Effect of Early Onset Angiotensin Converting Enzyme Inhibition on Myocardial Capillaries

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We investigated the preventive effects of long-term treatment with the angiotensin converting enzyme inhibitor ramipril on myocardial left ventricular hypertrophy and capillary length density in spontaneously hypertensive rats. Rats were treated in utero and subsequently up to 20 weeks of age with a high dose (1 mg/kg per day) or with a low dose (0.01 mg/kg per day) of ramipril. Animals given a high dose of ramipril remained normotensive, whereas those given a low dose developed hypertension in parallel to vehicle-treated controls. At the end of the treatment period, converting enzyme activity in heart tissue was inhibited dose-dependently in the treated groups. Both groups revealed an increase in myocardial capillary length density together with increased myocardial glycogen and reduced citric acid concentrations. Left ventricular mass was reduced only in high dose- but not in low dose-treated animals. Our results demonstrate that early onset treatment with a converting enzyme inhibitor can induce myocardial capillary proliferation, even at doses too low to antagonize the development of hypertension or left ventricular hypertrophy. We hypothesize that potentiation of kinins is responsible for this effect, probably by augmenting myocardial blood flow, which is a well-known trigger mechanism of angiogenesis in the heart. (Hypertension 1992;20:478-482)

KEY WORDS • angiotensin converting enzyme inhibitors • hypertrophy • capillaries • rats, inbred SHR

Cardiac left ventricular hypertrophy (LVH), a frequent consequence of arterial hypertension, is increasingly considered an independent cardiovascular risk factor giving rise to cardiac failure, ischemia, and arrhythmias.1 Spontaneously hypertensive rats (SHR) develop hypertension during their first 12 weeks of life. As in human hypertension, the increase in blood pressure is accompanied by vascular and cardiac hypertrophy. Whereas LVH induced by physical exercise or chronic hypoxia is associated with normal or even increased capillary density, hypertension-induced LVH usually features a reduced capillary supply that may eventually lead to cardiac ischemia.2

Antihypertensive treatment of SHR with angiotensin converting enzyme (ACE) inhibitors has been shown to reduce LVH as well as blood pressure.3-5 Linz et al recently reported that long-term treatment with a low or sub-antihypertensive dose of the ACE inhibitor ramipril (0.01 mg/kg per day) prevented LVH in rats with renal hypertension due to aortic banding, suggesting that early onset treatment with ACE inhibitors can induce structural changes of the heart independent of the blood pressure-lowering actions of these drugs. The question of whether a sub-antihypertensive dose of an ACE inhibitor can also prevent cardiac hypertrophy in genetic hypertension such as in SHR and whether these structural changes include an influence on myocardial capillary density or myocardial metabolism has not yet been examined.

In the present study we investigated the preventive effect of chronic high-dose (antihypertensive) and low-dose (sub-antihypertensive) treatment with the ACE inhibitor ramipril on cardiac hypertrophy, myocardial capillary length density, and parameters of myocardial metabolism in SHR.

The morphometric measurements were performed using the newly developed “orientator” method,7 specifically designed for an unbiased quantitative morphometric analysis of anisotropic structures such as myocardial capillaries.

Methods

SHR (n=36 per group) were obtained from Mollegard, Skensved, Denmark, and were treated both in utero and up to 20 weeks of age with the ACE inhibitor ramipril at doses of 1 mg/kg per day (group 1) and 0.01 mg/kg per day (group 2). Control SHR (group 3) received vehicle (H2O). Ramipril was obtained from Hoechst AG, Frankfurt/Main, FRG. The drug was added to the overnight drinking water (distilled H2O) and carefully adjusted to the individual drinking habits of the growing animals. Details of drug administration have been published previously.8-9

Dosage of ramipril during pregnancy and lactation was based on body weight of the dams under the assumption of sufficient distribution of the drug into
different body compartments including placenta and milk. Ramipril treatment at both doses had no influence on litter size and body weight of the offspring. Blood pressure was measured by tail plethysmography under light ether anesthesia at 2-week intervals. Measurements were begun when the animals were 6 weeks old.

**Morphometric Parameters**

At the end of the treatment period, 12 rats from each group were anesthetized with chloralhydrate, and the hearts were fixed by retrograde vascular perfusion with 3% glutaraldehyde in 0.2 M phosphate buffer at a pressure of 110 mm Hg.

Before fixation, the hearts were flushed with a dextran solution containing 0.5 g/l procaine-HCl. After perfusion, the wet weights of the left ventricles were determined.

The length density \(L_{cap/tiss}\) of myocardial capillaries was determined with the orientator method. Briefly, the orientator describes an approach to generate isotropic planes in biological specimens that allows the quantitative study of anisotropic structures. The left ventricle of each animal was partitioned systematically into parallel horizontal slices of equal thickness, parallel to the valvular plane of the heart. The slices were placed on a square lattice equipped with random numbers, which allowed strictly random selection of tissue for the stereological evaluation. After temporary embedding in Agar cylinders, two series of four sections per specimen were generated with the \(\theta\)-clock according to the systematic version I of the orientator method. The specimens were further prepared for light microscopic examination (after fixation, plastic embedding, microtomy, staining). The strong contrast between the optically empty vascular spaces and the surrounding cells allowed the automatic measurement of the stereological parameters \(Q_x\) and \(B_x\) with an IBAS image analyzer (Zeiss, Oberhausen, FRG), where \(Q_x\) and \(B_x\) are the sample means of number and boundary length of capillary profiles per unit reference area. The length density \(L_{cap}\) was calculated according to \(L_{cap} = 2Q_x\).

**Electron-Microscopic Stereological Investigations**

The volumetric composition of the myocardial cells was analyzed by stereological standard methods. From the technically best block of each of the two orientator series, as evaluated by light microscopy, ultrathin sections were cut with a Reichert OmU2 ultramicrotome (Leica, Stuttgart, FRG). After staining with lead citrate and uranyl acetate, the sections were evaluated with a Zeiss EM10 electron microscope to which a video camera was connected on-line. The electron microscopic image was projected by a monitor and a transparent sheet with a square lattice consisting of 100 test points was mounted. Twenty systematic visual fields with uniform random start within each section were evaluated. The volumes of myofibrils, mitochondria, and cytoplasmic matrix per unit volume of myocardial cell cytoplasm \(V_c\) were determined from the number of points hitting the respective compartments \(P_s\) according to the principle of Delesse: \(V_c = P_s\). This equation provides unbiased estimates of volume densities of arbitrary structures regardless of the direction of cutting.

**Biochemical Determinations**

At the end of the treatment period, rats not used for the morphometric studies \((n=24\) per group) were anesthetized with ether and exsanguinated. Hearts were rapidly frozen in liquid nitrogen and homogenized in 0.3% Triton X-100 (ACE activity, \(n=12\) per group) or 0.6 M HClO (glycogen, adenosine triphosphate [ATP], citric acid, \(n=12\) per group). ACE activity in plasma and heart tissue (apex) was measured by a fluorometric method. Glycogen, ATP, and citric acid concentrations of the heart were determined according to Keppler and Decker, Trautschold et al, and Möllering, respectively.

**Statistical Analysis**

Data are reported as mean±SEM. Statistical analysis was performed by two-way analysis of variance followed by appropriate post-hoc tests (CRISP) between groups.

**Results**

Oral treatment of SHR in utero and subsequently up to 20 weeks of age with the high dose (1 mg/kg per day) of ramipril completely prevented the development of hypertension, whereas SHR treated with the low dose (0.01 mg/kg per day) of ramipril developed hypertension in parallel to the control group (Figure 1).

Plasma ACE activity was significantly inhibited only after treatment with the high dose of ramipril, whereas ACE activity in heart tissue was dose-dependently reduced in both treated groups (Figure 2).

Figure 3 shows that the development of LVH was effectively prevented after high-dose but not after low-dose ACE inhibitor treatment. Cardiac capillary length density was increased in ramipril-treated SHR compared with untreated controls (Figure 4). This effect was observed not only after high-dose antihypertensive treatment, but also after low-dose treatment in animals in which LVH had developed.

Myocardial glycogen content was increased and citric acid content decreased after low- and high-dose treatment with ramipril (Figure 5). We also observed a
tendency for myocardial ATP content to increase after low- and high-dose treatment (12.58±0.38 after low dose; 12.90±0.47 after high dose versus 11.58±0.41 μmol/g dry weight in controls); however, it did not reach statistical significance (Figure 5).

The electron microscopic results are summarized in Table 1. No significant differences could be ascertained between the control group and the experimental groups. Descriptive examination of the electron micrographs disclosed essentially normal myocardium in all groups with no striking pathological alterations.

**Discussion**

Capillary proliferation is generally held to be a rare event in an adult mammalian heart. In SHR with cardiac hypertrophy, the only condition that so far has been reported to induce myocardial capillary growth is physical exercise. In a previous study, Clozel et al reported that in SHR with established hypertension, antihypertensive treatment with the ACE inhibitor cilazapril increased the density and the cross-sectional surface area of the myocardial capillaries. Our present data confirm and extend these observations, showing for the first time that early onset treatment of SHR with a sub-antihypertensive dose of an ACE inhibitor leads to a significant increase in myocardial capillary length density that according to Mall et al, most probably reflects neoformation of capillary branches in parallel connection.

After long-term treatment with the antihypertensive dose of the ACE inhibitor, capillary growth was associated with a reduction of LVM. This could be expected because reduction of left ventricular weight leads to a reduction of myofiber size, which in turn engenders a denser spacing of myocardial capillaries; thus, capillary length density increases. Surprisingly, however, the low dose of the drug also increased myocardial capillary length without having an effect on left ventricular mass. The volumetric composition of the myocardial cell can provide information on the functional status of the hypertrophied heart. We found no significant change in the ultrastructure of the myocardial cells from untreated rats as compared with those from the normotensive animals. In addition, none of the animals showed signs of myocardial insufficiency. Thus, neither descriptive examination of the myocardial ultrastructure nor the stereological data indicates that heart failure took place in this experiment.
ACE activity was significantly inhibited in the blood plasma after the hypertensive dose but not after the low dose of ramipril, whereas in tissue homogenates of the heart, both drug regimens caused a significant inhibition of the enzyme in a dose-dependent fashion. Similarly, both the high and the low dose of the ACE inhibitor significantly increased cardiac glycogen, decreased cardiac citric acid, and tended to increase cardiac ATP content. Thus, the inhibition of ACE in the blood reflected the preventive action of the drug on the development of hypertension and LVH, whereas inhibition of the enzyme in heart tissue was more closely associated with myocardial capillary proliferation and changes in cardiac metabolism.

How can the observed effects of long-term ramipril treatment on cardiac structure and metabolism be reconciled with the known mechanisms of action of ACE inhibitors?

First, several authors have suggested that angiotensin II (Ang II) acts as a promoter of vascular or myocardial hypertrophy in hypertension. In addition, Ang II has been implicated in the proliferation of vascular smooth muscle or heart cells. Inhibitors of ACE could, therefore, exert an antihypertrophic, anti-proliferative, or both, action by reducing the generation of Ang II in the heart and blood vessels even independent of their blood pressure-lowering effects.

However, our findings in SHR do not lend support to this contention: First, LVH was prevented only by the high dose of ramipril that prevented the development of hypertension but not by the low dose, which had no effect on the development of hypertension. This does not preclude the involvement of Ang II in cardiac hypertrophy in SHR because the low dose of ramipril may have been too low to be effective, but it also does not lend support to the idea that ACE inhibitors could prevent LVH independent of their hemodynamic actions by reducing cardiac Ang II concentrations. Our observation is at variance with the above-mentioned report by Linz et al. The discrepancy between the two studies could be explained by the fact that Linz et al used a renin-dependent model of experimental hypertension, which may respond to ACE inhibition more drastically than our model of genetic hypertension in SHR.

Furthermore, it is difficult to ascribe our observation of an ACE inhibitor-induced myocardial capillary proliferation to a partial or complete elimination of the putative growth factor Ang II. We did not measure Ang II levels in cardiac tissue in the present study. However, since ACE activity in the heart was inhibited by both doses of ramipril and since pathways of Ang II generation other than through ACE, especially the chymase pathway, have not been demonstrated in rat hearts, we assume that the local Ang II concentration in the heart was most likely reduced. The possibility that a compensatory increase of cardiac Ang II by a non-ACE-dependent pathway was responsible for myocardial capillary proliferation during ACE inhibitor treatment does, therefore, not seem to be a plausible explanation for our findings. Thus, it appears unlikely that ACE inhibitor-induced changes of the Ang II concentration in the blood or the heart were responsible for the increase in myocardial capillary length density.

Second, our study was designed to effectively prevent the development of hypertension in SHR by the high dose of the ACE inhibitor. Since subtle changes in blood pressure may occur very early in this genetic model of hypertension, this goal can be achieved best by commencing treatment as early as possible, i.e., during pregnancy. Under these conditions, we cannot rule out that ACE inhibitor treatment may have triggered myocardial capillary growth during fetal development, although to our knowledge such a mechanism has not yet been reported.

A third explanation for our findings on myocardial capillary length density resides in the bradykinin-potentiating effect of the ACE inhibitor. There are several pieces of evidence supporting this idea: First, the common denominator of all experimental conditions associated with myocardial capillary proliferation appears to be enhanced myocardial blood flow. Bradykinin has been shown to improve myocardial blood flow under different experimental conditions even at very low concentrations. Ramipril equally increased myocardial flow, and the effects of both bradykinin and the ACE inhibitor were abolished by a bradykinin B1-receptor antagonist. In our present study, high- and low-dose ramipril treatment inhibited the ACE activity in cardiac tissue, implying that the breakdown of bradykinin in the heart was inhibited and its actions potentiated. Thus, a bradykinin-mediated increase in myocardial perfusion was possible under our experimental conditions. Second, both treated groups exhibited increased cardiac glycogen and decreased citric acid concentrations together with a tendency toward increased ATP levels. These findings are compatible with bradykinin's known cardiac metabolic effects to enhance myocardial glucose.
uptake in normoxic isolated rat hearts, thus preserving cardiac glycogen stores. In addition, bradykinin, even at very low concentrations, was demonstrated to preserve myocardial glycogen and energy-rich phosphates under ischemic conditions. The same effect, observed under ramiplril treatment, could be abolished by a bradykinin B2 receptor antagonist.

In view of these previous observations, we propose the hypothesis that in our study, the ACE inhibitor-induced potentiation of cardiac bradykinin enhanced myocardial perfusion that in turn improved cardiac metabolism and also led to myocardial capillary proliferation. We further postulate that these bradykinin-related cardiac events were independent of the effect of the ACE inhibitor on blood pressure and LVH since they occurred after high- and low-dose ramiplril treatment. Further studies including specific Ang II and bradykinin receptor antagonists will have to examine the possible effect of an ACE inhibitor-induced kinin potentiation on myocardial capillary growth in more detail.

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