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Diverse effects of Ace inhibitors and angiotensin II receptor antagonists on prevention of cardiac hypertrophy and collagen distribution in spontaneously hypertensive rats

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Abstract

This study has compared the effects of two structurally different angiotensin converting enzyme inhibitors (ACEis) such as zofenopril (Zof, with sulfhydrylic group) and lisinopril (Lis, with carboxylic group) and an angiotensin II AT_1 receptor antagonist (losartan, Los) on the prevention of cardiac hypertrophy and collagen distribution in spontaneously hypertensive rats (SHRs). The SHRs were untreated or received: Zof (10 mg/kg/day), Lis (10 mg/kg/day) or Los (20 mg/kg/day) in drinking water starting at 4 weeks of age. At 8, 16 and 24 weeks of age, 8 rats/group were sacrificed for determination of blood pressure, cardiac hypertrophy and collagen distribution. All treatments significantly decreased blood pressure and cardiac indices, expressed as the ventricles to body weight ratio, both variables being significantly correlated. Total ventricular collagen content was similarly decreased in all treated groups. Zof significantly increased the expression of collagen type III and normalized the collagen type I/III ratio. These results suggest that the effects of these drugs on different types of collagen are independent from angiotensin II formation. Similar findings obtained with captopril seem to indicate that the antioxidant sulfhydrylic group of these ACEis can play a role in the distribution of collagen during cardiac hypertrophy.

Keywords: ACE inhibitors; Angiotensin; Collagen; Fibrosis; Hypertension; Hypertrophy

1. Introduction

Arterial hypertension is the most important aetiological factor for the appearance of symptomatic heart failure, and left ventricular hypertrophy (LVH) is the major risk factor [1]. The spontaneously hypertensive rat (SHR) is the analogous experimental model for primary hypertension in humans in comparison to its normotensive genetic control, the Wistar-Kyoto (WKY) rat [2]. LVH, which is mainly mediated by myocyte hypertrophy [3], develops progressively in SHRs [4] and is associated with a pathological accumulation of fibrous tissue within the cardiac interstitium [5]. This remodelling of the collagen network leads, such as in other experimental models and humans, to an increase in myocardial stiffness and abnormalities of cardiac function [6]. Therefore, spontaneous hypertension in rats offers a good experimental model to study the natural development of cardiac hypertrophy and myocardial fibrosis as well as their prevention/regression through pharmacological interventions.

Using in vitro and in vivo studies, it has been demonstrated that the renin–angiotensin–aldosterone system and its effector hormone, angiotensin II, are of major importance in regulating the myocardial collagen matrix structure in arterial hypertension [2]. The incubation of adult rat cultured cardiac fibroblasts with angiotensin II resulted in a dose-dependent increase in collagen synthesis [7]. Moreover, angiotensin II suppresses the collagenase activity that synergistically leads to progressive collagen accumulation within the cardiac interstitium [3]. Fibrillar types I and III collagens are major structural proteins of the myocardial collagen matrix. Type I collagen is usually present in the form of thick fibres and its concentration determines the tissue stiffness of the myocardium. Type III collagen forms fine reticular networks and is more distensible than type I

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collagen [2]. It has recently been suggested that the quality of collagen in the remodelling of myocardium in hypertensive heart disease is responsible for adverse cardiovascular events [8].

Angiotensin converting enzyme (ACE) inhibitors have been shown to successfully reverse LVH and myocardial fibrosis in SHRs [3,9–11]. These agents are characterized by a common 2-methyl propranolol-L-proline moiety and can be distinguished by their functional group (sulfhydrylic, carboxylic or phosphinylic) which allow the drugs to adhere to the zinc component of the active site of ACE [12].

The aim of this study was to investigate the effects of an ACE inhibitor containing a sulfhydryl-group (zofenopril) with a non-sulfhydryl ACE inhibitor (lisinopril, Lis) and an angiotensin II AT_1 receptor antagonist (losartan) on the prevention of hypertension, cardiac hypertrophy and collagen distribution in SHRs.

2. Methods

2.1. Experimental animals

A total of 96 male SHRs and 24 male WKY were purchased from Charles River Canada (St-Constant, Québec) at 4 weeks of age, immediately after weaning. The rats were housed individually and received a standard rat chow (Purina Rodent Lab Chow 5001, ICN Biochemicals, Cleveland, OH) and water ad libitum. They were maintained in a room at constant temperature (20–22 °C) and humidity (40%) with a 12h light–dark cycle. Following a 3-day acclimatization period, the animals were randomly divided into five groups ($n=24$ per group): (1) untreated WKY; (2) untreated SHRs (SHR); and SHRs receiving: (3) lisinopril, 10 mg/kg/day (SHR-Lis) [13]; (4) zofenopril, 10 mg/kg/day (SHR-Zof) [13]; and (5) losartan, 20 mg/kg/day (SHR-Los) [14–16]. All procedures were in accordance with the recommendations of the Canadian Council on Animal Care and monitored by an institutional animal care committee.

2.2. Drug administration

All drugs were administered in drinking water. Lisinopril was obtained from Sigma-Aldrich Chemie (Schnelldorf, Germany). Zofenopril was obtained from A. Menarini IFR (Florence, Italy) and was diluted in a solution of 800 mg/l of β-Cyclodextrin (Cycloheptaamylose, Sigma-Aldrich Canada, Oakville, Ontario) in distilled water [13]. Cozaar® tablets (losartan potassium, 100 mg, Merck Frosst, Canada) were crushed and dissolved in distilled water. The resulting solution was filtered by gravitation using a Whatman filter paper (2 qualitative) and diluted to obtain the required dosage. The concentration of the final solution was analysed using a spectrophotometer and pure losartan powder solution was used as reference. Untreated rats (SHR and WKY) received tap water. Water consumption and body weight were measured every week and drug concentrations were adjusted accordingly. Treatments began at 4 weeks of age and continued for 16 weeks.

2.3. Surgical procedure

At 8, 16 and 24 weeks of age, eight rats per group were anaesthetized with sodium pentobarbital (65 mg/kg i.p.). A small incision was made up to the dorsal nape of the neck and a ‘‘mixed’’ catheter consisting of pieces of welded PE-10 and PE-50 polyethylene tubing (Intramedic, Becton Dickinson and Company, Sparks, MD, USA) was fed, via a long trocar, from the neck to an incision site in the right leg. The left femoral artery was cannulated. The catheter was filled with heparinized saline (30 IU/ml) and sealed with a stainless steel plug. The rats were returned to their cages and allowed full recovery for 24h.

2.4. Hemodynamic determinations and tissue preparation

Rats, conscious and unrestrained, were left in their cage. The catheter was connected to a pressure transducer (Cobe, model CDX III, Fogg System Company, USA) via a PE-50 polyethylene tube, and the animals were allowed to calm down (≅15 min) before systolic and diastolic blood pressures were monitored with a Harvard oscillograph (model 50-8655). The mean arterial pressure (MAP) was calculated by adding one-third of pulse pressure to diastolic pressure. The heart rate was derived from the pressure readings. Rats were sacrificed with CO_2. The heart was immediately removed and placed in ice-cold saline to arrest in diastole and to remove blood. The atria and great vessels were dissected free from the ventricles and discarded. The ventricles were weighed and separated in two parts. A slice of approximately 2 mm thick was made from the upper section, placed in 10% formalin and kept for histological study. The right and left ventricles of the upper section were separated and kept. The lower section of the ventricles was also sliced (≅2 mm) for morphometric and collagen content evaluation. The right ventricle was then removed and discarded. All samples were frozen in liquid nitrogen and kept at −80 °C until assayed.

2.5. Cardiac hypertrophy evaluation

Cardiac index was calculated by dividing the weight of the ventricles by the body weight. The lower mid-level slices of the ventricles were scanned using an Agfa Snapscan (model 1212). The pictures were magnified hundredfold and morphometry evaluated. The left ventricular wall thickness was measured at seven points around the circular section and the average was calculated. The internal diameter of the left ventricle was measured from the farthest points of the major and minor internal diameters as already described by Tsoporis et al. [17].

2.6. Total ventricular collagen content

An estimate of the ventricular collagen concentration, the hydroxyproline concentration, was evaluated in left ventricular (LV) samples using a spectrophotometric technique as already described [18,19]. Briefly, approximately 20 mg of the lower LV slices was analysed ($n=8$ rats per group). The tissue was homogenized and hydrolysed with 6N HCl at 110 °C for 24 h. Hydrolysis samples were dried using a speed-vac evaporator and 1 ml of isopropanol was added. To this solution, 0.5 ml of oxidant (chloramine T) was added, vortexed and allowed to stand for 4 min 3.25 ml of Ehrlich's reagent (3 ml of Ehrlich's + 16 ml of isopropanol) were then added. The tubes were kept at 25 °C for 18 h and the absorbance was measured at 558 nm using a Beckman Spectrophotometer. The hydroxyproline concentration was determined from a standard curve. The collagen concentration was estimated by multiplying the hydroxyproline concentration by a correction factor of 8.2 [20]. The results are presented as total ventricular collagen content (total amount of collagen present in the ventricular tissue) and are expressed as mg of collagen (collagen concentration × ventricular weight).

2.7. Expression of ventricular collagen types I and III

The upper mid-level LV slices were dehydrated and embedded in paraffin. Two sequential 5-μm-thick sections were obtained for each heart ($n=8$ rats per group). Each section was stained with the collagen-specific picrosirius red [21]. Histological quantification of collagen was done under polarized light using an Olympus microscope. In such conditions, collagen type I appears in red and collagen type III appears in green. For each heart, collagen I and collagen III densities were evaluated in four optical sections by the quantification of the relative number of pixels classified as red or green by adjusting the threshold permitting a binary analysis. Collagen expression (%) was computed by the area of the pixels classified as collagen type I or collagen type III divided by the respective optical area.

2.8. Statistical analysis

Results are expressed as mean ± S.E.M. and were compared using one-way analysis of variance followed by a Bonferroni *t*-test if necessary. Correlations between mean arterial pressure and ventricles to body weight ratio or total ventricular collagen content were analysed using Pearson product moment correlation. The critical level of significance was set at P values ≤ 0.05.

3. Results

3.1. Body weights

The body weights were measured in 4-, 8-, 16- and 24-week-old rats. The body weights vs. time curves follow a similar pattern in all five experimental groups. At 16 and 24 weeks of age, the body weights were however significantly lower in the WKY and SHR-Lis groups compared to the other groups ($P \leq 0.05$).

3.2. Mean arterial pressure and heart rate

Fig. 1 shows the time course of MAP determined in conscious and unrestrained rats at 8, 16 and 24 weeks of age. Animals included in the SHR group had significantly sustained higher blood pressure compared to the WKY group. Throughout the treatment period, MAP was significantly decreased by zofenopril and losartan ($P \leq 0.05$ compared to SHR group), whereas significant lower values were obtained in the SHR-Lis group ($P \leq 0.05$ compared to WKY and SHR groups). Despite these marked falls in blood

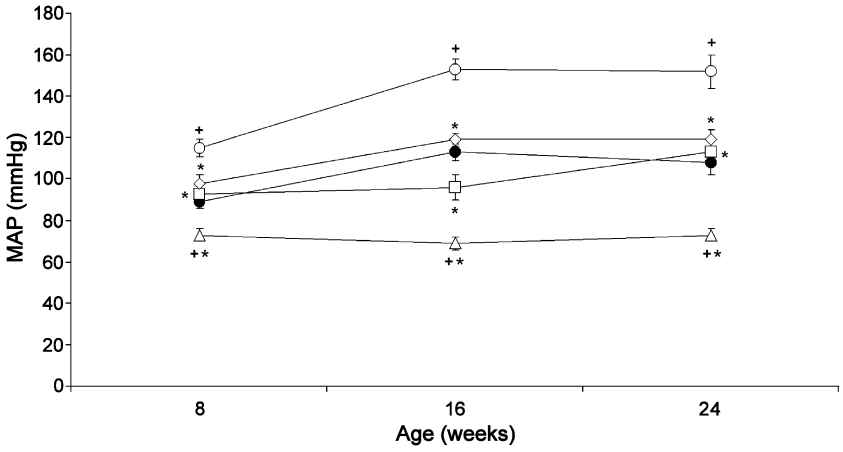

Fig. 1. Graph showing the mean arterial pressure (MAP) measured in conscious and unrestrained WKY rats (●), untreated SHRs (○) and SHRs treated with zofenopril 10 mg/kg/day (□), lisinopril 10 mg/kg/day (△) or losartan 20 mg/kg/day (◇) at 8, 16 and 24 weeks of age. The values are expressed as mean ± S.E.M., $n=8$ per group. $^{+}P \leq 0.05$ vs. WKY rats. $^{*}P \leq 0.05$ vs. untreated SHRs.

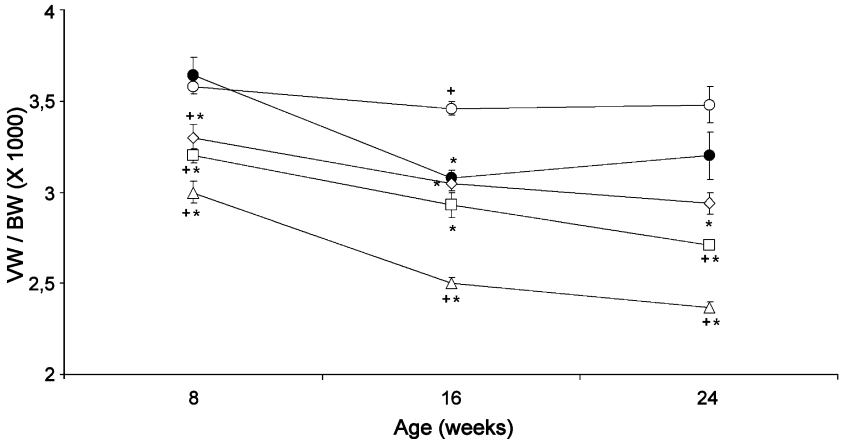

Fig. 2. Graph showing the ventricles to body weight ratio (VW/BW) of WKY rats (●), untreated SHRs (○) and SHRs treated with zofenopril 10 mg/kg/day (□), lisinopril 10 mg/kg/day (△) or losartan 20 mg/kg/day (◇) at 8, 16 and 24 weeks of age. The values are expressed as mean ± S.E.M., $n=8$ per group. $^{+}P \leq 0.05$ vs. WKY rats. $^{*}P \leq 0.05$ vs. untreated SHRs.

pressure, reflex tachycardia was not observed. Heart rate was similar in the five experimental groups during treatment (269 ± 12, 302 ± 14, 317 ± 15, 303 ± 8 and 306 ± 12 beats/min in the WKY, SHR, SHR-Zof, SHR-Lis and SHR-Los group, respectively, at the end of the treatment phase).

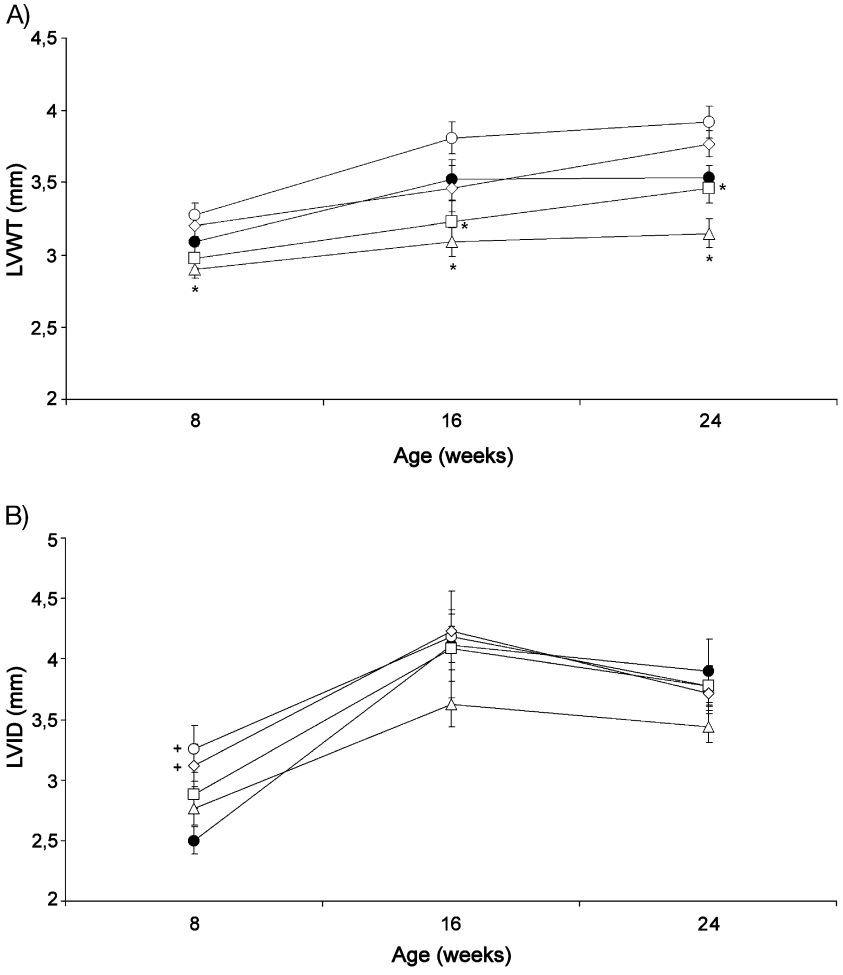

Fig. 3. Graph showing (A) the left ventricular wall thickness (LVWT) and (B) the left ventricular internal diameter (LVID) of WKY rats (●), untreated SHRs (○) and SHRs treated with zofenopril 10 mg/kg/day (□), lisinopril 10 mg/kg/day (△) or losartan 20 mg/kg/day (◇) at 8, 16 and 24 weeks of age. The values are expressed as mean ± S.E.M., $n=8$ per group. $^{+}P \leq 0.05$ vs. WKY rats. $^{*}P \leq 0.05$ vs. untreated SHRs.

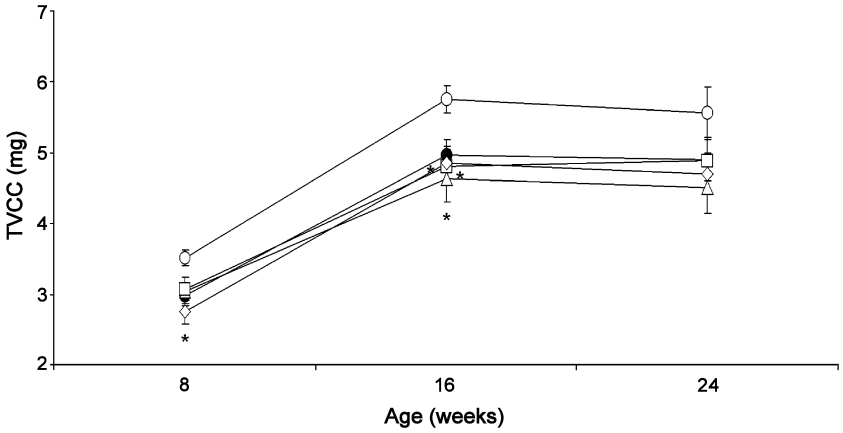

Fig. 4. Graph showing total ventricular collagen content (TVCC) of WKY rats (●), untreated SHRs (○) and SHRs treated with zofenopril 10 mg/kg/day (□), lisinopril 10 mg/kg/day (△) or losartan 20 mg/kg/day (◇) at 8, 16 and 24 weeks of age. The values are expressed as mean ± S.E.M., $n=8$ per group. $^{+}P \leq 0.05$ vs. WKY rats. $^{*}P \leq 0.05$ vs. untreated SHRs.

3.3. Cardiac hypertrophy

Fig. 2 presents the cardiac indices expressed as the ventricles to body weight ratio (VW/BW) in hearts of 8-, 16- and 24-week-old rats. Compared to the WKY group, the SHR group had significant higher VW/BW at 16 weeks of age (3.46 ± 0.05 vs. 3.08 ± 0.04, $P \leq 0.05$) which tended to stay higher at 24 weeks of age (3.48 ± 0.11 vs. 3.20 ± 0.13, $P=0.10$). Throughout the treatment period, cardiac indices were decreased by zofenopril and losartan ($P \leq 0.05$ compared to SHR group), whereas significantly lower values were obtained in the SHR-Lis group.

Fig. 3A shows the left ventricular wall thickness (LVWT) measured in hearts of 8-, 16- and 24-week-old animals. The animals included in the SHR group tended to have higher LVWT compared to the WKY group. Throughout the treatment period, LVWT was decreased by zofenopril and lisinopril ($P \leq 0.05$ compared to SHR group), whereas losartan had no significant effect when compared to the SHR group. Fig. 3B shows the left ventricular internal diameter (LVID) determined in hearts of 8-, 16- and 24-week-old animals. At 8 weeks of age, the animals included in the SHR group had higher LVID compared to the WKY group ($P \leq 0.05$). However, no significant differences were observed between these two groups at 16 and 24 weeks of age. Throughout the treatment period, zofenopril and losartan had no significant effect on LVID, whereas lisinopril tended to decrease this parameter when compared to the SHR group.

3.4. Ventricular collagen

Total ventricular collagen content (TVCC) determined in hearts of 8-, 16- and 24-week-old animals is shown in Fig. 4. In all groups, TVCC increased with aging with significant differences between 8 and 16 weeks of age. Animals included in the SHR group tended to have higher collagen accumulation compared to the WKY group. Throughout the treatment period, TVCC tended or was significantly decreased by all treatments compared to the SHR group.

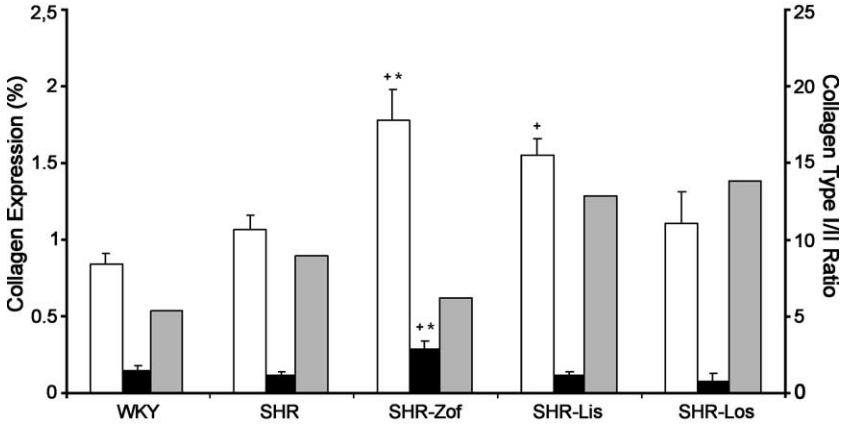

Fig. 5. Graph showing the expression of collagen type I (□) and III (■) and collagen type I/III ratio (▒) of WKY rats, untreated SHRs (SHR) and SHRs treated with zofenopril 10 mg/kg/day (SHR-Zof), lisinopril 10 mg/kg/day (SHR-Lis) or losartan 20 mg/kg/day (SHR-Los) at 24 weeks of age. The values are expressed as mean ± S.E.M., $n=4$ optical sections per heart, 8 rats per group. $^{+}P \leq 0.05$ vs. WKY rats. $^{*}P \leq 0.05$ vs. untreated SHRs.

Fig. 5 shows the expression of collagen types I and III evaluated in hearts of 24-week-old rats. The expression of collagen type I was similar in the WKY, SHR and SHR-Los groups, whereas a significant increase was observed in the SHR-Zof and SHR-Lis groups ($P \leq 0.05$ compared to WKY and/or SHR group). The expression of collagen type III was similar, with a slight tendency to decrease, in the SHR, SHR-Lis and SHR-Los groups compared to the WKY group, whereas a significant increase was noted in the SHR-Zof compared to SHR group. The collagen type I/III ratio was increased in all SHR groups compared to the WKY group except for the SHR-Zof group where the ratio was normalized (Fig. 5).

3.5. Correlations with mean arterial pressure

Fig. 6 shows the relationship of VW/BW to MAP (A) and the relationship of TVCC to MAP (B) for all experimental groups at all ages. Pearson correlations were calculated using data from all SHR-treated groups. There was a significant positive correlation (linear regression analysis: $y = 0.0055x + 2.35$; $R = 0.385$; $P < 0.001$; $n = 70$) between MAP and VW/BW, whereas there was no significant relationship ($R = 0.190$; $P = 0.115$; $n = 70$) between MAP and TVCC.

4. Discussion

It has been shown that ACE inhibitors [22–24] and angiotensin II AT_1 receptor antagonists [25–27] attenuate the development of hypertension in SHRs when the treatment is initiated at a young age. Accordingly, our findings show that all treatments significantly prevented the increase in MAP compared to the untreated SHR group. Zofenopril and losartan reduced blood pressure by 26% and 22%, respectively, while lisinopril produced an unexpected decrease of MAP by 52%. The oral dose (20 mg/kg/day) of losartan was determined according to the previous studies using SHRs [14–16]. The oral dose (10 mg/kg/day) of zofenopril and lisinopril was selected on the basis of the results obtained by Cushman et al. [13], who showed an

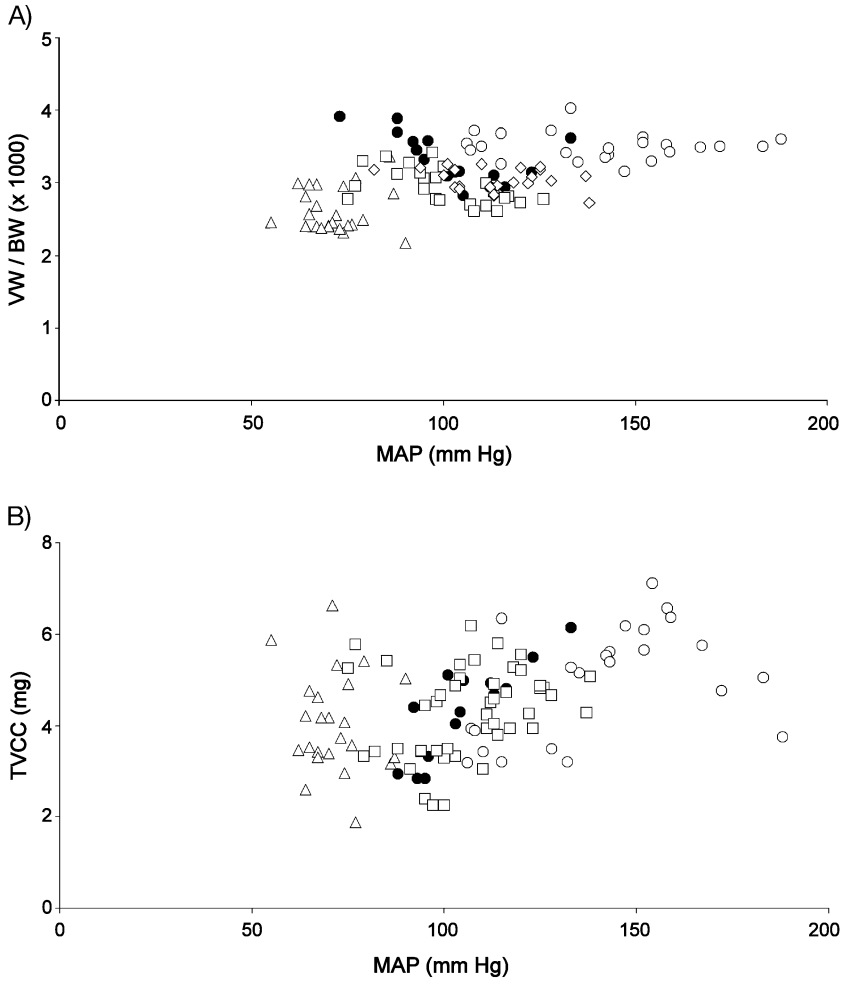

Fig. 6. Relationship of (A) ventricles to body weight ratio (VW/BW) to mean arterial pressure (MAP) ($n = 107$ observations) and (B) total ventricular collagen content (TVCC) to MAP ($n = 105$ observations) of WKY rats (●), untreated SHRs (○) and SHRs treated with zofenopril 10 mg/kg/day (□), lisinopril 10 mg/kg/day (△) or losartan 20 mg/kg/day (◇) at all ages.

equivalent reduction in MAP (− 20 mm Hg) after a single treatment with the two ACE inhibitors. Different experimental conditions and acute vs. chronic treatment may explain the different results obtained in decrease in blood pressure. This marked reduction in MAP produced by lisinopril had an impact also on the body weight curves. Thus, the body weights vs. time curves follow a similar and normal pattern in all experimental groups apart from lisinopril that significantly lowered the body weight of the rats compared to the other SHR groups. This finding has already been reported with lisinopril [4] as well as with other ACE inhibitors [11,28,29]. Clozel et al. [30] have suggested that this decrease could be related to a marked natriuresis produced by ACE inhibitors in case of a potent and prolonged ACE inhibition.

Although ACE inhibitors have been reported to regress LVH in hypertensive humans and various experimental models, the mechanisms underlying this process have not yet been fully elucidated. Linz et al. [31] have reported that ramipril, at a dose that did not decrease blood pressure, reversed LVH in aortic-banded rats. Moreover, Mori et al. [32] have observed that short-term treatment with lisinopril 3 mg/kg/day for 2 weeks inhibited the progression of hypertension and suppressed the development of LVH in SHRs, whereas treatment with lisinopril 0.5 mg/kg/day for 2 weeks suppressed the development of LVH without reducing blood pressure. Nevertheless, it certainly remains that the prevention of myocardial cell hypertrophy and therefore the regression of LVH depend, at least in part, on afterload reduction. In agreement with this, in the present study, all treatments significantly prevented the development of LVH compared to the untreated SHR group, the effect being well correlated ($R = 0.385$; $P < 0.001$) with the resulting decreased MAPs.

Cardiac hypertrophy is not only due to an enlargement of myocyte size. Myocyte hypertrophy is often accompanied by perivascular fibrosis of intramyocardial coronary arteries and interstitial fibrosis between myocytes. Previous studies in the SHR have shown an increase in interstitial collagen in addition to LVH [19,33]. Likewise in our study, the TVCC, measured by the hydroxyproline concentration, tended to be increased in the untreated SHR group compared to the WKY group. Weber et al. [34] have suggested that although the contribution of the muscle to myocardial mass is far greater than that of collagen (50–85% vs. 2–3%), even a minimal increase in collagen may markedly affect myocardial distensibility owing to its stiffness, which is considerably greater than that of muscle. It has been proposed that the excess of ventricular collagen seen in animals and humans with hypertensive LVH is a result of both exaggerated collagen synthesis and inadequate collagen degradation [35]. During the past decade, evidence has been provided that the circulating and local angiotensin–aldosterone systems promote the development of myocardial fibrosis in hypertensive heart disease [5]. Brilla et al. [7] have found in cultured adult rat cardiac fibroblasts that angiotensin II significantly increases collagen synthesis and inhibits matrix metalloproteinase (MMP-1) activity, the key enzyme of interstitial collagen degradation. Interestingly, the same group has observed an increase of the myocardial MMP-1 activity following oral lisinopril treatment in SHRs [36].

Other studies have clearly shown in SHRs that myocardial fibrosis is decreased by ACE inhibitors, such as lisinopril [32], enalapril [25] and trandolapril [37] and by angiotensin II AT_1 receptor antagonists such as losartan and candesartan [16,38]. Our data showed that the TVCC was similarly reduced by ACE inhibition and angiotensin II AT_1 receptor antagonism and this decrease was independent from the resulting decreased MAPs ($R = 0.190$; $P = 0.115$, N.S.). According to previous observations [5], it should be noted that the growth of cellular constituents of the various myocardial tissue compartments, namely cardiac myocytes and fibroblasts, which are responsible for myocardial collagen metabolism, may each have different regulatory mechanisms and that mechanical factors would not appear to account for the disproportionate accumulation of collagen that occurs with LVH in contrast to load-dependent growth of cardiac myocytes.

Types I and III are the two major types of collagen present in the myocardium in both normal and diseased myocardial tissue. Tissue with predominant collagen type I is characterized by strength and stiffness, whereas tissues containing large amounts of collagen type III are characterized by an increased elasticity [39]. Mukherjee and Sen [20] have reported a significant increase in collagen type I/III ratio in 30-week-old SHRs and they as others [2,7,8,21] have proposed that the quality rather than the quantity of the collagen is perhaps more important for predicting cardiac stiffness than the total amount of collagen present in the myocardium. The ratio of collagen types I and III would therefore be an important marker for determination of the quality of collagen and prediction of the stiffness of the heart muscle [40]. The studies so far realized to evaluate the effect of ACE inhibition or angiotensin II receptor antagonism on collagen type I/III ratio in SHRs indicate that factors other than angiotensin II seem to be involved in the distribution of collagen. Yonezawa et al. [25] showed that ACE inhibition (enalapril) or angiotensin II AT_1 receptor antagonism (FK-739) while causing regression of LVH, did not change collagen phenotypes in 20-week-old SHRs compared to untreated animals. Likewise, chronic treatment with losartan was unable to affect the ventricular pro-α_1(I) collagen messenger RNA [35] and quinapril did not change collagen type I and III in SHRs [41]. On the other hand, it has been reported by Murkherjee and Sen [18] that captopril reduced the total amount of collagen and reversed the altered augmented distribution of types I and III collagen in 34-week-old SHRs. In our study, we observed that while the other treatments did not differ among them, zofenopril group had a higher expression of collagen type III. This higher value of collagen type III expression normalizes the collagen type I/III ratio to a value comparable to that of the WKY group.

This last result is in agreement with the observation of Yang et al. [19] in 40-week-old SHRs showing that treatment with captopril and lisinopril produced a similar degree of reduction in blood pressure, regressed hypertrophy and reduced collagen, whereas a decrease in the collagen type I/III ratio (mainly due to an increase in collagen type III) was found with captopril, but not with lisinopril. Our observation seems to indicate that only sulfhydryl ACEis have an effect on the distribution of the collagen, probably due to the antioxidant properties of their SH groups. Further studies are needed to investigate and clarify the potential mechanism of action underlying this effect.

Evidence from both animal and human studies suggests that increased free radical formation and oxidative stress significantly contribute to left ventricular remodelling and heart failure after myocardial infarction [42]. Using post-myocardial infarction models created by ligating the coronary artery of mouse and rat hearts, beneficial effects of antioxidants such as vitamin E [43], dimethylthiourea [44] and probucol [42,45] have been reported and appeared to be related, at least in part, to reduced cardiac fibrosis. Zofenopril has been reported to act as a free radical scavenger of various reactive oxygen species and to have antioxidant properties in vitro [46,47] as well as in vivo [48–50]. These properties, together with zofenopril's high lipophilicity determining an enhanced cardiac tissue penetration and its uniqueness in producing a selective and long-lasting inhibition of heart tissue ACE [51] can be involved in its beneficial effect afforded in patients with myocardial infarction [52] or chronic heart failure [53]. Moreover, it has been shown in these studies [52,53] that the benefit of 6-week treatment is still significantly present after 1 year, indicating an effect of zofenopril on cardiac remodelling. The mechanism by which zofenopril significantly increases the expression of collagen type III and normalizes the collagen type I/III ratio remains unknown. The role of its sulfhydryl group in this beneficial effect needs further investigation.

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