Long-Term Angiotensin II Type 1 Receptor Blockade With Fonsartan Doubles Lifespan of Hypertensive Rats

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***Abstract*—** In this study, we investigated the outcome of lifelong treatment with the angiotensin II type 1 receptor (AT_1) blocker fonsartan (HR 720) in young stroke-prone spontaneously hypertensive rats (SHR-SP). In addition to the primary end point, lifespan, and to determine the mechanisms involved in the treatment-induced effects, parameters such as left ventricular hypertrophy, cardiac function/metabolism, endothelial function, and the expression/activity of endothelial nitric oxide synthase and of angiotensin-converting enzyme (ACE) were also investigated. Ninety 1-month-old SHR-SP were allotted to 2 groups and treated via drinking water with an antihypertensive dose of fonsartan ($10\ mg \cdot kg^{-1} \cdot d^{-1}$) or placebo. Fonsartan doubled the lifespan to 30 months in SHR-SP, which was comparable to the lifespan of normotensive Wistar-Kyoto rats. After 15 months, a time when ≈80% of the placebo group had died, left ventricular hypertrophy was completely prevented in fonsartan-treated animals. Furthermore, cardiac function and metabolism as well as endothelial function were significantly improved. These effects were correlated with increased endothelial nitric oxide synthase expression in the heart and carotid artery and with markedly decreased tissue ACE expression/activities. Lifespan extension and cardiovascular protection by long-term AT_1 blockade with fonsartan led to similar beneficial effects, as observed with long-term ACE inhibition. **(*Hypertension*. 2000;35:908-913.)**

Key Words: angiotensin II ■ angiotensin-converting enzyme ■ fonsartan ■ nitric oxide synthase ■ rats, stroke-prone spontaneously hypertensive

Recently, we have reported that lifelong antihypertensive angiotensin-converting enzyme (ACE) inhibitor treatment doubled the maximal lifespan in stroke-prone spontaneously hypertensive rats (SHR-SP), being identical to the maximal lifespan of placebo-treated normotensive Wistar-Kyoto rats (WKY). This effect correlated with preservation of endothelial function, cardiac function/size, and cardiac metabolism.[1]

The beneficial effects of chronic ACE inhibition have been attributed to reduction of local angiotensin II (Ang II) production and increased accumulation of local kinin concentrations. Reduction of local Ang II concentrations induced by ACE inhibition was mainly related to antihypertensive and antihypertrophic actions. Increased local kinin concentrations by inhibition of ACE/kininase II, stimulating B_2 kinin receptors, were associated with increased synthesis and release of nitric oxide (NO).[2] This mechanism most likely contributed to the preservation of vascular and cardiac function by inhibiting or scavenging superoxide production.[3]

Different from ACE inhibition, under which Ang II may be formed via ACE-independent pathways,[4] Ang II type 1 receptor (AT_1) blockade fully prevented the vasoconstrictor, hormone-stimulating, and growth-promoting effects of Ang II. Human studies with AT_1 blockers are, so far, limited. The compounds have proved to be effective for the treatment of hypertension[5] and congestive heart failure.[6,7]

Up to now, experimental investigations with AT_1 blockers revealed no uniform outcome, although in many studies with experimental hypertension, these drugs share with ACE inhibitors the same beneficial effects. Left ventricular hypertrophy (LVH) and impaired cardiac function and metabolism in SHR-SP were equally prevented by early-onset long-term treatment by AT_1 blockers and ACE inhibitors.[8] Hypertrophy after hypertension induced by inhibition of endothelial NO synthase (eNOS) activity was also prevented to a similar extent by both long-term treatment regimens.[9] The same was true for hypertrophy in the 2-kidney, 1-clip rat model with an activated renin-angiotensin system.[10] However, in rats with persistent systolic pressure overload due to ascending aortic stenosis, long-term AT_1 blockade did not regress LVH,[11] whereas long-term ACE inhibition regressed LVH, normalized survival, and improved cardiac function in the same model.[12] AT_1 antagonism and ACE inhibition displayed similar inhibitory effects on hypertrophic remodeling in rats after acute myocardial infarction[13] and acute ischemia/reperfusion injuries,[13,14] whereas in the chronic situation, only the ACE inhibitor treatment was effective.[15]

Experimental studies concerning the effect of AT_1 blockers on survival are very scarce. A 1-year study[16] in rats with coronary ligation–induced chronic heart failure showed no difference in survival after losartan or captopril treatment;

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however, in that study, no control group was included. In another study[17] involving the AT_1 blocker irbesartan, a dose-dependent increase in survival in the rat postinfarction model of congestive heart failure was shown.

Because no data exist concerning prevention of mortality with early-onset long-term AT_1 blocker treatment in genetically hypertensive rats, we investigated the effects of fonsartan (HR 720)[18] in an antihypertensive dose on maximal lifespan extension in SHR-SP. In addition, and also to determine the mechanisms involved in the treatment-induced effects, we investigated cardiac function/size and metabolism, endothelial function, expression of eNOS, and ACE expression and activity.

Methods

Animals

Male 1-month-old SHR-SP with equal body weights were purchased from Møllegrad (Skvensgaard, Denmark). They were housed 3 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

Ninety animals were randomly allotted to 2 groups, with 45 animals in each group, which were treated, via drinking water, with placebo or an antihypertensive dose ($10\ mg \cdot kg^{-1} \cdot d^{-1}$) of fonsartan.[18] Treatment started immediately after randomization and was adjusted to the actual fluid consumption. Body weights and systolic blood pressures (tail plethysmography) were determined every 3 months. Deaths were recorded as they occurred.

Interim Analysis

Interim analysis was scheduled when ≈80% of the placebo-treated animals had died, which was after 15 months. Ten animals each were randomly selected and anesthetized (hexobarbital, $80\ mg \cdot kg^{-1}$ IP) for direct recording of mean arterial blood pressure in the left carotid artery. Thereafter, blood samples were drawn, and thoracic aortas, carotid arteries, and hearts were removed for molecular, biochemical, and/or functional analyses. Renin activity and concentrations of aldosterone and Ang II were determined in plasma.[1] ACE activities in plasma, thoracic aorta, and right cardiac ventricle were radioenzymatically measured by use of [^{3}H]Hip-Gly-Gly as substrate (Hycor ACE-activity test).

Expression of eNOS in Carotid Artery (Western Blotting)

Vessels were thawed and extracted with guanidinium isothiocyanate/phenol/chloroform.[19] Western blot analyses using 100 μg of the total protein extracts were performed as previously described.[20]

Expression of mRNA of eNOS and ACE in Left Cardiac Ventricle

Total mRNA was isolated from apex sections of the left ventricles by guanidinium isothiocyanate/phenol/chloroform extraction.[19] RNAse protection experiments for ACE and eNOS, with the use of 20 mg of total RNA, were performed as previously described.[1,21]

Isolated Working Heart

Hearts were perfused with a constant perfusion pressure of 65 mm Hg.[22] Left ventricular pressure, left ventricular dP/dt_{max}, and heart rate were measured via a balloon catheter. Coronary flow was determined with an electromagnetic flow probe. After a washout phase of 5 minutes, 1 mL of the coronary effluent was sampled for measuring lactate concentration, the activities of lactate dehydrogenase and creatine kinase,[22] and concentrations of kinins. The antibody used in the kinin radioimmunoassay did not distinguish among bradykinin, lysyl-bradykinin, and methionyl-lysyl-bradykinin.[23] Thereafter, the hearts were gently blotted to dryness for determination of total and left and right ventricular heart weights.

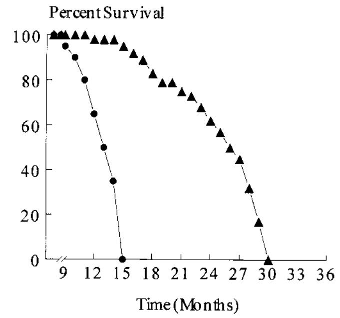

Figure 1. Effect of early-onset lifelong treatment with fonsartan on cumulative percent survival in SHR-SP. ● indicates placebo; ▲, fonsartan ($10\ mg \cdot kg^{-1} \cdot d^{-1}$). Percent survival was significantly enhanced with fonsartan vs placebo in SHR-SP (Kaplan-Meier analysis followed by Cox-Mantel log rank test; $\chi^2=43.92$, $P<0.05$).

Isolated Rings of Thoracic Aorta

The method used was the same as previously described.[1] Briefly, in a temperature-regulated (37°C) 10-mL organ bath with modified Tyrode's solution, each strip of the aorta was mounted vertically between 2 fine stainless steel pins. The upper pin was connected to an isometric strain-gauge transducer. The transducer signal was recorded with a computer-assisted biosignal analyzer. Aortic strips were suspended under a passive tension of 4.9 mN. After an equilibration period of 1 hour, the strips were contracted by 20 mmol/L KCl. At the plateau of KCl-induced contraction, acetylcholine (10^{-8} to 10^{-5} mol/L) was added in a cumulative manner to relax the vessel strips. Acetylcholine-induced relaxations were related to the respective KCl contractions.

Statistical Analysis

The data are given as mean±SE. Cumulative survival was analyzed for differences according to Kaplan-Meier followed by Cox-Mantel log rank test. ANOVA or ANOVA on ranks, as appropriate, followed by multiple pairwise comparisons according to Student-Newman-Keuls was used. Null hypotheses were rejected at $P<0.05$.

Results

Measurements of Body Weight and Systolic Blood Pressure

Body weights increased from 97±4 g (at 1 month) to 370±4 g (at 15 months) in placebo-treated SHR-SP. Fonsartan treatment did not significantly affect body weight. Systolic blood pressure (plethysmographic tail-cuff method) of 103±4 mm Hg in 1-month-old placebo-treated SHR-SP was significantly increased (157±5 mm Hg) after 3 months and reached the highest value (243±6 mm Hg) after 12 months. Fonsartan treatment completely prevented the rise in blood pressure (107±3 mm Hg).

Cumulative Survival

All placebo-treated SHR-SP survived within the first 9 months of age. Thereafter, the animals successively died, revealing a maximal lifespan of 15 months. Fonsartan treatment doubled the life expectancy to 30 months (Figure 1).

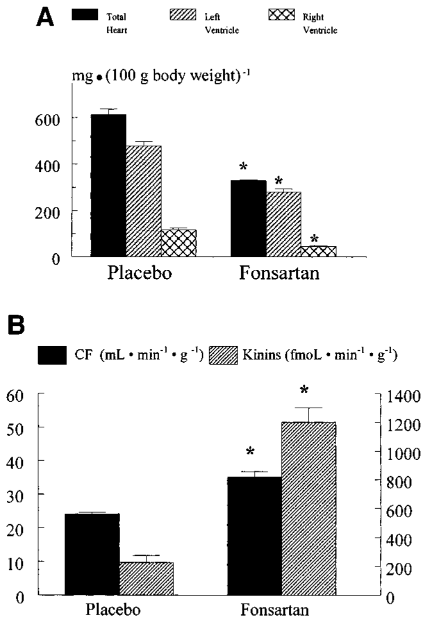

Figure 2. A, Total and left and right ventricular heart weights at the interim analysis after 15 months of treatment with fonsartan (10 mg · kg^{-1} · d^{-1}) vs placebo. **P*<0.05 vs matching placebo (n=10 per group). B, Changes in coronary flow (CF, mL · min^{-1} · g heart wet wt^{-1}, left ordinate) and kinins in the coronary effluent (fmol · min^{-1} · g heart wet wt^{-1}, right ordinate) of isolated working hearts at the interim analysis after 15 months of treatment with fonsartan (10 mg · kg^{-1} · d^{-1}) vs placebo. **P*<0.05 vs matching placebo (n=10 per group).

Interim Analyses After 15 Months

In SHR-SP, fonsartan treatment reduced mean arterial blood pressure (to 98±5 mm Hg) compared with placebo treatment (155±5 mm Hg). These values are in line with those found by plethysmographic tail-cuff determination.

Compared with placebo treatment, fonsartan treatment significantly reduced total and left and right ventricular heart weights (Figure 2A); heart weights in SHR-SP after fonsartan treatment were similar to those of normotensive WKY.[1]

Significantly increased plasma renin activity and plasma Ang II concentration were observed in fonsartan-treated animals compared with placebo-treated animals. Plasma aldosterone concentration and plasma ACE activity as well as ACE activities in the thoracic aorta and right cardiac ventricle were significantly reduced by fonsartan treatment (Table). In the left cardiac ventricle, a significant reduction of ACE mRNA expression was observed with fonsartan treatment (Table).

Isolated Working Heart Preparation

Compared with hearts from placebo-treated SHR-SP, hearts from fonsartan-treated SHR-SP revealed a significant increase in left ventricular pressure and left ventricular contraction rate (dP/dt_{max}) (2334±101 [placebo] versus 4005±99 [fonsartan] mm Hg · s^{-1}). Also, heart rate was significantly increased by fonsartan treatment (135±7 [placebo] versus 177±7 [fonsartan] bpm). In hearts from fonsartan-treated rats, coronary flow and release of kinins into the coronary effluent were significantly enhanced (Figure 2B). The activities of cytosolic enzymes and the release of lactate into the coronary effluent were significantly lower in hearts from fonsartan-treated rats (respective values for placebo versus fonsartan were as follows: creatine kinase, 1.0±0.1 versus 0.76±0.08 mU · min^{-1} · g heart wet wt^{-1}; lactate dehydrogenase, 1.48±0.12 versus 1.02±0.1 mU · min^{-1} · g heart wet wt^{-1}; and lactate release, 25.2±1.1 versus 5.5±0.2 μmol · min^{-1} · g heart wet wt^{-1}).

Isolated Thoracic Aorta

The strongly impaired endothelium-dependent relaxation in response to acetylcholine in placebo-treated SHR-SP was significantly prevented by fonsartan treatment (Figure 3A).

Expression of eNOS in Cardiac Left Ventricle and Carotid Artery

Expression of eNOS mRNA was slightly but significantly increased in hearts from fonsartan-treated rats (Figure 3B). Expression of eNOS protein assessed by densitometric Western blot analysis showed a slight increase in the carotid artery of fonsartan-treated SHR-SP: respective intensity of placebo versus fonsartan was 75±36 versus 152±90 (optical density · mm^2)$^{-1}$.

Markers of the Renin-Angiotensin System in SHR-SP After 15-Month AT_1 Blockade With Fonsartan

	Placebo	Fonsartan (10 mg · kg^{-1} · d^{-1})
PRA, ng Ang I · mL^{-1} · 10 min^{-1}	3.1±0.3	12±2.6*
Ang II, pg · mL^{-1}	69±9.1	761±70*
Aldosterone, pg · mL^{-1}	380±16	288±15*
ACE activity		
Plasma, nmoL · mL^{-1} · min^{-1}	166±7.7	113±8.2*
Thoracic aorta, nmoL · mg protein^{-1} · h^{-1}	1646±59	528±89*
Right cardiac ventricle, nmoL · mg protein^{-1} · h^{-1}	83±12	28±2.2*
ACE expression in left cardiac ventricle, mRNA signal intensity−ACE/γ-actin) · 10^{-4}	258±31	169±10*

Values are mean±SE. PRA indicates plasma renin activity.
**P*<0.05 vs placebo.

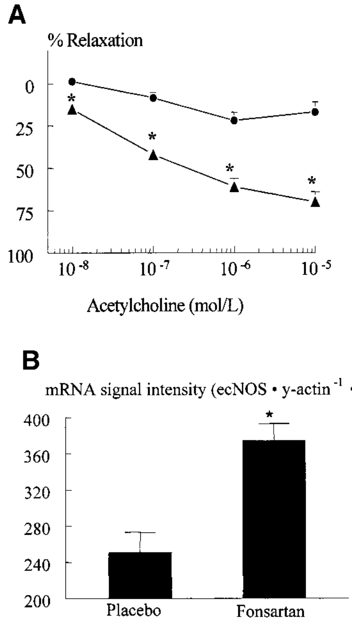

Figure 3. A, Endothelium-dependent relaxations by acetylcholine expressed as percent reversal of KCl (20 mmol/L)–elicited contractions in aortic rings at the interim analysis after 15 months. ● indicates placebo; ▲, fonsartan (10 mg · kg^{-1} · d^{-1}). *$P<0.05$ vs matching placebo (n=10 per group). B, Expression of eNOS (mRNA signal intensity) in the left cardiac ventricle at the interim analysis after 15 months of treatment with fonsartan (10 mg · kg^{-1} · d^{-1}) and placebo. *$P<0.05$ vs matching placebo (n=10 per group).

Discussion

Effect of AT_1 Blockade on Lifespan Extension

Lifelong treatment with the AT_1 blocker fonsartan in a blood pressure–lowering dose of 10 mg · kg^{-1} · d^{-1} doubled life expectancy in SHR-SP, which corresponded with the lifespan of normotensive WKY.[1] This lifespan extension by fonsartan appeared to be the result of the concert of beneficial effects that the drug exerted on blood pressure, development of cardiac hypertrophy, cardiac pump function and metabolism, and endothelial function.

Effects of AT_1 Blockade on Cardiac LVH

Previously, we showed that compared with age-matched normotensive WKY, senescent SHR-SP with cardiac hypertrophy exhibited significant increases in cardiac ACE mRNA expression and activity.[1] Consistent with these findings are data in failing human hearts in which ACE expression of the cardiac left ventricle was upregulated.[24] Surprisingly, lifelong treatment with fonsartan significantly decreased myocardial ACE mRNA expression (−38%) and ACE activity (−67%) in SHR-SP. In another model of hypertension induced by N^G-nitro-L-arginine methyl ester, the increased cardiac ACE activity was also reduced by an AT_1 blocker.[9] This effect might be explained by unknown indirect counterregulating mechanisms in response to increased circulating renin as well as Ang II levels under long-term AT_1 blockade.[25,26] However, some insight might be gained from the findings that NO competitively inhibited the activity of purified ACE and that stimulated endothelial NO release from rat carotid arteries physiologically reduced the conversion of angiotensin I to Ang II.[27] No acute (60-minute incubation) inhibitory action on ACE activity in plasma and cardiac tissue from untreated rats could be observed in the presence of fonsartan (data not shown).

The strong inhibition of cardiac ACE activity by long-term fonsartan treatment provides for an enhanced local accumulation of kinins, which seems to be related to an antihypertrophic action. Recently, it was shown that bradykinin abolished the Ang II–induced hypertrophy in adult myocytes cocultured with endothelial cells but not in myocytes in the absence of endothelial cells.[28] Therefore, it seems that besides AT_1 blockade, the strongly decreased cardiac ACE activity observed in response to the long-term AT_1 blocker treatment also contributed to the antihypertrophic effect.

Effect of ACE Inhibition on Heart Function and Metabolism

Isolated hearts from placebo-treated SHR-SP showed impaired cardiac function (left ventricular pressure/dP/dt_{max}) and increased metabolic markers for ischemia (activities of creatine kinase and lactate dehydrogenase as well as lactate content). In the present study, fonsartan treatment prevented the impairment of myocardial metabolism. Furthermore, this treatment evoked an increased coronary flow, probably mediated by blocked Ang II activity and increased kinins. The enhanced kinin accumulation, in turn, is most likely due to the strong inhibition of cardiac ACE activity by fonsartan treatment.

Similar beneficial effects were seen in isolated rat hearts with ischemia/reperfusion under AT_1 blockade with losartan. In the present study, the cardioprotective effects were dependent on bradykinin receptor activation, because cardioprotection by losartan was blunted by the B_2 kinin receptor antagonist icatibant.[29] An improvement of coronary flow by kinins has been also shown in isolated normoxic and ischemic working rat hearts.[30] Cardiac pump function seems also to be mediated by kinins. AT_1 blockade significantly improved pump function in rats with congestive heart failure. Cotreatment with the B_2 kinin receptor antagonist icatibant partially reversed this effect. AT_1 blockade seems to be also related to the activation of AT_2 receptors whose blockade reversed the beneficial effects of AT_1 blockers.[31] Stimulation of AT_2 receptors has been shown to activate the kinin/NO system.[32,33] Thus, these data indicate that the decrease in afterload is not the only cause of cardioprotective effects induced by AT_1 blockers.

Effect of AT_1 Blockade on Isolated Thoracic Aorta

Our results confirm previously published results showing that the strongly impaired endothelium-dependent relaxation in aortas from hypertensive rats became ameliorated by chronic AT_1 blockade.[8,34] This improvement of endothelial dysfunction can be attributed to a variety of mechanisms. The reduction of elevated blood pressure by AT_1 blockade could be considered to be a primary nonspecific mechanism. On the other hand, in vitro treatment with losartan of aortas from untreated 20-month-old SHR also reduced vascular dysfunction in response to acetylcholine.[35]

The present study revealed that endothelial preservation by lifelong treatment with fonsartan was positively correlated with a slightly increased aortic eNOS expression, which, in turn, was most likely associated with an enhanced NO synthesis and release. This was supported by experiments showing that Ang II stimulated eNOS protein levels in the rat kidney[36] and NO synthesis and release in cultured endothelial cells.[32,37]

There are some data indicating that like ACE inhibitors, AT_1 blockers were also able to interact with the kinin system.[32,33] Strongly increased plasma Ang II levels as a result of AT_1 blockade[25,26] obviously activate AT_2 receptors; this activation, in turn, results in the local formation of kinins and, consequently, NO synthesis.[32,38]

Conclusion

Lifelong antihypertensive AT_1 blockade prevents the development of LVH and dysfunction of the heart and endothelium, resulting in a doubling of the life expectancy of rats with a genetic form of hypertension. These beneficial cardiovascular effects can be attributed to various molecular/biochemical mechanisms. The deleterious actions of Ang II are abolished by specific AT_1 blockade and also, concomitantly, by inhibition of tissue ACE activity. The latter increases local kinin concentrations. As a result of AT_1 blockade, increased levels of Ang II in the plasma and tissue lead to an upregulation of eNOS and, presumably, stimulation of endothelial AT_2 receptors, evoking kinin release with subsequent NO formation. Thus, the mechanisms by which chronic AT_1 blockade and ACE inhibition act in SHR-SP are similar, in that both block the renin-angiotensin system and both enhance the release of NO via kinins, leading to a comparable beneficial outcome in the respective survival of hypertensive animals.

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