Effects of Captopril and Losartan on Cardiac Stereology in Rats with Renovascular Hypertension

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What's Known

• Hypertension is associated with increased arterial wall thickness, extracellular matrix component synthesis, cardiomyocyte hypertrophy, and cardiac fibroblast proliferation.
• Captopril, an angiotensin-converting enzyme inhibitor, and losartan, an angiotensin II receptor blocker, reduce blood pressure, hypertrophy of left ventricular and aortic smooth muscle cells, and vascular fibrosis.

What's New

• Renovascular hypertension is associated with higher heart weights and total volumes of the heart, myocardium, endocardium, and vessels and lower numbers of cardiomyocytes, and Purkinje cells.
• Captopril or losartan decreases the systolic blood pressure, heart weight, and total volumes of the heart, myocardium, endocardium, and vessels, but captopril has the greatest inhibitory effect.

Abstract

Background: Captopril, an angiotensin-converting enzyme inhibitor, and losartan, an angiotensin II receptor blocker, are used for the treatment of hypertension, but their effects on cardiac stereology are unknown. This study, therefore, aimed to examine their effects on cardiac stereology in rats with renovascular hypertension.

Methods: This study was conducted at Histomorphometry and Stereology Research Centre, and Cardiovascular Pharmacology Research Lab, Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, in August 2015 to August 2016. Fourty-eight rats were allocated to six groups (n=8 per each group): a sham group, which received a vehicle (distilled water) and five renal artery-clipped groups, which received the vehicle, captopril (50 or 100 mg/kg/day), or losartan (25 or 50 mg/kg/day). After four weeks, the animals' systolic blood pressures (mm Hg) were measured, and the total volumes of their heart, myocardium, endocardium, matrix, and myocardial vessels (mm^3), as well as the number of their cardiomyocytes, and Purkinje fibers were determined. Data were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P value of equal to or less than 0.05 was considered significant.

Results: The renal artery-clipped rats receiving the vehicle had a significantly higher systolic blood pressure (P<0.001); heart weight (g) (P<0.001); and total volume of the heart (P<0.001), myocardium (P=0.020), endocardium (P=0.009), and myocardial vessels (P=0.008); as well as a significantly lower number of cardiomyocytes (P=0.010) and Purkinje cells (P=0.005), than did the rats in the sham group. The renal artery-clipped rats receiving captopril or losartan had a significantly lower systolic blood pressure (P<0.001), heart weight (P=0.007), and total volume of the heart (P<0.001), myocardium (P<0.001), endocardium (P=0.027), and myocardial vessels (P=0.004) than did the renal artery-clipped rats receiving the vehicle. Neither captopril nor losartan prevented a reduction in the number of Purkinje cells, but captopril at the higher dose attenuated cardiomyocyte loss (P=0.010).

Conclusion: Captopril and losartan lowered the systolic blood pressure and cardiac hypertrophy but failed to prevent Purkinje cell loss. Captopril only at the higher dose prevented cardiomyocyte loss. Captopril exerted a greater inhibitory effect on cardiac stereology, which warrants further research.

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Introduction

Hypertension is considered a risk factor for stroke and cardiovascular and renal diseases. It is one of the main causes of end-organ damage and disabilities and accounts for an enormous share of healthcare costs worldwide.[1, 2]

Hypertension is associated with cardiac and vascular hypertrophy.[3] In cardiac hypertrophy, myocardial cells grow in diameter and length as a compensatory response to increased arterial resistance in a bid to improve cardiac pumping function.[4, 5] Vascular changes, which occur in hypertrophied hearts not only affect blood pressure but also beget morphological changes in the myocardium, and render the heart more prone to ischemia.[6] In advanced stages of hypertrophy, myocardial cells dwindle and suffer fibrosis, which leads to deleterious changes in cardiac systolic and diastolic functions. These changes are the major risk factors for myocardial infarction, cardiac arrhythmias, congestive heart failure, and sudden death.[7]

The rat model of two-kidney, one-clip (2K1C) renovascular hypertension, induced by narrowing one renal artery, has been widely used to study the pathophysiology of hypertension and the effects of antihypertensive drugs. The model is associated with the activation of the renin-angiotensin-aldosterone system and the consequential increase in angiotensin II, and aldosterone syntheses.[8] By acting on the angiotensin II type I receptor, angiotensin II increases the activity of matrix metallopeptidases (MMPs), particularly MMP_2. Such an increase leads to augmented vasoconstriction and thrombosis, thickened arterial walls, amplified extracellular matrix component synthesis, stimulated neural interfaces,[9, 10] hypertrophied cardiomyocytes, and proliferated cardiac fibroblasts.[11]

The renin-angiotensin–aldosterone system can be blocked at various levels such as angiotensin-converting enzyme (ACE) inhibition and the angiotensin II type 1 receptor blockade. The blockade of the system by captopril, an ACE inhibitor, reduces the systolic blood pressure, and attenuates the hypertrophy of left ventricular and aortic smooth muscle cells.[12-14] Moreover, the blockade of angiotensin II type 1 receptor by losartan prevents vascular fibrosis and metalloproteinase synthesis and leads to a gradual decrease in left ventricular hypertrophy and blood pressure.[15-17]

There is currently a paucity of stereological research in the existing literature on the effects of the inhibition of the renin-angiotensin–aldosterone system by ACE inhibitors and angiotensin II receptor blockers on cardiac hypertrophy in a rat model of 2K1C hypertension. Accordingly, we designed the present study to examine the effects of captopril, an ACE inhibitor, and losartan, an angiotensin II receptor blocker, on cardiac structures in such a model.

Materials and Methods

Ethical Approval

The protocol of the study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1393.S7166).

Animals

Male Sprague–Dawley rats (weight=200–250 g) were obtained from the Laboratory Animal Breeding Center, Shiraz University of Medical Sciences, and kept under standard conditions (12 hour light/12 hour dark, temperature=20–24 °C) with food and water *ad libitum*.[18]

Experimental Design and Protocol

Forty-eight animals were allocated to six groups (n=8 per each group): a sham group, which received distilled water (1 mL/day) as the vehicle (Sham-V); a renal artery-clipped group, which received the vehicle (RAC-V); two renal artery-clipped groups, which received captopril (Tehran Darou, Tehran, Iran) at 50 (RAC-C50) or 100 (RAC-C100) mg/kg/day; and two renal artery-clipped groups, which received losartan (Tehran Darou, Tehran, Iran) at 25 (RAC-L25) or 50 (RAC-L50) mg/kg/day.

The animals were anesthetized with 60 mg/kg ketamine (Merck, Germany) and 8 mg/kg xylazine (Merck, Germany), and were subjected to the placement of plexiglass clips (internal diameter=0.20–0.22 mm) on the left renal arteries or sham operation as described previously.[19] Briefly, incisions were made in the left flanks. Then, the left renal arteries and veins were located, separated, and cleaned of the surrounding tissues. Afterwards, plexiglass clips were placed on the left renal arteries in the closest possible proximity to the abdominal aortas. Then, the abdominal wall and skin incisions were sutured with catgut and silk sutures (SUPA, Tehran, Iran), respectively. The sham-operated animals were subjected to the same procedure, but no clip was placed on the left renal arteries. As from the first postoperative day, the rats were administered the vehicle, captopril, or losartan daily by oral gavage for four weeks.

By the fourth week, the animals' blood pressures were measured via the noninvasive tail-cuff method using Chart 5.0 software, PowerLab 4.30 (AD Instruments Inc, MA, Australia).[20]
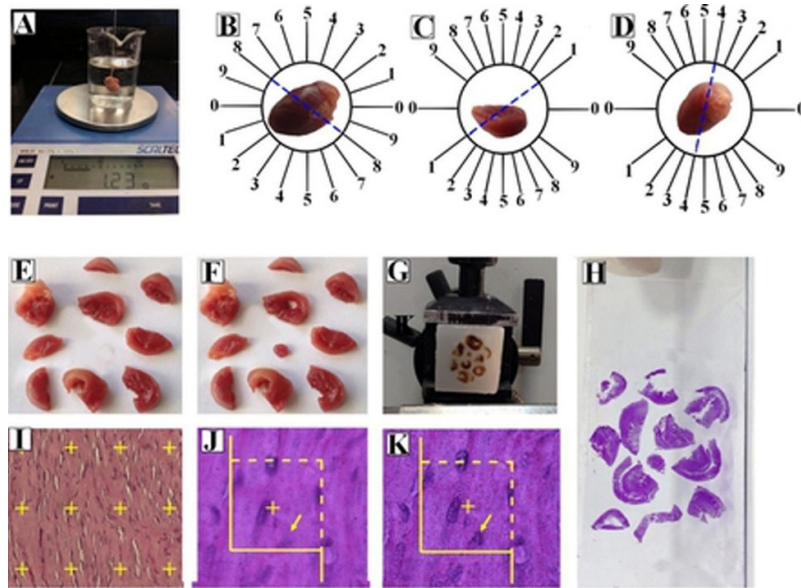
Figure 1: The micrographs demonstrate the summary of the stereological techniques employed in the present study. **A.** The volume of the heart was measured via the immersion method. **B.** Isotropic uniform random sections of the heart according to the oriented method were obtained by placing the heart on an equally-divided circle and sectioning it into two parts in a random direction (here 8). **C and D.** The parts of the heart were sectioned in new random directions (here 1 and 4) after being placed on the cosine-weighted divided circle. **E.** The slabs were collected. **F.** A circle was punched out. **G.** The slabs and the circle were embedded in a block, and were cut with a microtome. **H.** A stained section is illustrated on one microscopic slide. **I.** The point-counting method was used to estimate the volume density of the favored structures (myocardium, matrix, and vessels) on the PAS staining sections. **J and K.** The dissector method was utilized to estimate the numerical density of the nuclei of the cardiomyocytes. The arrowheads indicate the nuclei, which have not appeared in the reference section but can be observed in the look-up section and follow the unbiased counting rules.

Thereafter, the rats were anesthetized with ketamine and xylazine, and their hearts were removed. The hearts were then weighed, and their primary volumes were determined via the immersion method (figure 1A).[21] They were subsequently fixed in a 4% formaldehyde buffered solution (Merck, Germany), and used for stereological studies.

Stereological Studies

The oriented method was utilized to obtain isotropic uniform random (IUR) sections. The paraffinized hearts were cut serially in 5-µm- and 25-µm-thick sections, and stained with periodic acid-Schiff (PAS) (Merck, Germany). The 5-µm- and 25-µm-thick sections were used for volume estimation and cell counts, respectively. The sections were analyzed using a video-microscopy system that consisted of a microscope (E-200, Nikon, Tokyo, Japan) linked to a video camera (SSC, Sony, Tokyo, Japan), a computer, and a flat-screen monitor.

Estimating the Shrinkage and the Total Volume of the Heart

Shrinkage was calculated through the use of a trocar and preparation of single slices from the incision. Via the IUR method,[22] the two perpendicular diameters were measured (figure 1B, C, and D), and the mean values of their radii were calculated as pre-fixing radii (r_{before}). After tissue processing (figure 1E, F, and G) and staining (figure 1H), the two perpendicular diameters in the above pieces (section-part) were re-measured, and the mean values of their radii were calculated as the post-fixing radii (r_{after}). The shrinkage volumes of the tissues were calculated using the following formula:[21]

volume shrinkage $= 1 - (r_{after}^2/r_{before}^2)^{1.5}$

The final volume was then calculated using the following formula:

V final heart $= V$ *primary* $\times (1 - $ *volume shrinkage*$)$.

Estimation of the Volumes of the Myocardium, Endocardium, Matrix, and Myocardial Vessels

The volumes of the structures were determined via the point-counting method. A point grid (point tool or system) was randomly placed on 15 scopes of each 5-µm slide at certain and equal distances. Then, the number of points intersecting with the incision surface was counted (figure 1I). Volume density was calculated using the following formulae:[23]

$$Vv\,(structure/heart) = \frac{\sum Pstructure}{\sum Ptotal}$$

where ΣP (total) was the number of grid intersection points with the chosen total scope, and ΣP (structure) was the number of grid intersection points with each of the heart structures.

The absolute volume was determined through the following formula:[23]

V (structure)=Vv (structure/heart)×V final (mm^3).

Estimation of the Number of Cardiomyocytes and Purkinje Cells

The nuclei of cardiomyocytes and Purkinje cells were counted in 25-µm-thick sections via the optical dissector method (figure 1J and K). The number of cells was obtained using the following equation:[23]

$$N_V = \frac{\sum \bar{Q}}{\sum p \times [a(f) \times h]}$$

where N_V was the numerical density based on the number per volume unit (mm^3), $\Sigma\bar{Q}$ was the set of counted cells, ΣP was the number of applied dissectors (with the associated point), a (f) was the area associated with each dissector (mm^2), and h was the dissector height (mm [0.01]).

The total number (N) of cells was estimated through the multiplication of numerical density (N_v) by reference volume ($N=N_V \times V_{ref}$).[23]

Statistical Analysis

Data, which were presented as mean±SD, were examined for the normality of distribution using the one-sample Kolmogorov–Smirnov test. The normally-distributed data were analyzed using the one-way analysis of variance (ANOVA). In cases with significant differences with ANOVA, pair-wise comparisons were performed using the least significant difference (LSD) test. The data analyses were performed using Statistical Package for Social Sciences (SPSS) software version 22.0, (IBM, USA) at a P value of equal to or less than 0.05.

Results

Systolic Blood Pressure

The systolic blood pressure (mm Hg) of the RAC-V group was significantly higher than that of the Sham-V group (P<0.001) (figure 2). The systolic blood pressure of the renal artery-clipped groups receiving captopril (RAC-C50 and RAC-C100) or losartan (RAC-L25 and RAC-L50) was significantly lower than that of the RAC-V group (P<0.001) (figure 2). Moreover, the systolic blood pressure was significantly higher in the RAC-C50, RAC-C100, RAC-L25, and RAC-L50 groups than in the Sham-V group (P=0.040, P=0.007, P=0.030, and P=0.080, respectively).

Body and Heart Weights

There were no significant differences in terms of body weight gain (g) between all the groups
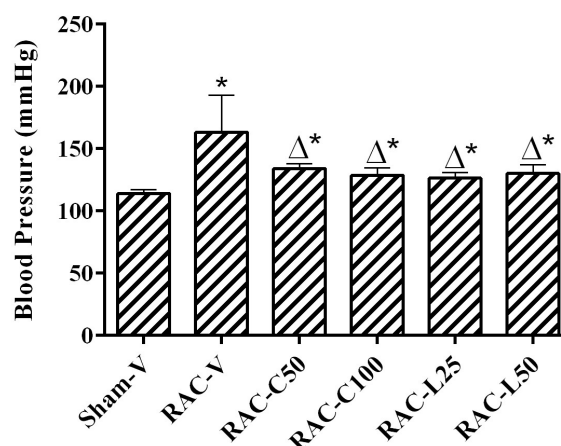

Figure 2: The systolic blood pressures of all the groups after four weeks of treatment are indicated in this figure. The bars represent mean±SD (n=8 per each group). Comparisons were made using the one-way analysis of variance (ANOVA), followed by the least significant difference test. *Indicates a significant (P≤0.05) difference from the Sham-V group; ΔIndicates a significant difference (P≤0.05) from the RAC-V group; Sham-V: Sham-operated group receiving the vehicle (distilled water); RAC-V: Renal artery-clipped group receiving the vehicle; RAC-C50 and RAC-C100, Renal artery-clipped groups receiving captopril at 50 and 100 mg/kg/day, respectively; RAC-L25 and RAC-L50: Renal artery-clipped groups receiving losartan at 25 and 50 mg/kg/day, respectively

during the study (figure 3A). The heart weight (g) of the RAC-V group was significantly higher than that of the Sham-V group (P<0.001). However, the heart weight was significantly lower in the groups receiving captopril (RAC-C50 and RAC-C100) or losartan (RAC-L25 and RAC-L50) than in the RAC-V group (P<0.001). The RAC-C100 group had a significantly lower heart weight than did the RAC-C50 group (P=0.007) (figure 3B). There were no significant differences concerning the heart weight between the Sham-V group and the RAC-C50, RAC-C100, RAC-L25, and RAC-L50 groups.

Volumes of the Heart, Endocardium, Myocardium, Matrix, and Myocardial Vessels (mm^3)

The heart volume was significantly larger in the RAC-V group than in the Sham-V group (P=0.012) (figure 4A). Nonetheless, the heart volume was significantly lower in the groups receiving captopril (RAC-C50 and RAC-C100) or losartan (RAC-L25 and RAC-L50) than in the RAC-V group (P<0.001). There was no significant difference regarding the myocardial volume between the groups receiving captopril (RAC-C50 and RAC-C100) and the groups receiving losartan (RAC-L25 and RAC-L50) (figure 4A). The difference vis-à-vis the heart volume between the RAC-C50, RAC-C100, RAC-L25, and RAC-L50 groups, and the Sham-V group failed to constitute statistical significance.
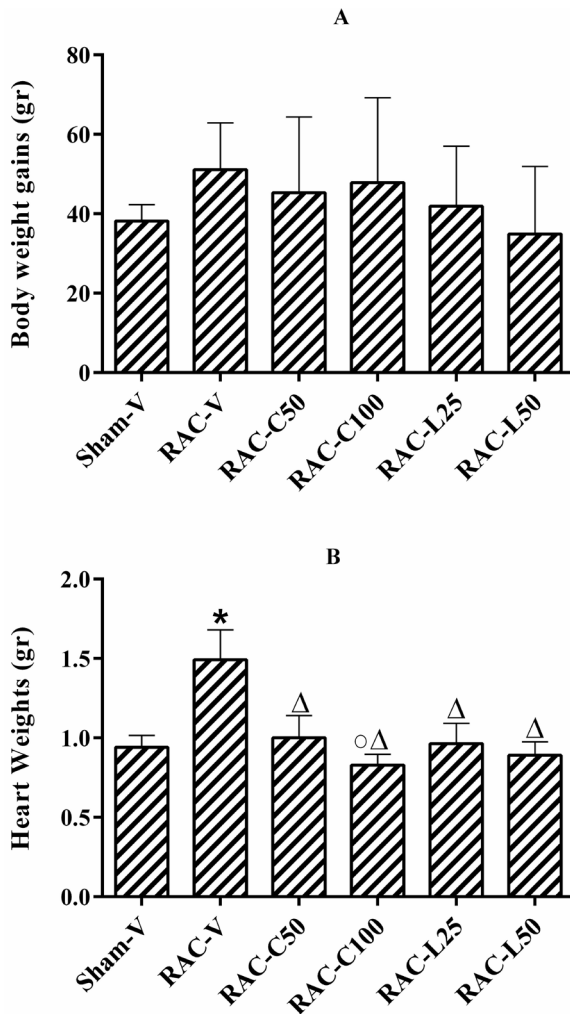
A

B

Figure 3: The figure indicates (A) the body, (B) heart weights of all the groups after four weeks of treatment. The bars represent the mean±SD (n=8 per each group). Comparisons were made using the one-way analysis of variance (ANOVA), followed by the least significant difference test. *Indicates a significant (P≤0.05) difference from the Sham-V group; ΔIndicates a significant (P≤0.05) difference from the RAC-V group; °Indicates a significant difference from the RAC-C50 group; Sham-V: Sham-operated group receiving the vehicle (distilled water); RAC-V: Renal artery-clipped group receiving the vehicle; RAC-C50 and RAC-C100: Renal artery-clipped groups receiving captopril at 50 and 100 mg/kg/day, respectively; RAC-L25 and RAC-L50: Renal artery-clipped groups receiving losartan at 25 and 50 mg/kg/day, respectively

The myocardial volume of the RAC-V group was significantly larger than that of the Sham-V group (P=0.021) (figure 4B). Nevertheless, the myocardial volume was significantly lower in the groups receiving captopril (RAC-C50 and RAC-C100) or losartan (RAC-L25 and RAC-L50) than in the RAC-V group (P<0.001). No significant difference was detected concerning the myocardial volume between the groups receiving captopril (RAC-C50 and RAC-C100) and the groups receiving losartan (RAC-L25 and RAC-L50) (figure 4B). The differences regarding the myocardial volume between the

Sham-V group and the RAC-C100 and RAC-L25 groups were not significant. Still, the myocardial volume was significantly lower in the RAC-C50 and RAC-L50 groups than in the Sham-V group (P=0.010 and P=0.030, respectively).

The volume of the endocardium in the RAC-V group was significantly larger than that of the Sham-V group (P=0.009) (figure 4C). However, the endocardial volume was significantly smaller in the RAC-C50, RAC-C100, and RAC-L50 groups than in the RAC-V group (P=0.008, P=0.006, and P=0.027, respectively). Additionally, the difference in terms of the endocardial volume between the groups receiving captopril (RAC-C50 and RAC-C100), and the groups receiving losartan (RAC-L25 and RAC-L50) was neither statistically significant (figure 4C), nor was there any statistically significant difference with respect to the total endocardial volume between the Sham-V group and the RAC-C50, RAC-C100, RAC-L25, and RAC-L50 groups.

Concerning the matrix volume, no difference of statistical significance was revealed between the Sham-V group and the RAC-V, RAC-C50, RAC-C100, RAC-L25, and RAC-L50 groups (figure 4D).

The myocardial vessel volume of the RAC-V group was significantly larger than that of the Sham-V group (P=0.008) (figure 4E). The vessel volume was significantly smaller in the RAVC-50, RAC-C100, and RAC-L50 groups than in the RAC-V group (P<0.001, P<0.001, and P=0.006, respectively). The volume of the myocardial vessels was significantly larger in the RAC-L25 group than in the captopril-receiving groups (RAC-C50, P=0.004; and RAC-C100, P=0.006) (figure 4E). No statistically significant difference was demonstrated concerning the myocardial vessel volume between the Sham-V group and the RAC-C50, RAC-C100, and RAC-L50 groups. Nonetheless, the myocardial vessel volume of the RAC-L25 group was significantly larger than that of the Sham-V group (P=0.030).

Number of Cardiomyocytes and Purkinje Cells

The number of cardiomyocytes in the RAC-V group was significantly lower than that of the Sham-V group (P=0.010) (figure 5A). There were significantly more cardiomyocytes in the RAC-C100 group than in the RAC-V group (P=0.050). With regard to the number of cardiomyocytes, the difference between the groups receiving captopril (RAC-C50 and RAC-C100), and the groups receiving losartan (RAC-L25 and RAC-L50) failed to reach statistical significance (figure 5A). There was no significant difference concerning the number of cardiomyocytes
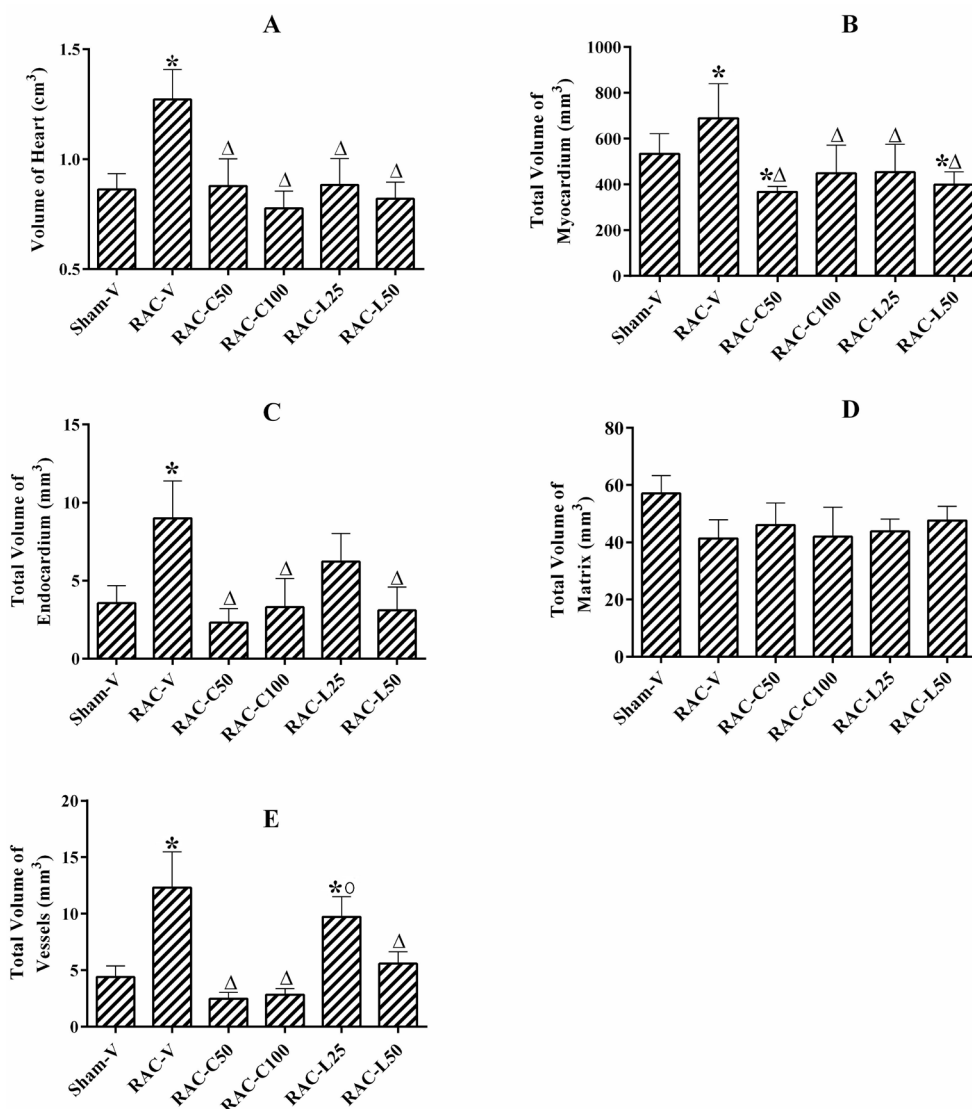
Figure 4: The total volume of the heart (A), myocardium (B), endocardium (C), matrix (D), and myocardial vessels (E) of all the groups are indicated after four weeks of treatment. The bars represent the mean±SD (n=8 per each group). Comparisons were made using the one-way analysis of variance (ANOVA), followed by the least significant difference test. *Indicates a significant (P≤0.05) difference from the Sham-V group; ΔIndicates a significant (P≤0.05) difference from the RAC-V group; ºIndicates a significant (P≤0.05) difference from the RAC-C50 and RAC-C100 groups; Sham-V: Sham-operated group receiving the vehicle (distilled water); RAC-V: Renal artery-clipped group receiving the vehicle; RAC-C50 and RAC-C100: Renal artery-clipped groups receiving captopril at 50 and 100 mg/kg/day, respectively; RAC-L25 and RAC-L50: Renal artery-clipped groups receiving losartan at 25 and 50 mg/kg/day, respectively

between the RAC-C50 and RAC-C100 groups and the Sham-V group. Still, the RAC-L25 and RAC-L50 groups had significantly fewer cardiomyocytes than did the Sham-V group (P=0.007 and P=0.009, correspondingly).

The number of Purkinje cells was significantly lower in the RAC-V group than in the Sham-V group (P=0.005) (figure 5B). However, there was no significant difference concerning the number of Purkinje cells between the groups receiving captopril (RAC-C50 and RAC-C100) or losartan (RAC-L25 and RAC-L50) and the RAC-V group (figure 5B). In addition, there were significantly fewer Purkinje cells in the RAC-C50, RAC-C100, RAC-L25, and RAC-L50 groups than in

the Sham-V group (P<0.001).

Discussion

The findings of the present study showed that the narrowing of left renal arteries using plexiglass clips resulted in renovascular hypertension, characterized by increased systolic blood pressure, heart weight, and total volumes of the heart, myocardium, endocardium, and myocardial vessels. We also found that treatment with different doses of captopril and losartan reduced the systolic blood pressure, the heart weight, and the total volumes of the heart, myocardium, endocardium, and myocardial vessels.
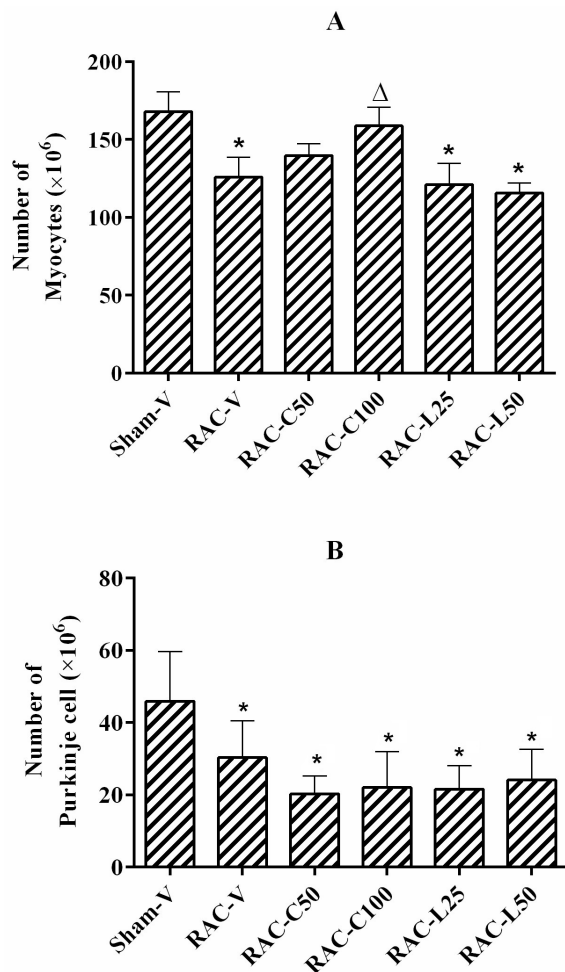
A

B

Figure 5: The numbers of cardiomyocytes (A) and Purkinje cells (B) are indicated in the hearts of all the groups after four weeks of treatment. The bars represent the mean±SD (n=8 per each group). Comparisons were made using the one-way analysis of variance (ANOVA), followed by the least significant difference test. *Indicates a significant difference from the Sham-V group; ΔIndicates a significant difference from the RAC-V group; Sham-V: Sham-operated group receiving the vehicle (distilled water); RAC-V: Renal artery-clipped group receiving the vehicle; RAC-C50 and RAC-C100: Renal artery-clipped groups receiving captopril at 50 and 100 mg/kg/day, respectively; RAC-L25 and RAC-L50: Renal artery-clipped groups receiving losartan at 25 and 50 mg/kg/day, respectively

Our findings as regards renovascular hypertension secondary to the narrowing of the left renal artery were in the same line with the findings of some previous investigations.[19, 24] This model has been widely used to study the effects of drugs on hypertension.[24, 25] In the current study, the model was associated with cardiac hypertrophy, characterized by increased heart weight (37%) and volume (32.1%). These findings are similar to those of previous reports using renovascular or other models of experimental hypertension.[26, 14] Increased cardiac hypertrophy might be a compensatory response to elevated arterial pressure, and afterload in the present model. Accordingly,

cardiac hypertrophy occurs in a bid to maintain cardiac output and body perfusion in the face of increased afterload.

The findings of the present study indicated that cardiac hypertrophy was reflected in increased volumes of the myocardium and myocardial vessels (congestion and perivascular fibrosis), and a decreased number of cardiomyocytes. Such changes might be attributed to an elevated serum level of angiotensin II,[27] which occurs in such a model and is believed to increase cardiac and vascular hypertrophy, and remodeling by enhancing the expression of proto-oncogenes B, production of growth factors C, and synthesis of extracellular matrix proteins.[28] These findings are concordant with those of previous reports using the same model[6, 24, 29] or other models[14] of hypertension. Previous studies have attributed these changes to cardiomyocyte hypertrophy,[30] perivascular fibrosis,[6, 31] necrosis, and cardiomyocyte apoptosis.[29, 32, 33] Although hypertrophy initially involves myocytes, it progressively extends to the perivascular and then interstitial matrices.[6] We detected no significant difference in the matrix between the renovascular hypertensive group receiving the vehicle, and the sham-operated group receiving the vehicle; consequently. The elevated myocardial volume is probably due to the increased volume of myocardial vessels and cardiomyocyte hypertrophy despite a reduction in the number of cardiomyocytes. In all probability, more time was needed for the fibrosis of the interstitial matrix. From hemodynamic and functional viewpoints, such changes are an attempt on behalf of the heart to cope with the new reality imposed by increased arterial resistance and afterload.

We also showed that in agreement with a previous report,[34] the present model of renovascular hypertension was associated with an increased endocardial volume and a reduced number of Purkinje cells. It is likely that the increased thickness of the endocardial layer together with the decreased number of Purkinje cells contributed to subendocardial fibrosis.[35] As far as the literature is concerned, the present study is the first of its kind, which idicate a decrease in the number of Purkinje cells in renovascular hypertension. However, the significance of such a finding is unknown at present. A previous investigation, however, demonstrated that in comparison with cardiomyocytes, Purkinje cells with fewer transverse tubules (t-tubules), and more glycogen were more susceptible to injury in ischemia.[36] Thus, the further reduction in the number of Purkinje cells in relation to the cardiomyocytes (roughly 1.5-fold) in the present

study may be indicative of the higher vulnerability of such cells to increased arterial resistance and afterload. Despite the therapeutic measures for hypertension, tachyarrhythmias, and arrhythmias commonly lead to heart failure and death, which are due to a disorder of Purkinje cells as the cardiac conduction system.[37] Further studies are required to reveal the mechanism of the pressure overload/increased loss of Purkinje cells in the present model of hypertension, and its likely role in cardiac hypertrophy-induced arrhythmias.

The current study showed that captopril and losartan, at the doses used, lowered the systolic blood pressure. Such findings correspond to those reported in earlier studies on experimental,[12-14, 16, 17] and human[15, 38] hypertension models. While reducing blood pressure significantly relative to hypertensive rats receiving the vehicle, captopril and losartan at neither dose could lower blood pressure to a level comparable to that of our sham group. The level of the systolic blood pressure achieved in the present study is in the range of stage II and grade II hypertension of the American[39] and European[40] guidelines for the management of hypertension, respectively. Both guidelines suggest that in hypertension with such severity, the use of a single drug is not enough to reduce blood pressure to a normal level, and recommend that a combination of two drugs be used to normalize blood pressure. Hence, it may not be irrational to conclude that in line with the established guidelines,[39, 40] the inability of captopril or losartan to normalize blood pressure is related to the severity of hypertension.[41]

This study also showed that captopril and losartan inhibited a rise in the volume of the heart, myocardium, and endocardium. However, losartan at 25mg/kg/day failed to prevent an increase in the volume of the endocardium. The antihypertensive activity of such drugs has been attributed to the blockade of the renin-angiotensin–aldosterone system at different levels, resulting in diminished angiotensin II-mediated effects. As was mentioned earlier, angiotensin II causes cardiac and vascular hypertrophy, remodeling, and fibrosis.[28, 41] Therefore, it may not be unreasonable to suggest that by reducing angiotensin II-mediated effects, these drugs not only prevent an increase in the systolic blood pressure and cardiac volume and weight but also attenuate cardiac hypertrophy. The inability of losartan at 25 mg/kg/day to prevent an increase in endocardial volume may be related to the difference in the mechanisms of action between captopril and losartan. Captopril acts by the inhibition of angiotensin II synthesis

and the inhibition of bradykinin, an endogenous vasodilator, whereas losartan acts only by inhibiting the angiotensin II effects.[28] Moreover, the varied effects of the two drugs on the total volume of the myocardium as compared with our sham group may be related to their mechanisms of action and need further investigation.

Neither captopril nor losartan had any effect on the volume of the matrix in the present study. Unfortunately, the lack of similar studies using either captopril or losartan, or drugs from their classes, precludes proper comparisons and contrasts. Nevertheless, our findings indicate that such drugs appear to mainly act on the cellular segments of the myocardium, rather than the matrix. Our findings also showed that captopril and losartan reduced the myocardial vessel volume. Such an effect might be due to the attenuation of angiotensin II-induced angiogenesis.[42] We found that captopril was more potent at reducing the volume of myocardial vessels. The difference in the potency of the two drugs as well as their effects relative to our sham group may be related to their mechanisms of action and needs to be further investigated.

The results of the current study also showed that captopril at the higher dose was able to prevent a reduction in the number of cardiomyocytes. When compared with the sham group, captopril, but not losartan, compensated for the hypertension-induced cardiomyocyte loss, which is in concordance with the findings of some previous investigations,[43, 44] and in discordance with the results of some other studies.[17, 33] The effects of captopril on cardiomyocytes may be attributed to its mechanism of action in that it acts on the renin-angiotensin-aldosterone system and prevents bradykinin metabolism.[43] We also found that while lowering blood pressure, not only did captopril and losartan fail to prevent a reduction in the number of Purkinje cells but also they amplified their loss in the present model of hypertension. The reasons for the absence of an effect by captopril or losartan on Purkinje cells are not known, since we are the first to report such an effect. The use of a renin inhibitor, which prevents the conversion of angiotensinogen to angiotensin I in the cascade leading to the synthesis of angiotensin II, could shed more light on the role of the renin-angiotensin-aldosterone system in the hypertension-induced histological changes observed in the present study. Essential hypertension comprises the majority of the cases of human hypertension and is represented in animal models of spontaneous hypertension. The use of such animal models, which were unavailable in our settings, could further clarify the stereological changes of the heart in a more

prevalent form of human hypertension.

Conclusion

The findings of the present study indicated that renovascular hypertension was associated with cardiac hypertrophy, characterized by increased volumes of the heart, myocardium, endocardium, and myocardial vessels, as well as reduced numbers of cardiomyocytes and Purkinje cells. Such effects are most likely mediated by angiotensin II inasmuch as the model is the result of elevated serum angiotensin II levels. The findings also showed that captopril and losartan prevented an increase in the volumes of the heart, myocardium, endocardium, and myocardial vessels but failed to inhibit a decrease in the volume of the matrix and the number of cardiomyocytes and Purkinje cells. Additionally, the study showed that captopril at the higher dose attenuated myocardial loss. The differential effects of captopril and losartan may be related to their mechanism of action. Further studies with longer durations and varied drug doses are required to shed more light on the nature and mechanisms of the effects of captopril and losartan on the volume of the matrix and the number of cardiomyocytes and Purkinje cells in renovascular as well as other experimental models of hypertension.

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