The effect of AT\textsubscript{1} receptor antagonist on chronic cardiac response to coronary artery ligation in rats

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Received 6 July 1995; accepted 18 October 1995

Abstract

Objective: The aim was to study the effect of the AT\textsubscript{1} receptor antagonist losartan on hemodynamic and morphometric changes following experimental infarction. Methods: Experimental infarction was produced in adult male rats by ligating the coronary artery. Treatment with losartan was compared to untreated controls, in rats with experimental infarction and sham-operated animals. Results: Infarcted hearts were characterized by significant decreases in left ventricular developed pressure, as well as positive and negative (dP/dt\textsubscript{max}), whereas left ventricular end-diastolic pressure (LVEDP), relaxation constant \( \tau \) and right ventricular systolic pressure (RVSP) significantly increased. Treatment with losartan decreased the LVEDP, the relaxation constant \( \tau \) and RVSP in the infarcted hearts. Right ventricular weight significantly increased in rats with infarction; this was attenuated by losartan. Infarct size was not significantly influenced by losartan treatment. Morphometric data revealed decreased capillary supply in infarcted hearts, especially in regions close to infarction; the decrease was less pronounced after losartan treatment. Capillary density in near infarct region decreased from 2826/mm\(^2\) to 1471/mm\(^2\) in untreated animals but in the treated animals it decreased from 2982/mm\(^2\) to only 2037/mm\(^2\). Simultaneous significant decrease in myocyte-to-capillary ratio in treated animals compared to untreated rats (0.87 to 0.67) seems to indicate formation of new capillary channels after losartan treatment. LVEDP was dependent on the size of infarction in untreated but not in treated animals. A close correlation between LVEDP and capillary density was found. Conclusions: Decreased ventricular contractility, prolonged relaxation and decreased coronary capillary density in rat experimental cardiac infarction confirm and amplify previous reports dealing with this experimental model. Moreover, we have found evidence of improved hemodynamics and coronary angiogenesis after losartan treatment.

Keywords: Myocardial infarction; Hypertrophy; Microcirculation; Angiotensin receptor; RAAS; Angiogenesis; Losartan; Rat, anesthetized

1. Introduction

Survival after acute myocardial infarction is dependent on the size of the infarcted tissue, the degree of its stretching, and on the reaction of the surviving tissue. With improving treatment of acute myocardial infarction, chronic changes in myocardial tissue, loosely termed ventricular remodeling, are becoming increasingly recognized as important determinants in ultimate patient survival. Some of these changes are similar to alterations reported in cardiac hypertrophy due to pressure overload [1]. Both the increase in ventricular volume as well as hypertrophy of the remaining viable tissue are, initially, compensatory mechanisms which provide a functional advantage. At some point, however, these responses may become deleterious and may contribute to congestive heart failure [2–4].

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Elsevier Science B.V.
SSDI 0008-6363(95)00244-8

Time for primary review 26 days.
Both the circulating as well as local renin-angiotensin systems (RAS) are known to be involved in the development of cardiac and vascular hypertrophy, repair and remodeling [5]. Beneficial effects of treatment with angiotensin converting enzyme (ACE) inhibitors following myocardial infarction have been well documented in numerous studies on experimental animals, as well as in clinical studies (e.g. [6-8]). These effects may be either due to reduced tissue angiotensin II content or to a concomitant increase in the local levels of bradykinin. On the other hand, experiments utilizing angiotensin II receptor antagonists mimic exclusively the lack of local angiotensin II. In our study, we used the specific nonpeptide antagonist, losartan, which has been shown to inhibit the binding of angiotensin II at the AT1 receptor subtype. As a result of its high substrate specificity, losartan has little to no effect on the kallikrein-kinin system and does not increase the local concentration of bradykinin and related peptides.

One of the prominent features of ventricular remodeling following myocardial infarction is the rarefaction of the capillary bed [9,10]. The renin-angiotensin system plays an important, though not fully clarified role in the regulation of angiogenesis. For instance, angiotensin II itself was reported to stimulate vascular formation in cremaster muscle of rats [11], and rabbit cornea [12]. On the other hand, the use of ACE inhibitors, which would lead to a decrease in local angiotensin II, has been found to attenuate the rarefaction of capillaries commonly occurring with cardiac pressure overload hypertrophy [13,14]. In this case, the angiogenic effect of ACE inhibitors may be also attributable to bradykinin potentiation. This direct vasodilatory stimulus, with resulting increase in blood flow may act as the mechanical factor triggering the angiogenic response. Schieffer and co-workers [15], however, reported increased capillary density in infarcted hearts after treatment with losartan, which led to a decrease in local angiotensin II without influencing bradykinin levels. In contrast, Fan and Hu [16] found that losartan blocked the angiogenic effect of angiotensin II.

The aim of the present study was to reexamine the potential cardioprotective role of chronic treatment with losartan in rats with experimental infarction. Hemodynamic measurements were combined with morphometric studies at the tissue level (myocyte size and capillary supply).

2. Materials and methods

2.1. Experimental animal preparation

Adult male Sprague-Dawley rats (Charles River breeding farm) were used in the study. The protocol used in the present investigation conforms with the Guide for the care and use of laboratory animals published by the US National Institutes of Health and was approved by the University of Ottawa Animal Care Committee. The animals were divided in four groups: infarct-untreated rats (14 animals), infarct-treated rats (13 animals), sham-treated rats (11 animals) and sham-untreated rats (10 rats).

Thirty minutes before surgery, atropine sulphate was injected subcutaneously at a dose of 1 mg/kg. Then, following anesthesia by intraperitoneal injection of ketamine (50 mg/kg) and sodium pentobarbital (30 mg/kg), the rats were intubated and artificially ventilated with a Harvard rodent ventilator. While in a supine position, the animals were placed on a thermal insulation pad. Afterwards, a cranio-caudal incision approximately 3 cm parallel to the sternum was made through the skin. The pectoral muscles were cut to expose the ribs, and the thorax was opened at the 4th intercostal space. After placement of ligatures around the sternum to tie off the internal mammary arteries and veins, the sternum was severed, the pericardium was incised and the heart exposed. The left coronary artery was encircled with a curved needle of 3-0 silk surgical suture inserted between the left atrial appendage and pulmonary artery. In infarcted animals, the ligature was tied off, thereby occluding the left coronary artery, while in sham-operated animals it remained loose. The thoracotomy was then closed in layers. Negative intrathoracic pressure was restored by inserting thin tygon tubing between the 6th and 7th ribs and imposing a negative pressure of 10 cm H2O. The animals were allowed to recover for a period of 3 weeks. During the first 3 days post-surgery, the rats were treated with penicillin (30000 U/kg) and streptomycin (40 mg/kg) i.m.

Prior to surgery, treated animals received 15 mg/kg losartan by gastric gavage. During the 3 week recovery period treated animals were given losartan 15 mg/kg per day in drinking water. This is a dose which normalizes elevated blood pressure and prevents heart hypertrophy in rats with spontaneous hypertension [17]. The initial concentration of the drug (0.075 mg/ml) was chosen based on an average fluid intake of 200 ml/kg per day. Actual fluid intake was verified every other day, and losartan concentrations were adjusted to the individual drinking habits of the animals. The fresh solution of losartan for the next two days was prepared as follows: mg of drug to be dissolved in volume on hand = 15 mg × body weight in kg × volume in ml prepared for 2 days/water consumption in ml per animal per day based on the previous period. Average body weight at the beginning of the experiment was 359 g, which remained essentially the same in groups with experimental cardiac infarction (356 g), while it increased to 391 g in sham-operated rats. Three weeks after surgery, rats with infarction increased their body weight to 427 g and sham-operated rats to 445 g (see Fig. 1). Body weights were not significantly influenced by the treatment.

2.2. Functional measurements

Three weeks after surgery, the rats from all experimental groups were anesthetized as described above. Blood
pressure in the left (LV) and right ventricles (RV) was measured using a Millar catheter-tip transducer connected to a Grass 7PIF Polygraph. The catheter was inserted into the left ventricular chamber via the right carotid artery under continuous pressure monitoring. Measurements were recorded after a 15 min stabilization period. Similarly, a catheter was introduced into the RV via the right jugular vein.

The analog pressure signal was digitized with a sampling frequency of 1 kHz and stored on computer for later processing. The following parameters were derived: systolic pressure (LVSP, RVSP), left ventricular end-diastolic pressure (LVEDP), developed pressure (LVDevP) and the maximal rates of pressure development (+dP/dt)\text{max} and fall (−dP/dt)\text{max}. In addition, the time constant of relaxation (τ) was calculated on the basis of an experimental model of isovolumetric pressure decay, as the time required for the pressure at (−dP/dt)\text{max} to be reduced by 1/e [18]. Heart rate was calculated from the left ventricular pressure signal.

2.3. Morphometric analysis

At the endpoint of the experiments, the anesthetized animals were sacrificed by infusion of saturated potassium chloride solution and the hearts were removed. The ventricles were isolated from the atria, the right ventricular free wall was separated leaving the septum and left ventricle intact. Both right and left ventricles were weighed and their weights were normalized by the length of tibia. Afterwards, the left ventricle including the interventricular septum was cut, from base to apex, into four transverse slices perpendicular to the long axis of the ventricle. The apex was used for estimation of the myocardial dry weight. The three other slices were frozen in liquid nitrogen for cryotomic preparation of two 16 μm sections from each slice. The first section, used for determination of infarct size was fixed in formalin and stained using Avallone’s modification of Jones’ silver methenamine method [19]. The second section, used for capillary and muscle cell morphometry, was stained by Lojda’s method for detection of alkaline phosphatase and dipeptidyl peptidase IV activities in capillary endothelium [20]. This double-staining procedure ensured that all capillaries were visualized.

Morphometric measurements were done in eight animals from each experimental group with the aid of an Olympus BHS microscope at a magnification of 400×. The measurements were done by a single investigator (T.S.) in a blind fashion. Twelve fields, each covering 62500 μm² were selected from the subendomyocardium of each heart. Six fields were located close to the site of infarction, while the remaining six fields were remote. In sham-operated controls the six fields were randomly selected from the subendocardial region. In each field, the number of capillaries and muscle cells was counted using the correction of edge effect. These counts were used to calculate the myocyte-to-capillary ratio. The heterogeneity of capillary spacing was evaluated by the method of capillary domains [19,21]. A Summagraphic digitizing tablet was used to record the positions of capillaries and our “Capillary Domain” program was used to calculate equidistant border lines between each capillary. The resulting polygonal region around each vessel was defined as the domain area, and the equivalent radius of the Krogh tissue cylinder with the same area as the capillary domain was calculated by an on-line computer. The frequency distribution of these radii was approximately log-normal. The standard deviation of the log radii (SDlog) was used as the heterogeneity index [21].

Area fractions occupied by muscle cells, capillaries and interstitial tissue in selected cross-sectional fields were determined using the point counting method. A grid with 121 points per field was used. Average myocyte and capillary profiles were calculated, from the area occupied by these two tissue components divided by their respective densities.

Myocyte diameter measurements were made in 25–30 muscle cells selected per field and drawn with a drawing cylinder attached to a microscope. Cell diameters were digitized and analyzed on a graphic tablet linked to an image analysis software program (Bioquant IV, R and M
Biometrics, Inc., Nashville, TN). Since the majority of muscle cells were not cut exactly at right angles, their cross sections often assumed an ellipsoid shape. In this case, the minor axis corresponded to the diameter of an original circle. Accordingly, actual cell diameters were measured at the midpoint of the major axis of the cell's ellipsoid cross-section. Analysis of sampling and of reproducibility of morphometric methods used in the present study may be found in our previous publications [19,22].

For estimation of infarct size, we employed the morphometric method of Fishbein et al. [23]. Infarct size was expressed as the average length fraction (in %) of the endocardial perimeter occupied by the infarcted tissue. Histological sections stained by silver methenamine and hematoxylin-eosin were projected at a magnification of 17.3 x by a microprojector (Zeiss Jena, Germany). Whole section contours as well as the infarct scar area were manually traced on paper. The internal perimeter of the left ventricle and its center of gravity was measured using a semiautomatic image analyzer (Leitz ASM 68K). The infarct was projected on a transection of the left ventricular cavity by means of lines drawn from the center of gravity to both lateral margins of the scar. The length of the endocardial infarct projection was then measured. Infarct transmurality was defined and expressed as the average area fraction (in %) of infarct segment occupied by the scar. The infarcted areas were estimated using the point counting method with the aid of a test grid containing a field of uniformly spaced sampling points.

2.4. Statistical analyses

All results are expressed as mean ± standard error of the mean (SEM). Statistical evaluation of basic anatomical and physiological data was performed using a 2-way ANOVA and least squares pairwise contrasts. Morphometric measurements were assessed by a 2-way incomplete block factorial and subsequent pairwise contrasts. Since capillary domain data followed a log-normal distribution, these data were logarithmically transformed before analysis. Subsequently a 2-way nested factorial ANOVA was applied as well as least squares pairwise contrasts when applicable. To examine relationships between specific functional and morphometric parameters, analysis of covariance (ANCOVA) and regression modeling was used to predict values of specific dependent variables from one or more independent (predictor) continuous variables.

3. Results

Body weights and heart weights are summarized in Fig. 1. The average body weight at the end of the experiment was similar in all four groups of experimental animals. Animals with myocardial infarction had significantly higher right ventricular mass compared to sham-operated animals (P < 0.01), losartan treatment significantly attenuated this increase (P < 0.01). No significant differences were apparent for left ventricular mass among the four groups. The percentage of dry tissue was significantly lower (P < 0.01) in samples from infarcted hearts (21.4 ± 0.7 untreated and 21.3 ± 0.4 treated) when compared to hearts from sham-operated animals (23.7 ± 0.4 untreated and 24.1 ± 0.3 treated). Losartan-treated animals had smaller indices of infarct size but none of these reached the level of statistical significance: infarct size 57 ± 5% versus 65 ± 4%, and infarct transmurality 45 ± 2 versus 51 ± 1%.

Table 1 contains the summary of our functional measurements. In rats with myocardial infarction, the left ventricular systolic pressure was lower than in sham-operated animals, but this effect did not reach the level of statistical significance. Left ventricular end-diastolic pressure was significantly increased and thus, the developed pressure decreased in the infarcted group (P < 0.01). This was accompanied by significant decrease in (+dP/dt)max in this group. Both early and late pressure relaxations were impaired in hearts with experimental infarction, as indicated by a decrease of (−dP/dt)max and by a rise in the constant τ (P < 0.01). Finally, the right ventricular pressure was significantly increased in the infarcted animals. Treatment with losartan did not noticeably influence any of the measured hemodynamic parameters in the sham-op-

<table>
<thead>
<tr>
<th>Hemodynamic measurements</th>
<th>Sham-ununtreated</th>
<th>Sham-losartan</th>
<th>Infarct-ununtreated</th>
<th>Infarct-losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mmHg)</td>
<td>125 ± 4</td>
<td>114 ± 4</td>
<td>117 ± 5</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mmHg)</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
<td>16 ± 3 *</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Left ventricular developed pressure (mmHg)</td>
<td>120 ± 4</td>
<td>109 ± 4</td>
<td>101 ± 6 *</td>
<td>99 ± 5 *</td>
</tr>
<tr>
<td>(+dP/dt)max (mmHg/s)</td>
<td>7767 ± 434</td>
<td>7812 ± 356</td>
<td>5407 ± 370 *</td>
<td>5867 ± 460 *</td>
</tr>
<tr>
<td>(−dP/dt)max (mmHg/s)</td>
<td>7136 ± 388</td>
<td>6514 ± 343</td>
<td>4181 ± 410 *</td>
<td>4423 ± 293 *</td>
</tr>
<tr>
<td>Constant τ (ms)</td>
<td>10.5 ± 0.4</td>
<td>10.4 ± 0.5</td>
<td>16.8 ± 1.3 *</td>
<td>13.1 ± 0.8</td>
</tr>
<tr>
<td>Right ventricular systolic pressure (mmHg)</td>
<td>26 ± 1</td>
<td>26 ± 1</td>
<td>39 ± 6 *</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>331 ± 11</td>
<td>344 ± 10</td>
<td>349 ± 18</td>
<td>350 ± 11</td>
</tr>
</tbody>
</table>

* Mean ± SEM significant at P < 0.01 when compared to control groups.
† Significant at P < 0.02 when compared to treated infarct group.
erated rats. In the infarcted animals, however, the increase in left ventricular end-diastolic pressure and the prolongation of pressure relaxation (constant $\tau$) were significantly attenuated with losartan treatment ($P < 0.02$). Losartan partially prevented the rise in right ventricular systolic pressure after myocardial infarction, but the difference between treated and untreated animals did not reach the level of statistical significance, probably due to a large variability in the untreated group. Finally, heart rate was similar in all four groups of animals.

Morphometric data are presented in Tables 2 and 3 and in Figs. 2 and 3. Table 2 contains capillary and myocyte density values (no./mm$^2$) for all four groups. For capillary density, ANOVA revealed significant overall effects of group ($P < 0.001$, with sham-operated rats having higher capillary densities), treatment ($P < 0.001$, losartan increasing capillary density in the infarct group) and zone ($P < 0.001$, lower values in regions close to infarction relative to regions further removed). In the case of myocyte density, similar effects of group and zone were noted, while no significant effect of treatment was observed. Finally, the myocyte-to-capillary ratio was significantly influenced by treatment in the infarcted hearts ($P < 0.001$), with losartan decreasing the ratio in the zone located close to the infarction. The presence of infarction itself, however, did not influence the myocyte-to-capillary ratio.

Changes in capillary domain area paralleled those in capillary density (see Fig. 2): hearts from sham-operated animals had smaller domain areas, whereas in infarcted hearts, the domain areas were larger in regions close to the infarction relative to regions further removed. Losartan decreased the capillary domain areas in infarcted hearts both near and far from the infarct. The index of heterogeneity of capillary spacing (SDlog) was highest in the

![Table 2](https://academic.oup.com/cardiovascres/article-abstract/31/4/568/300320)

<table>
<thead>
<tr>
<th>Capillary (no./mm$^2$)</th>
<th>Myocyte (no./mm$^2$)</th>
<th>Myocyte/capillary ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (untreated) control</td>
<td>2826 ± 76</td>
<td>2229 ± 84</td>
</tr>
<tr>
<td>Sham (losartan) control</td>
<td>2982 ± 78</td>
<td>2264 ± 70</td>
</tr>
<tr>
<td>Near-infarct untreated</td>
<td>1471 ± 103 * *</td>
<td>1258 ± 81 * *</td>
</tr>
<tr>
<td>Far-infarct untreated</td>
<td>1949 ± 72 *</td>
<td>1533 ± 62 * *</td>
</tr>
<tr>
<td>Near-infarct losartan</td>
<td>2037 ± 76 * *</td>
<td>1366 ± 61 * *</td>
</tr>
<tr>
<td>Far-infarct losartan</td>
<td>2318 ± 54 * *</td>
<td>1754 ± 70 * *</td>
</tr>
</tbody>
</table>

Each experimental group contains eight animals.

For capillary and myocyte densities:
* $P < 0.001$ vs sham-control.
* $P < 0.001$ vs far-infarct untreated.
* $P < 0.02$ vs far-infarct treated.
* $P < 0.01$ vs the same region untreated.

For myocyte/capillary ratio:
* $P < 0.001$ vs near infarct untreated.
* $P < 0.02$ vs sham-control.

![Fig. 2](https://academic.oup.com/cardiovascres/article-abstract/31/4/568/300320)

Fig. 2. Bar graph illustrating capillary domain areas ($\mu m^2 \times 100$) in sham (solid bars) and infarcted rat hearts (near infarct—hollow bars; far infarct—grey bars) with and without losartan treatment. Values are mean ± SEM.

![Table 3](https://academic.oup.com/cardiovascres/article-abstract/31/4/568/300320)

<table>
<thead>
<tr>
<th>Cell diameter (CDIAM, $\mu m$), average myocyte profile area (AMP), and average capillary profile area (ACP, $\mu m^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDIAM ($\mu m$)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Sham (untreated) control</td>
</tr>
<tr>
<td>Sham (losartan) control</td>
</tr>
<tr>
<td>Near-infarct untreated</td>
</tr>
<tr>
<td>Far-infarct untreated</td>
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<tr>
<td>Near-infarct losartan</td>
</tr>
<tr>
<td>Far-infarct losartan</td>
</tr>
</tbody>
</table>

Each experimental group contains eight animals.

Mean ± SEM.

CDIAM, sig. effect of GRP, TRT, ZONE ($P < 0.001$).
AMP, sig. effect of GRP ($P < 0.001$), TRT ($P < 0.03$), ZONE ($P < 0.001$).
ACP, sig. effect of GRP ($P < 0.001$).
GRP — sham versus infarct.
TRT — losartan versus untreated.
ZONE — near versus far.
regions close to the infarcted tissue. Both significant effect of group (i.e. presence or absence of infarct) and zone (close or far from infarction) were found \( (P < 0.01) \), while no effect of treatment was detected (see Fig. 3).

The percentage of space occupied by myocytes, capillaries and interstitial tissue was also assessed. Area fraction occupied by myocytes varied between 79 and 84%, the area was significantly lower in the infarcted hearts \( (P < 0.01) \). The area occupied by capillaries varied between 6.3 and 9.3%. It was significantly larger in the hearts from sham-operated animals when compared to infarcted hearts \( (P < 0.01) \), and it was larger in regions located far from infarction. The remaining, interstitial space fraction was significantly larger in the infarcted hearts \( (P < 0.01) \), especially in the regions close to the infarcted tissue \( (P < 0.01) \). In control hearts percentages were: 7.4 (untreated) and 7.7 (treated). For infarcted hearts percentages were: 13.9 close and 11.2 far from infarction (untreated) and 12.8 and 10.5% respectively (treated).

Table 3 contains data on cell diameters measured directly, and average myocyte profile area and capillary profile area derived from the myocyte and capillary densities. Cell diameter was significantly greater in infarcted hearts than in control \( (P < 0.001) \). Within the infarcted hearts it was significantly greater in the region close to the infarct than in more distant regions \( (P < 0.001) \). Losartan treatment significantly attenuated increases in cell size in infarcted hearts \( (P < 0.001) \). These changes were also reflected by similar changes in area of the average myocyte profiles. Finally, the average capillary profile was significantly larger in the infarcted hearts. Fig. 4 summarizes the relationship between infarct size and left ventricular end-diastolic pressure (LVEDP). As expected, the LVEDP increased with increasing size of the infarct in untreated hearts. In the group of infarcted hearts treated with losartan, this correlation disappeared. ANCOVA indicated that both treatment \( (P < 0.01) \) and infarct size \( (P < 0.01) \) were useful predictors of changes in LVEDP. Finally, a close correlation between capillary density near the infarct site and LVEDP was also found (see Fig. 5). This correlation was highly significant for capillary density close to the infarcted zone \( (P < 0.0001, r = 0.91) \) and significant in the zone far from the infarction \( (P < 0.001, r = 0.73) \).

4. Discussion

The heart responds to overload following myocardial infarction with an adaptational hypertrophic reaction, which is aimed to restore the myocardial mass and to normalize the increased wall stress. This was clear from our results concerning cardiac weights and morphometric indices. In spite of the presence of sizeable infarction, the left ventricular weights, both absolute and relative, remained unaffected. The right ventricular weight increased in untreated rats with cardiac infarction which is in agreement with several previous studies using the same animal model [2,4,9,10]. The most probable explanation for right ventric-
ular hypertrophy is the presence of pulmonary hypertension due to congestive left ventricular failure. This is also supported by our hemodynamic measurements (reduced left ventricular developed pressure, increased end-diastolic pressure). The increase was more pronounced in the untreated animals when compared to rats treated with losartan. This may also explain the differences in right ventricular weights between these two experimental groups as a sign of attenuated congestive heart failure in treated animals. The comprehensive time constant of relaxation, \( \tau \), was increased more in untreated than treated animals. Prolonged relaxation may have contributed to elevated diastolic pressure and a progressive chamber dilatation accompanying the infarction.

Our finding of reconstitution of left ventricular tissue in infarcted hearts is in agreement with the reports of Olivetti et al. [24] and Nishikimi et al. [25]. These authors also reported an increase in left ventricular end-diastolic pressure and a reduction of \( \frac{dP}{dr}_{\text{max}} \) in rats with myocardial infarction. Therefore, we reconfirmed the reconstitution of left ventricular mass and impaired ventricular function, reported previously. In our study, treatment with losartan resulted in better functional characteristics of the infarcted hearts, which may result from improved mechanics of the surviving muscle tissue, as the treatment did not influence significantly the infarct size. This is also supported by our findings of minimal correlation between the left ventricular end-diastolic pressure and infarct size in the treated group (Fig. 4). We have no information however, concerning the effect of losartan on the elastic properties of the scar tissue.

Several authors have reported an improvement in cardiac function as a result of angiotensin-converting enzyme inhibition. It was Pfeffer and coworkers who originally showed in rats with experimental myocardial infarction that the administration of captopril improved hemodynamics and increased survival [6], and his results have been confirmed by many subsequent investigators. The effects of ACE inhibitors are usually abolished by chronic bradykinin receptor blockade, suggesting that angiotensin II may not be involved [8,26]. Recently, however, Raya and coworkers [27] and Nishikimi et al. [25] described a similarly reduced end diastolic pressure in infarcted hearts treated with either captopril or losartan. Capasso described less depression of both positive and negative \( \frac{dP}{dr} \) in infarcted hearts treated with losartan, while captopril caused no change [28].

The presence of cardiac infarction in our study was associated with decreases in capillary and myocyte densities, which were more pronounced in the near-infarct regions. Similar results for a single region of the infarcted heart were reported previously by Turek et al. [10] and Anversa et al. [9]. Olivetti and coworkers [24] also reported higher numbers of capillaries in the remote regions of the infarcted hearts when compared to near-infarct areas.

Treatment with losartan moderated the decreases in capillary density observed in the infarcted hearts: regions near the infarction had 38% higher capillary density than in untreated animals, while in the regions far from the infarction the capillary density was only 14% higher. In contrast, the effect of losartan on myocyte density was only marginal and thus the resulting myocyte-to-capillary ratio was lower in infarcted treated hearts indicating additional formation of capillaries. The combination of these two measurements clearly indicates formation of new capillaries in treated animals.

Fig. 3 describes the changes in SDlog, which is an index of heterogeneity of capillary spacing. SDlog is an independent determinant of tissue oxygenation: the higher the heterogeneity of capillary spacing the greater the chance for appearance of anoxic foci [21]. SDlog was found to be significantly greater in infarcted hearts than in control hearts and significantly greater in the region close to the infarction than in the regions further away. Treatment with losartan did not significantly influence the SDlog values. The index of heterogeneity of capillary spacing as reported here, is the first such observation reported for infarcted hearts.

Morphometric data clearly showed an improved capillary supply of infarcted hearts treated with losartan when compared to untreated infarcted hearts. This effect was more pronounced in regions bordering the infarcted tissue where the capillary supply is impaired the most. Formation of new capillaries is especially supported by a decrease in myocyte-to-capillary ratio, because the alternative explanation, i.e. decreased number of myocytes, is not supported by measurements of myocyte densities. Thus, the increased capillary density cannot result simply from less pronounced myocyte hypertrophy but it must also be associated with a proliferation of new capillaries. Recently, Schieffer et al. [15] also reported capillary densities in infarcted hearts treated with losartan or enalapril to be higher when compared with the untreated infarct group, but still lower than that of the sham group. These authors did not measure myocyte densities nor did they provide a hypothesis explaining their results.

A close link between angiogenesis and the renin-angiotensin system has been known for some time. Our experiments were prompted by the studies of Canby and Tomanek [13] and Unger and coworkers [14] who reported an increase in the capillary density of hypertrophic hearts treated with ACE inhibitors. These results, however, may be also explained by the presence of increased local levels of bradykinin, which is caused by a co-inhibition of kininase II and ACE with subsequent increase in blood flow — a stimulus for angiogenesis. A bradykinin mechanism cannot be expected in losartan-induced angiogenesis. Losartan treatment in myocardial infarction, however, has been found to be associated with decreased minimal coronary resistance [15]. This may be the result of an improvement in endothelial function of coronary resistance vessels, due...
to a reduction in end-diastolic pressure and enhanced myocardial relaxation, or as a direct response to decreased local levels of angiotensin. An alternative explanation may be either direct effect of AT₁ inhibition or the indirect stimulation of AT₂ receptors. Long-term inhibition of AT₂ receptors results in a feedback increase of angiotensin II plasma levels. It is of interest that AT₂ receptors are more pronounced in the early stages of development [29] which also corresponds to the period of increased angiogenesis in the mammalian heart [19]. In the adult left ventricle, approximately 20% of the angiotensin receptors are AT₂ [30]. A recent report by Stoll et al. [31], however, makes this possibility less likely. These authors observed the antiproliferative effect of AT₁ receptors on coronary endothelial cells in vitro. Finally, one can speculate about increased expression in this situation of prostaglandin synthase-2, leading to increased production of prostaglandins, which in turn, modulate cell growth and differentiation [32].

The relationship between cardiac capillarization and cardiac function is not fully elucidated. Our results demonstrate a surprisingly close correlation between left-ventricular end-diastolic pressure and capillary density in the cardiac tissue close to the infarcted zone (Fig. 5). Obviously, this finding does not represent proof of a causal relationship between these two parameters but it may suggest a possible link between angiogenesis and hemodynamic improvement after losartan treatment. Further investigation of this relationship is necessary.

In conclusion, our results indicate that the AT₁ receptor antagonist losartan significantly improves cardiac mechanics after coronary artery ligation, attenuates the hypertrophic response in this situation and acts as an angogenic stimulus. Based on all of these properties, this class of drug has promising therapeutic potential.

Acknowledgements

This study was supported by grants from the Ontario Heart and Stroke Foundation, and personal support to Dr. T. Sladek from the Medical Research Council of Canada. Losartan has been supplied by the DuPont Merck Pharmaceutical Company. We would like to thank Mrs. Ching Kuo and Mrs. Barbara Hebert for their valuable technical assistance, Marcia Heron for her comments on an earlier version of the manuscript and Mrs. Denyse Blais for typing the manuscript.

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