Long-term Effect of Losartan Administration on Blood Pressure, Heart and Structure of Coronary Artery of Young Spontaneously Hypertensive Rats

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Summary

Alterations in geometry and structure of coronary arteries have marked consequences on supplementation of the respective area. We evaluated long-term effect of losartan on blood pressure (BP), heart weight/body weight (HW/BW), geometry and structure of coronary artery (RS) of young SHR and Wistar rats. Four-week-old Wistar rats and SHR were used. Losartan was administered (20 mg/kg/day) in drinking water by gavage for 5 weeks. BP was measured by plethysmographic method. Cardiovascular system was perfused with a fixative (120 mmHg). RS was processed for electron microscopy. Wall thickness (intima+media)-WT, inner diameter-ID, cross sectional area (intima+media)-CSA, volume densities (VD) of endothelial cells-EC, extracellular matrix-ECM of intima, smooth muscle cells-SMC, and ECM of media were evaluated. BP of 4-week-old SHR did not differ from that in Wistar rats. In 9-week-old SHR were increased: BP, HW/BW, WT, CSA, WT/ID, CSAs of SMC, ECM of media and decreased: VD and CSA of EC. Losartan administration decreased BP and HW/BW in both groups. Geometry of RS was affected only in SHR (decreased: WT, CSA, WT/ID and increased: ID, circumferential tension, VD and CSA of EC). Losartan administration reduced BP and myocardial mass in both groups and beneficially affected geometry and structure of coronary artery in SHR.

Key words

SHR, losartan, ultrastructure, coronary artery

Introduction

There is unequivocal evidence that the pathological background of hypertension and accompanied alterations in function and structure of the cardiovascular system in SHR are of multifactorial origin. Nevertheless, the individual systems do not seem to participate equally. In our previous studies, we observed that either nitric oxide (NO) deficiency in adult (Kristek et al. 2003) and/or young SHR (Kristek et al. 2007), or its substrate L-arginine (Kristek 1998) are very probably not the sufficient cause for development and maintaince of hypertension. Moreover, either long-term phospodiesterase-5 inhibition with sildenafil (due to this increase of cGMP) from prehypertensive period did not prevent alterations in function, geometry and structure of conduit arteries typical for adult SHR (Kristek et al. 2007). Thus, the different underlying pathophysiological mechanisms seem to be involved in hypertension in SHR.

The studies from other laboratories and also therapeutic effectiveness of either angiotensin converting enzyme inhibitors or angiotensin II (Ang II) receptor blockers suggest an important role of renin-angiotensin system in these processes. Ang II is intimately involved in the many regulatory mechanisms in the cardiovascular system beside blood pressure increase, it stimulates proliferation of smooth muscle cells in the arterial wall (Freeman et al. 1995), enhances collagen deposition, matrix components (Lopez et al. 2001, Rosendorf 1996), alters structure and thickness of the arterial wall both resistant and conduit arteries, modulates sympathetic activity (Ruiz-Gayo et al. 2000), stimulates endothelin-1 release from vascular endothelium and tunica adventitia (An et al. 2006), influences bioavaibility of NO and endothelial functions (Yokoyama et al. 2005). Pathological alterations in the majority of these regulatory systems were observed in spontaneous hypertension. Most known Ang II effects are mediated via the Ang II type 1 receptor (AT1) and therefore administration of

angiotensin receptor blocker offers the possibility to modify multiplicity of Ang II actions on the cardiovascular system.

The effect of Ang II itself and the effect of AT1 receptor inhibition are relatively well documented in resistant part (especially mesenteric bed) of the cardiovascular tree. Only very small attention was addressed to the effect of AT1 receptors blockade on the geometry and structure of conduit arteries in SHR. Moreover, data about the effect of AT1 receptor blockade from the prehypertensive period through adulthood on the geometry and structural composition of coronary arteries of SHR have not come to our attention.

The aim of our study was to evaluate whether losartan, an Ang II AT1 receptor antagonist, administered to both normotensive Wistar rats and SHR influences (i) blood pressure, (ii) heart weight/body weight ratio, (iii) geometry of coronary artery, and (iv) volume densities and cross sectional areas of respective parts of the coronary artery wall. Having in mind the fact that pathological changes evoked by hypertension are more difficult to be influenced when they become stabilized we administered losartan from the early prehypertensive period of ontogenic development through adulthood.

Material and methods

All procedures and experimental protocoles were approved by the Ethical Committee for Experimental Work of the Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use.

The animals were housed at a temperature of 22-24 ºC, under a 12 h light: dark cycle and fed a regular pellet diet.

Four-week-old Wistar rats and SHR were taken for the study. The animals were divided into four groups of ten animals each: 1) Wistar rats, 2) SHR, 3) Wistar rats treated by losartan,

4) SHR treated by losartan. Losartan was daily administered in a dose 20 mg/kg/day dissolved in drinking water by gavage. The experiment lasted 5 weeks and in all groups blood pressure (BP) was measured indirectly of pre-warmed animals by the plethysmographic method on the tail artery.

At the end of the experiment the animals (nine weeks old) were sacrificed by an overdose of anaesthesia, the chest was opened and the cardiovascular system was perfused at a constant pressure of 120 mm Hg for 10 min via a cannula placed in the left ventricle. As a fixative 300 mM glutaraldehyde in 100 mM phosphate buffer was used. After perfusion the hearts were excised and weighed. The proximal part of the the septal branch of the left descending coronary artery (RS) was excised, cleaned, divided into three segments (about 1 mm), fixed with the same fixative, postfixed with 40 mM OsO4, stained en block with uranylacetate, dehydrated through ascending concentration of alcohol and embedded in Durcupan ACM (Sigma). Two randomly selected blocks of the artery were cut perpendicularly to the longitudinal axis. The inner diameter (ID) and arterial wall thickness - tunica intima and tunica media (WT) were measured in light microscopy. The arterial wall thickness was measured at about 45^{o} intervals around the circumference of the artery. The cross sectional area - tunica intima and tunica media (CSA) and circumferential tension (BP x ID/WT) were calculated.

In the coronary artery volume densities (VD) of endothelial cells, subendothelial matrix (space between endothelial cells and first layer of smooth muscle cells, including elastic lamina), smooth muscle cells, and extracellular matrix among smooth muscle cells were measured by the point counting method (Weibel et al., 1966) using ultrathin sections. The same blocks used for light microscopic measurements were used for morphometric measurements at electron microscope level. Volume density was calculated: total number of points falling on a particular structure/total number of points falling on the entire vessel wall

(tunica intima + tunica media), expressed as $\mu m^3/\mu m^3$ vessel wall (tunica intima + tunica media). From the values of volume densities, appropriate CSAs were calculated.

Values are given as mean ± S.E.M. Anova and Bonferroni test for unpaired variables were used for statistical evaluation. Results were considered significantly different when $P<0.05$.

Results

General Parameters

At the 4th (the beginning of the experiment) and 5th week of the postnatal life the BP of untreated SHR did not differ from the BP of control (untreated) Wistar rats. In the SHR group BP continually increased from the 5th week of age. In 9-week-old SHR it was increased by 37%. Administration of losartan to Wistar rats evoked decrease of BP in the 9th week of age by 7%. BP in SHR after losartan treatment was significantly reduced from the 7th week and in 9-week-old SHR, it was in comparison to untreated SHR decreased by about 10%; but it was still higher (24%) than in control Wistar rats. The values of weekly measured BP are documented in Fig. 1.

Body weight of the control Wistar rats was in the whole course of the experiment higher in comparison to SHR, nevertheless, the differences were decreasing with age and in the 9th week it was only 8%. Administration of losartan to Wistar rats evoked an increase of body weight, at 9th week by 11%. In SHR, after losartan treatment, the body weight was increased (in 9th week by 14%) even after first week of treatment and it did not differ from the body weight of control Wistar rats. All values are illustrated in Fig. 2.

Heart weight (heart weight after perfusion with fixative is higher than heart weight without perfusion - due to fixative in the open arterial tree) of SHR did not differ from control Wistar rats. Administration of losartan evoked a decrease of heart weight in both Wistar rats

(24%) and SHR (15%). No differences in this respect was observed between control Wistar rats and SHR receiving losartan (Fig 3).

Heart weight/body weight (HW/BW) ratio in SHR was increased (26%) compared to Wistar rats. Losartan administration resulted in decrease of HW/BW ratio in both groups, in Wistar rats by 35%, in SHR by 28%. No differences in this regard were found between Wistar rats and SHR receiving losartan (Fig. 3).

Geometry of the Coronary Artery

The inner diameter of the artery in SHR did not differ from that in Wistar rats. No difference was also found between Wistar rats and Wistar rats treated with losartan. Compared to untreated SHR, losartan administration to SHR resulted in an increase of inner diameter (18 %), the difference was also significant in comparison with Wistar rats (Fig. 4).

Wall thickness of the artery was increased in SHR (39 %) when compared with Wistar rats. Administration of losartan to Wistar rats did not evoke any changes in the wall thickness. On the other hand significant decrease of wall thickness was found in SHR after losartan treatment (25 %) and the decrease was up to the control level (Fig. 5).

The cross sectional area (arterial wall mass) of the arterial wall (tunica intima + tunica media) of SHR in comparison with Wistar rats increased (41 %). No difference in this respect was observed between Wistar rats and Wistar rats receiving losartan. The significant decrease was found between SHR and SHR administered losartan (13 %). Nevertheless, the arterial wall mass in treated SHR was still higher (19 %) than in control Wistar rats (Fig. 5).

The value of wall thickness/inner diameter ratio in SHR group was increased (37 %) when compared to control Wistar rats. Long-term administration of the losartan to Wistar rats did not evoke significant effect. Losartan administered to SHR evoked a decrease of the ratio

(36 %) and at the end of the experiment the wall thickness/inner diameter ratio did not differ from the controls (Fig. 4).

Circumferential stress in the coronary artery of the SHR did not differ from that in Wistar rats. Administration of losartan to Wistar rats did not result in any effect. Losartan administration to SHR significantly increased circumferential stress in the artery in comparison with both untreated SHR (49 %) and Wistar rats (53 %) (Fig. 6).

Tunica Intima

In the coronary artery of SHR we observed decrease of VD and related CSA of tunica intima Evaluation of cellular and extracellular parts of the tunica intima showed that there were no differences in the extracellular matrix between SHR and Wistar rats either in VD or CSA. The difference between tunica intima of SHR and Wistar rats was due to decreased VD and CSA of endothelial cells compared with Wistar rats (Table 1, 2).

Five weeks of losartan administration to Wistar rats did not evoke changes in both VD and related CSA in tunica intima. Also no effect of losartan was observed in VD and CSA of endothelial cells and extracellular matrix. On the other hand administration of losartan to SHR evoked pronounced effect on the intima. VD and CSA of tunica intima were significantly increased and at the end of the experiment they did not differ from those of normotensive Wistar rats. The analysis of the intima revealed that whereas both VD and CSA of extracellular matrix were not affected by losartan administration the VD and related CSA of endothelial cells were significantly increased (Table 1, 2).

Tunica Media

The tunica media occupied significantly higher volume density and CSA in SHR than in normotensive Wistar rats. Analysis of the media showed that CSA of both SMC and extracellular matrix was increased in SHR (Table 1, 2).

Administration of losartan to Wistar rats did not evoke changes in both VD and related CSAs in the tunica media and also no effect of losartan was observed in volume density and CSA of smooth muscle cells and extracellular matrix (Table 1, 2). Opposite to Wistar rats losartan administration to SHR decreased VD and CSA of media and at the end of the experiment both did not differ from those in normotensive Wistar rats. The analysis of the media revealed that losartan prevented in a similar extent increase of VD and CSA of both SMC and extracellular matrix. At the end of the experiment there were no differences in this respect between SHR+losartan and normotensive Wistar rats (Table 1, 2).

Discussion

General Parameters

Blood pressure increase and cardiac hypertrophy in 9-week-old SHR, as well as the process of BP increasing from the prehypertensive period observed in the present study confirm developing phase of hypertension. The findings are fully in agreement with reports from other laboratories (Lee et al. 1983, Cunha et al. 1997) including the original data presented by Okamoto et al. (1963).

Five weeks of losartan therapy resulted in significant decrease of BP and heart weight/body weight ratio in both normotensive Wistar rats and SHR. The preventive effect of losartan against blood pressure elevation in SHR could be connected with (i) inhibition of postjunctional Ang II effects via receptors and/or via increased plasma Ang II level with subsequent stimulation of AT2 receptors (Gohlke et al. 1998), (ii) affection of a variety of vasoconstrictor mechanisms including endothelin-1 release (An et al. 2006), (iii) reduction of superoxide anions production (Dantas et al. 2004), decrease of adrenergic vasoconstriction (Paulis et al. 2007), all result in prevalence of vasodilatory mechanisms. Ang II was also shown to be one of the most potent mitogens and its reduced operation due to AT1 receptors

inhibition is probably responsible for the decrease of myocardium mass. It is noteworthy that a lower dose of losartan (15 mg/kg) administered to 3 weeks old SHR for 4 weeks, in spite of blood pressure decrease, did not influence either vascular or cardiac hypertrophy. The regression of vascular and cardiac hypertrophy was observed when treatment was extended to 10 weeks (Morton et al. 1992). The hypotensive effect of losartan and decrease of heart weight after losartan administration in both normotensive and SHR is in good consent with the observations of Ruiz-Gayo et al. (2000), Soltis (1993), Soltis et al. (1993), Suo et al. (2002), Li et al. (1997), Silva-Antonialli et al. (2000). Compared with our study a more pronounced decrease of BP (up to control level) after losartan administration was observed by Soltis et al. (1993) and Kaneko et al. (1996). Higher effect of losartan in those experiments was achieved very probably due to application of losartan (the same dose as in our experiment) by injection s.c. (Soltis et al. 1993) and/or administration of higher doses of losartan – 30 mg/kg/day (Kaneko et al. 1996).

At the end of the experiment heart weight/body weight ratio was in treated SHR even beneath the value of untreated Wistar rats. BP was in treated SHR significantly lower than in untreated SHR yet it was still significantly higher in comparison to Wistar rats. Thus, our results imply higher antiproliferative (about -30%) as antihypertensive (about -8%) effect of losartan in SHR. The question arose whether a higher effect of losartan on heart weight/body weight ratio compared with BP in both groups is a real beneficial effect of losartan, since relative decreased mass of myocardium has to surmount relatively higher resistance than in untreated rats. Moreover, the finding also indicates that these two parameters are not necessarily coupled. Similar results were observed after NO donors and/or sildenafil administration to young and/or adult SHR (Kristek et al. 2003, Cebova et al. 2006, Kristek et al. 2007).

Geometry of the Coronary Artery.

In SHR we observed alterations in the geometry of the coronary artery signalizing hypertrophy of the arterial wall - increased wall thickness, cross sectional area, and wall thickness/inner diameter ratio. No difference was found in inner diameter. Hypertrophy of the arterial wall at this age was documented by many authors in various arteries (Cunha et al. 1997, Rizzoni et al. 1998). But on the other hand Lee et al. (1983) observed hypertrophy of the arterial wall in the first order branch and small arterioles of the superior mesenteric artery (SMA) in 10 – 12 – week- old SHR but not in SMA itself.

In spite of pronounced alterations in the geometry of the coronary artery in SHR circumferential stress (BP x radius/wall thickness) did not differ from that in Wistar rats. It means that increase of BP in SHR was properly balanced by increased WT/ID ratio. No differences in circumferential stress between 12 - week-old SHR and age matched Wistar rats was also observed by Cunha et al. (1997) in the carotid artery.

Losartan administration evoked a higher effect on the geometry of the coronary artery in SHR compared with Wistar rats. We suggest that increased ID (ID in SHR was increased at about 18%, contrary to about 3% in Wistar rats) and higher antiproliferative (WT and CSA were reduced at about 25% resp. 13 % in SHR, vs. about 10% resp. 5% in Wistar rat) than antihypertensive effect in SHR after losartan administration disturbed the balance among the parameters participating on circumferential stress and due to this WT/ID ratio was too low for a given intraluminal pressure, and it resulted in significant increase of circumferential stress. Increased circumferential stress appears to be one of the most important stimuli leading to hypertrophy of the arterial wall. Having in mind the findings of Thubrikar and Robicsek (1995) that decrease of circumferential stress inhibits atherogenesis, we suppose that the opposite could aggravate the pathological processes in the arterial wall. Since the

effect of losartan on the geometry of the coronary artery seems to be greater than that expected on the basis of the BP reduction, our data suggest that losartan may also induce structural changes in the heart and blood vessels by a pressure-independent mechanism.

Volume Densities and CSAs

Evaluation of volume densities of the coronary artery wall components and calculation of related CSAs revealed some differences between SHR and Wistar rats. In SHR in spite of hypertrophy of the arterial wall CSA of the intima did not reach CSA of that in normotensive Wistar rats. More detailed analysis of the intima in SHR revealed that only volume density and CSA of endothelial cells were significantly lower compared with Wistar rats. The findings support the suggestion that hypertension in SHR may consist, at least partially, on compromised function of endothelial cells (Pourageaud and Freslon 1995, Liu et al. 2002). This coupled with our previous findings documenting decreased endothelium dependent relaxation to acetylcholine in the iliac artery from SHR (Gerova et al. 2005). Since in a parallel series of experiments we observed that long term increase of either NO level or cGMP (from prehypertensive period through 9th week) in SHR had no effect on the BP, heart/body weight ratio, and geometry of the coronary and carotid arteries (Kristek et al. 2007). We hypothesised that other regulatory mechanisms than nitric oxide should be engaged.

The tunica media represents about 95% of the arterial wall in SHR and it is responsible for increase of the arterial wall mass. The analysis of the tunica media revealed that both parts – smooth muscle cells and extracellular matrix participate on the hypertrophy of the arterial wall equally. Since we did not observe mitotic activity of smooth muscle cells in the coronary artery wall of SHR it is likely that the increase of wall thickness was due to hypertrophy of these cells rather then hyperplasia. The finding is according with suggestion that in slow-

developing models of hypertension (including SHR) the major growth response in large arteries was mainly due to hypertrophy of smooth muscle cells (Olivetti et al. 1982).

To our knowledge there is no literary data to compare volume densities and CSAs of individual components of the coronary artery wall in 9-week-old Wistar rats and age-matched SHR. Increased tunica media and decreased mass of endothelial cells support experimental studies suggesting that the presence of a functional endothelium is essential for maintaining smooth muscle cells in a nonproliferative state (Garg and Hassid 1989). Increased CSA of smooth muscle cells is in accordance with greater active tension of SHR arteries compared with vessels from Wistar rats (Mulvany et al. 1980; Ruiz-Gayo et al. 2000). Lee et al. (1983) observed significant differences in volume densities and CSAs of majority of components in large mesenteric arteries and to a less extent also in small vessels of 10-week-old SHR when compared with Wistar Kyoto rats. On the other hand, they did not find differences in volume densities and CSAs of any components in media of the superior mesenteric arteries. Inconsistent results among individual arteries suggest different answers of various parts of the arterial tree to the similar stimulus thus it is impossible to transform results from one vessel to another.

Five weeks of losartan administration to Wistar rats did not result in any effect on volume densities and corresponding CSAs of individual components of arterial wall.

On the other hand, losartan administration to SHR evoked pronounced effect on the arterial wall but different on intima in comparison with media. The analysis of the intima revealed increased mass of endothelial cells in the arterial wall. We suggest that it could be responsible for improving of the physiological regulatory mechanisms as it was observed in vessels after losartan treatment. We found enhanced endothelium dependent relaxation to acetylcholine in the thoracic aorta after losartan administration (Torok et al. 2006). Acetylcholine-induced relaxation also observed Soltis (1993) in aorta after 2 weeks of

losartan (10 mg/kg s.c.) treatment. The findings are consistent with observation of Olson et al. (2004) who observed that losartan enhanced NO-synthase mRNA levels, protein expression, and NO production in pulmonary artery endothelium.

The analysis of the media revealed that both smooth muscle cells and extracellular matrix were decreased approximately to a similar extent. The preventive effect of losartan administration on arterial wall mass increasing may have been reasonably explained by protective effect of losartan on endothelial cells (Garg and Hassid 1989, 1997), and inhibition of proliferative action of Ang II on SMC. Tea et al. (2000) found AT2 receptors mediated vascular mass regression by stimulating SMC apoptosis in vivo, an effect seen during AT1 receptor blockade. Decreased mass of SMC in the vessel wall may participate in the antihypertensive effect of losartan. Our results revealed that losartan administration also affects extracellular matrix production. Thus, close relationship between matrix production and mass of SMC seems to be present. Present findings (decreased volume density and CSA of SMC and extracellular matrix) are in good agreement with observationss of Lopez et al. (2001), Rosendorf (1996), and Varo et al. (2000). They observed that Ang II enhances collagen deposition and matrix components and alters structure and thickness of the arterial wall both resistant and conduit arteries.

In conclusion, the present study has shown the course of blood pressure and morphological differences between coronary artery of SHR and normotensive Wistar rats at 9^{th} week of age (myocardial and vessel wall hypertrophy, decrease of volume density and CSA of endothelial cells). Long-term losartan administration resulted in reduction of BP and myocardial mass in both SHR and Wistar rats. In opposite to Wistar rats losartan prevented increase of arterial wall mass and beneficially affected mainly volume density and related CSA of endothelial cells (up to control level) in SHR.

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Table 1. Volume densities of individual parts of arterial wall of Wistar rats, Wistar rats administered losartan (Wistar+Los), SHR, and SHR administered losartan (SHR+Los). Values are means±S.E.M. **p<0.01 vs Wistar rats, +p<0.05, ++p<0.01 vs SHR.

Volume density (%)	Wistar rats	Wistar+Los	SHR	SHR+Los
Tunica intima	11.49±1.64	10.85±2.85	5.40±0.97**	9.09±0.99^{+}
Endothelial cells	7.44±1.16	5.85±0.95	2.64±0.61**	6.66±0.84^{++}
Extracellular matrix	4.04±0.52	3.52±1.12	2.74±0.49	2.41±0.24**
Tunica media	88.51±1.64	90.92±2.08	94.64±0.97**	90.97±1.01^{+}
Smooth muscle cells	73.30±1.94	73.75±2.30	76.93±1.28	74.20±1.23
Extracellular matrix	15.21±1.59	17.13±1.19	17.71±0.78	16.71±1.33

Table 2. Cross sectional areas of individual parts of arterial wall of Wistar rats, Wistar rats administered losartan (Wistar+Los), SHR, and SHR administered losartan (SHR+Los).Values are means±S.E.M. *p<0.05, **p<0.01 vs Wistar rats, +p<0.05, ++p<0.01 vs SHR.

Cross sectional areas (μm²)	Wistar rats	Wistar+Los	SHR	SHR+Los
Total	7 300±500	6 900±800	10 300±500**	9 000±500*$^{+}$
Tunica intima	863±130	629±162	546±83*	825±91^{+}
Endothelial cells	557±86	395±91	266±55**	597±69^{++}
Extracellular matrix	304±49	235±76	280±45	227±31
Tunica media	6883±994	6156±1090	10346±1191*	8420±625
Smooth muscle cells	5 665±765	5036±971	8446±1042*	6 860±501
Extracellular matrix	1 219±279	1118±145	1899±164*	1 600±177

Fig. 1

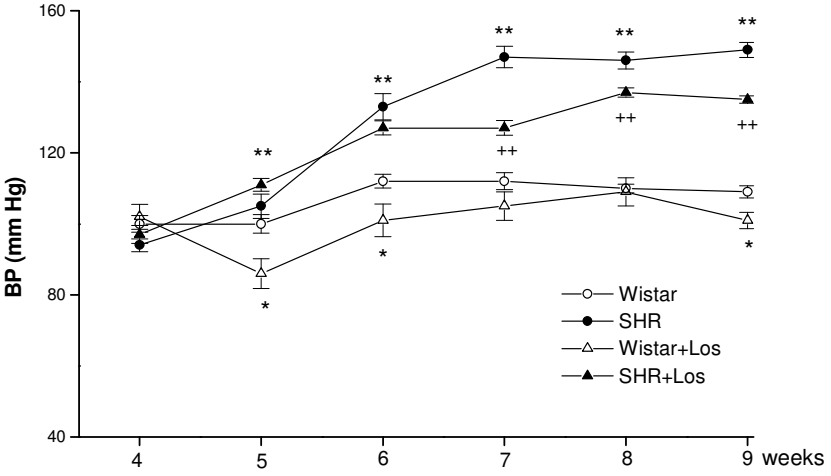

Fig. 1. Blood pressure value (BP) during the course of the experiment. Wistar rats (Wistar), Wistar rats administered losartan (Wistar+Los), spontaneously hypertensive rats (SHR), and SHR administered losartan (SHR+Los).

*p<0.05, **p<0.01 vs. control Wistar rats, ++p<0.01 vs. SHR.

Fig. 2

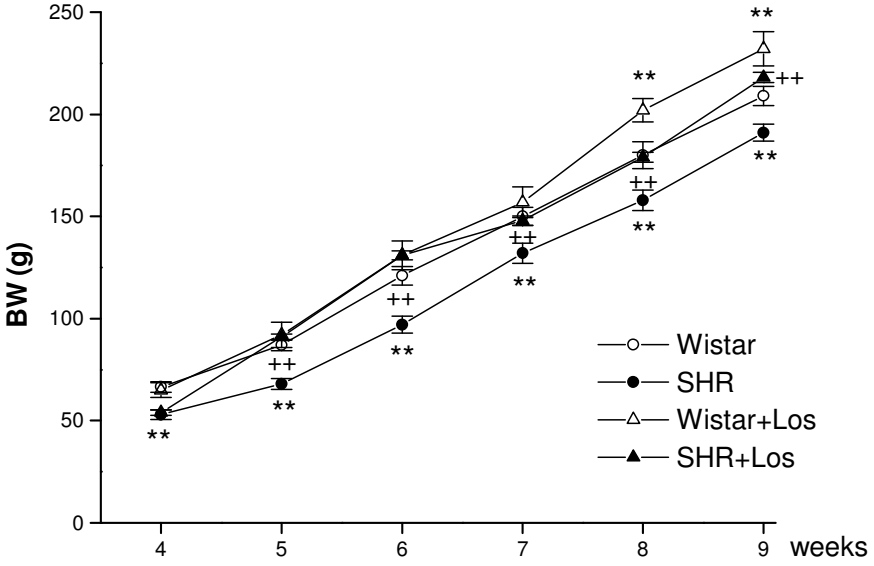

Fig. 2. Body weight (BW) value during the course of the experiment. Wistar rats (Wistar), Wistar rats administered losartan (Wistar+Los), spontaneously hypertensive rats (SHR), and SHR administered losartan (SHR+Los).

**p<0.01 vs. Wistar, ++p<0.01 SHR vs. SHR+Los.

Fig. 3.

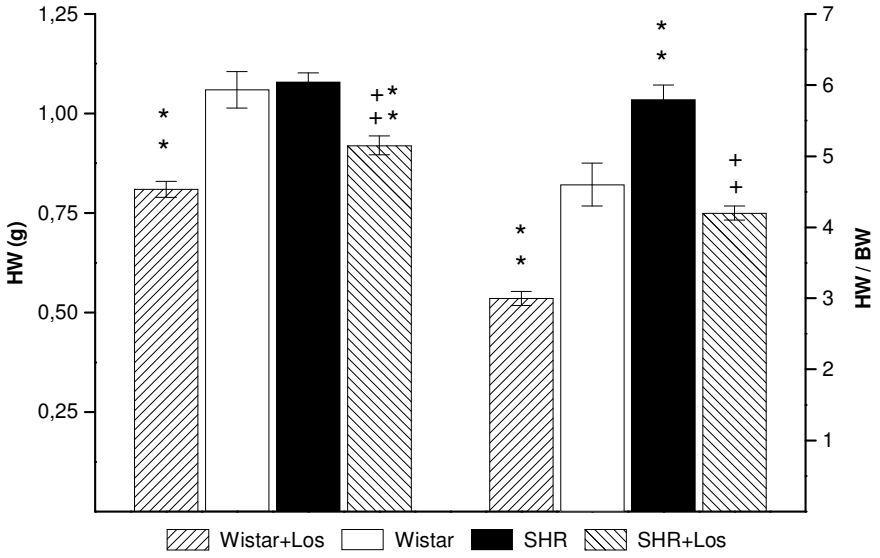

Fig. 3. Heart weight and heart weight/body weight ratio of Wistar rats (Wistar), Wistar rats administered losartan (Wistar+Los), spontaneously hypertensive rats (SHR), and SHR administered losartan (SHR+Los).

**p<0.01 vs. control Wistar rats, ++p<0.01 vs. SHR.

Fig. 4.

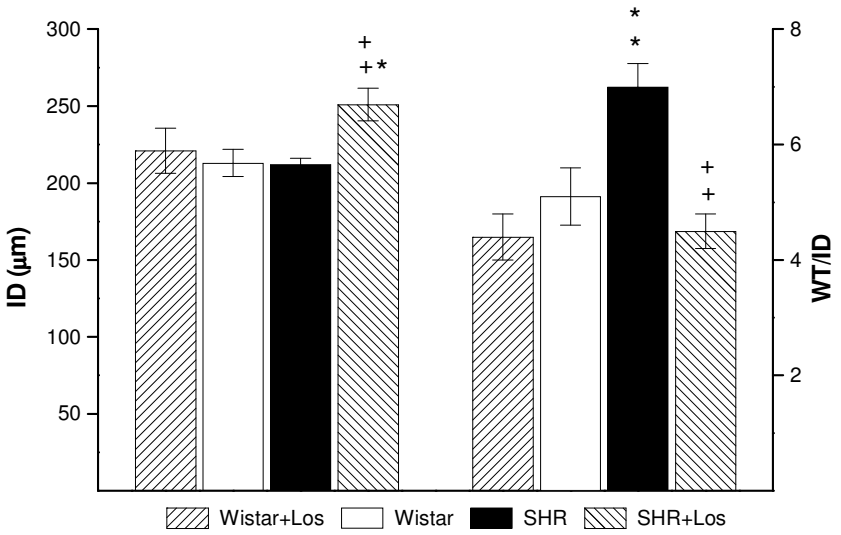

Fig. 4. Inner diameter (ID) and wall thickness/inner diameter ratio (WT/ID) of Wistar rats (Wistar), Wistar rats administered losartan (Wistar+Los), spontaneously hypertensive rats (SHR), and SHR administered losartan (SHR+Los).

**p<0.01 vs. control Wistar rats, ++p<0.01 vs. SHR.

Fig. 5.

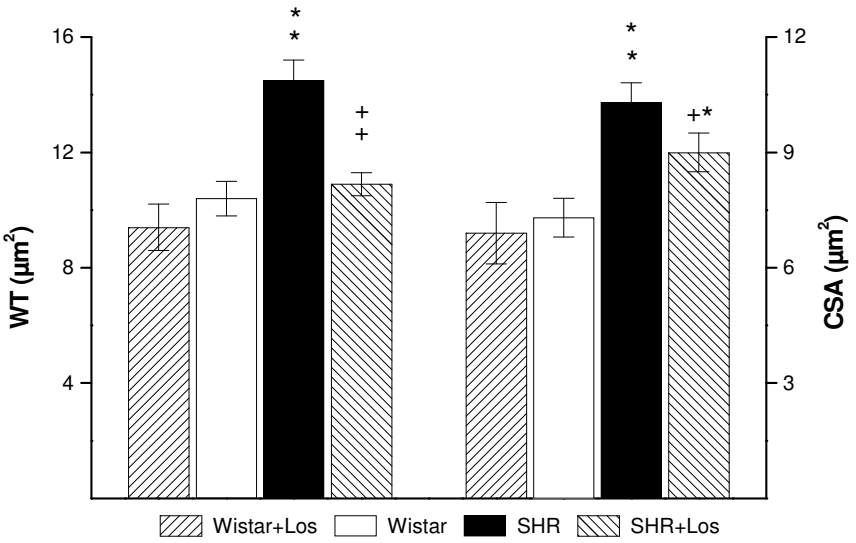

Fig. 5. Wall thickness (WT) and cross sectional area (CSA) of Wistar rats (Wistar), Wistar rats administered losartan (Wistar+Los), spontaneously hypertensive rats (SHR), and SHR administered losartan (SHR+Los).

*p<0.05, **p<0.01 vs. control Wistar rats, +p<0.05, ++p<0.01 vs. SHR.

Fig. 6.

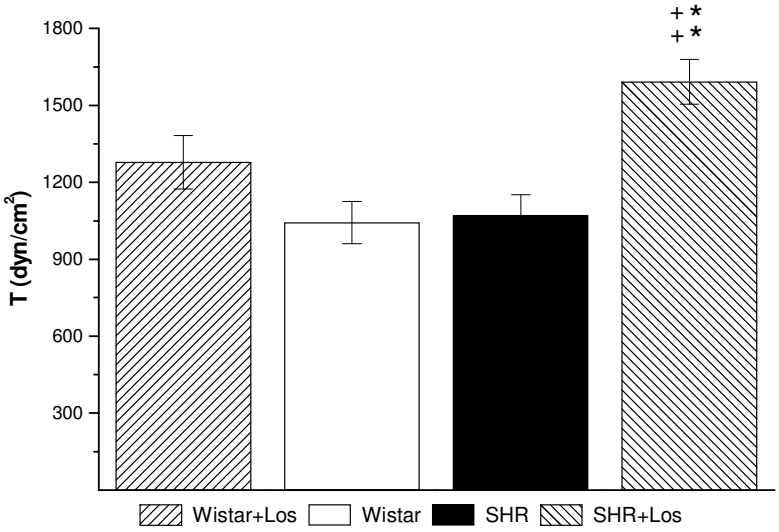

Fig. 6. Circumferential tension (T) in the coronary artery. Wistar rats (Wistar), Wistar rats administered losartan (Wistar+Los), spontaneously hypertensive rats (SHR), and SHR administered losartan (SHR+Los).

**p<0.01 vs. control Wistar rats, ++p<0.01 vs. SHR.