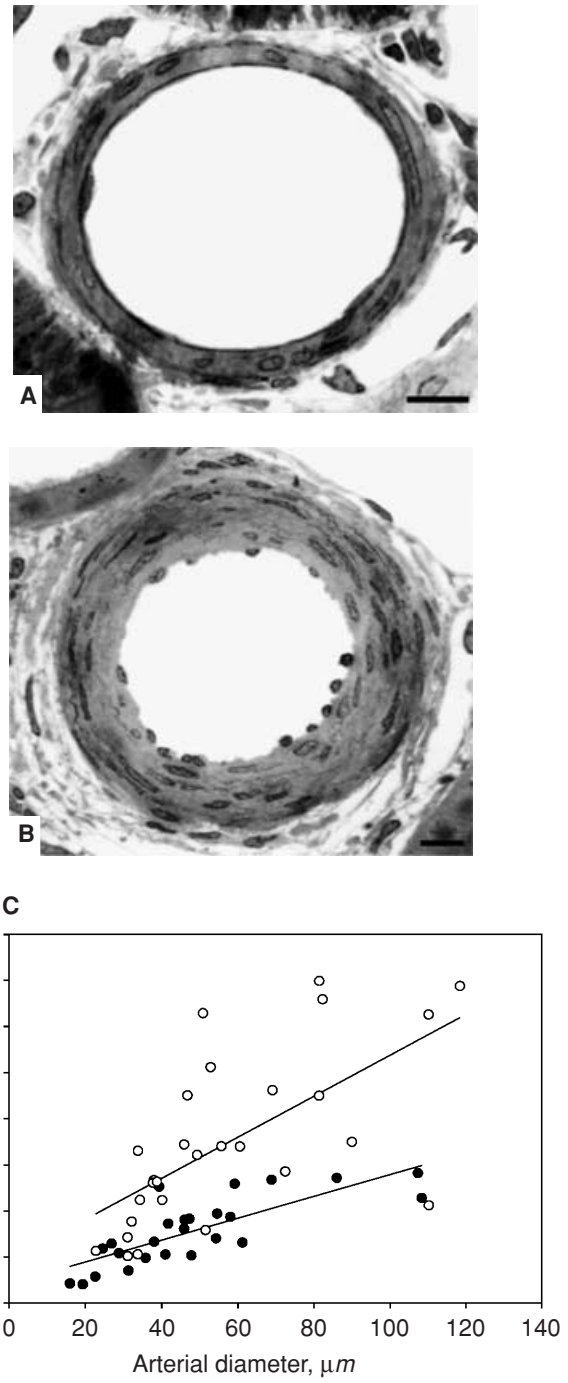

Fig. 3. Cross sections of cortical radial arteries in 8-week-old male control (*A*) and losartan-treated spontaneously hypertensive rats (SHR) (*B*) (1 μm Epon section, bar length 10 μm). (*C*) Wall thickness in relation to arterial diameter in arteries in male control SHR (•) and losartan-treated SHR (○) at 8 weeks of age.

Findings in SHR in the current study suggest that, although normal blood pressure was maintained by captopril treatment throughout the period of observation in the study by Lee et al [2], this probably occurred in the

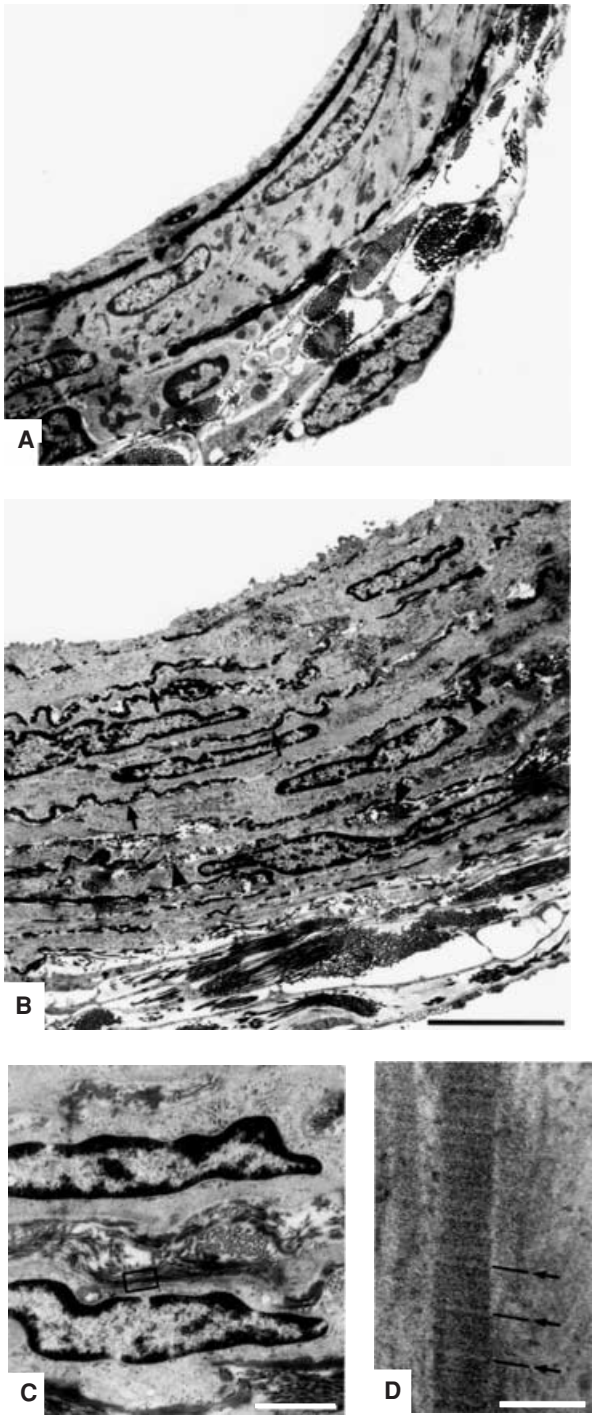

Fig. 4. Transmission electron micrographs of cortical radial artery wall structure in (*A*) control male spontaneously hypertensive rats (SHR) and (*B* to *D*) losartan-treated male SHR at 8 weeks of age. The artery in (B) has six to seven layers of smooth muscle cells compared to two layers in (A). Moreover (B) lacks a clear internal and external elastic layer as seen in (A); instead elastic tissue (arrow) together with collagenous fibers (arrowhead) are found throughout the entire media between the smooth muscle cells (bar length 5 μm). (C) shows an area between two smooth muscle cells (corresponding to an area labeled in (B) by an arrowhead) with abundant type I collagen fibers (bar length 1 μm). The high power electron microscope in (D), quadrangle in (C), clearly demonstrates the banded pattern of type I collagen (60 to 70 nm periodicity) (bar length 0.1 μm).

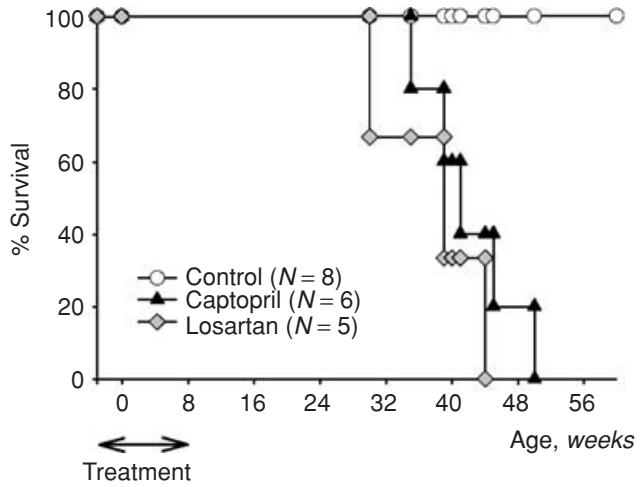

Fig. 5. Survival in female control spontaneously hypertensive rats (SHR) and SHR treated for 2 weeks in utero and up to 8 weeks of age.

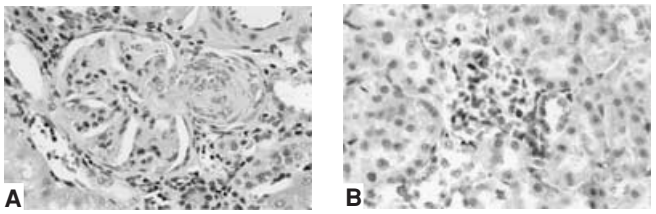

Fig. 6. Malignant hypertension lesions in female spontaneously hypertensive rats (SHR) treated for 2 weeks in utero and up to 8 weeks of age or 30 weeks and older, displaying very severe hypertrophy of renal arterioles (*A*) as compared to control female SHR of 48 weeks of age (*B*).

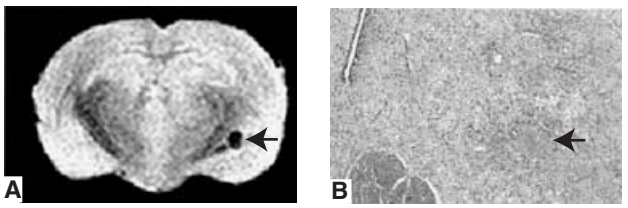

Fig. 7. Cerebral magnetic resonance imaginag (MRI) and histology. Postmortem analysis of the cerebrum of a female spontaneously hypertensive rat (SHR) of 40 weeks treated with captopril for 2 weeks in utero and up to 8 weeks of age. MRI revealed hypointense areas (arrows) in the basal ganglia (*A*). This is caused by the T2 shortening effect of iron. The presence of hemorrhage at these locations (arrow) was confirmed by histology (*B*).

presence of profound intrarenal vascular changes. In some of these previous studies [13, 15] groups were included where captopril treatment was stopped at 2 months of age, a design quite similar to the present study. However, these rats were also not followed until spontaneous death, but sacrificed at 8 to 10 months of age. In contrast to the present study, these studies reported that blood pressure was 30 mm Hg lower after cessation of treatment and no morbidity was observed. It is possible

that a longer period of observation would have exposed pathology similar to what we observed in the present study. Indeed, Lan et al [17] reported rebound of hypertension and 100% mortality by 52 weeks of age in SHR administered an ACE inhibitor, perindopril, from either 4 to 10 or 4 to 20 weeks of age. However, the latter study also lacks information on renal or extrarenal pathology. Thus the present study uncovers an important paradox in SHR. On the one hand, interference with angiotensin II formation and AT_1 receptor-mediated actions early in life resulted in long-term beneficial systemic hemodynamics. On the other hand, severe intrarenal vascular abnormalities developed.

Inhibition of the renin-angiotensin system in young SHR induced a defect in the capacity to concentrate urine, confirming previous observations in normotensive rats and piglets [4, 5, 18, 19]. However, this defect is much milder than the lack of concentrating ability observed in ACE knockout mice [8, 20]. The treated SHR also did not show obvious abnormality of the renal papilla, as is common in the knockout mice, and in human neonates exposed to ACE inhibitors [21–23]. Whether this partial maintenance of medullary function in our study is related to the species or to residual angiotensin activity in this compartment is not clear.

The pathogenesis of the intrarenal arteriolar changes is unknown. The most notable change found was pronounced hyperplasia of smooth muscle cells and disrupted architecture of the media. This is paradoxic because stimulation of the AT_1 receptor on vascular smooth muscle cells has well-known proliferative and hypertrophic effects [24–27]. Up-regulation of the number of AT_1 binding sites in the neonatal SHR kidney [28, 29] may explain the marked sensitivity to these deleterious effects. Apparently, inhibition of the generation or effects of angiotensin in a crucial developmental stage stimulates the uncontrolled production of a different trophic factor that is normally down regulated by angiotensin. The nature of this factor is enigmatic.

Perinatal exposure of normotensive rats to ACE inhibitors at very high doses also causes hypertrophy of intrarenal arteries [4, 5]. However, detailed morphologic information is not available. Intima hyperplasia of intrarenal arteries was observed in renal allografts in rats treated for 34 weeks with an ACE inhibitor or an AT_1 antagonist [30]. Similar gross changes have been found in knockout mice lacking angiotensinogen, ACE, or AT_1 receptors [6–9, 20]. Recently, the ultrastructure of intrarenal arteries of adult knockout mice lacking AT_{1a} receptors was studied [10]. Although wall thickening was also observed, this was mainly caused by the appearance of additional populations of luminal and abluminal cells outside more or less intact elastic laminas, although there were some irregular intercalated elastic fibers among the smooth muscle cells. Thus the phenotype of the intrarenal arteries in SHR treated at an early age with AT_1 blockade was more disturbed than that in knockout mice lacking AT_{1a} receptors. Besides the species difference, another obvious difference is blood pressure, which at 8 weeks in the losartan-treated SHR, although reduced to 120 ± 9 mm Hg, was considerably higher than the 87 ± 8 mm Hg measured in AT_1 knockout mice [31]. Recent findings, however, have shown that in ACE knockout mice the renal structural defects are not necessarily associated with hypotension. Selective expression of germinal ACE in ACE knockout mice restored normal kidney structure despite persistent hypotension [32].

Intracardiac injection of ACE and AT_1 receptor antisense cDNA to 5-day-old SHR has successfully been applied to prevent the development of hypertension, renal, and cardiovascular pathophysiologic changes on a long-term basis [33–36]. Due to the retrovirus-mediated transfer, the antisense cDNA was even incorporated into the germ line and transmitted to the offspring that also demonstrated marked reduction of blood pressure [37]. Nevertheless, both the neonatally treated parents as well as the F_1 and F_2 offspring demonstrated blood pressures that were still higher than those found in normotensive Wistar-Kyoto (WKY) rats and showed a reduction of blood pressure after an intravenous injection of losartan. Combination of these findings in SHR with data obtained in the present study indicates that some limited degree of AT_1 receptor activation is needed for the development of normal vasculature.

CONCLUSION

In summary, despite initial normalization of blood pressure, transient exposure to ACE inhibitors or AT_1 antagonists in young SHR lead to severe smooth muscle cell hyperplasia, and disruption of the intrarenal vascular architecture. Ultimately this resulted in malignant hypertension. Furthermore, this study revealed that interference with angiotensin II formation and AT_1 receptor-mediated actions early in life leads to a paradoxic situation of intrarenal vascular smooth muscle hyperplasia, suggesting another trophic factor that is inhibited by angiotensin II under physiologic conditions.

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