Increased expression of cardiac angiotensin II type 1 (AT₁) receptors decreases myocardial microvessel density after experimental myocardial infarction

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Abstract

Objective: To study the effects of increased levels of myocardial angiotensin II type 1 (AT₁) receptor on microvascular growth following myocardial infarction (MI). Methods: MI was created in transgenic rats (TGR) with a cardioselective overexpression of the AT₁ receptor. We used Sprague–Dawley (SD) rats as controls. Some of the rats were treated with the selective AT₁ receptor blocker losartan (Los). Rats were sacrificed after 3 weeks. Results: MI caused left ventricular (LV) hypertrophy and LV dysfunction in both SD and TGR, which was prevented by AT₁ receptor blockade. Furthermore, MI decreased microvessel density in the non-infarcted myocardium (SD MI: 1653 ± 37/mm², P < 0.01 vs. sham-operated controls), however, microvessel density decreased significantly more in TGR with MI (1298 ± 33/mm², P < 0.01 vs. SD MI). AT₁ receptor blockade restored microvessel density (SD MI Los: 2046 ± 195/mm²; TGR MI Los: 1742 ± 47/mm²; P < 0.01 vs. untreated). The differences in microvessel density were still present after correction for LV hypertrophy. The increase in microvessel density after AT₁ receptor blockade was not accompanied by increased myocardial vascular endothelial growth factor (VEGF) levels. Microvessel density correlated with parameters of myocardial stretch, such as LV end-diastolic pressure (r = 0.681, P < 0.001) and N-ANP (r = 0.424, P = 0.01). Conclusions: Microvessel density after MI is decreased when the AT₁ receptor is overexpressed, and this is amenable to AT₁ receptor blockade. This suggests that efficacy of AT₁ receptor blockers post-MI may not only be due to attenuation of LV remodeling, but also to a stimulatory effect on angiogenesis.

Keywords: Angiotensin; Growth factors; Infarction; Microcirculation; Receptors; Renin angiotensin system

1. Introduction

After myocardial infarction (MI), the renin–angiotensin system (RAS) becomes activated. Specifically, the angiotensin II type I (AT₁) receptor seems to play a prominent role in post-MI remodeling, since AT₁ receptor expression is increased after MI [1] and in LV dysfunction [2]. Although microvessel growth (angiogenesis) within the infarcted zone is a component of this remodeling process, it has been established that the microvessel network becomes unable to support the greater demands of the spared, hypertrophied myocardium, which may result in ongoing loss of viable tissue, infarct extension, and

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replacement fibrosis. Therefore, the maintenance and formation of microvessels may be important potential targets for treatment.

The activated RAS promotes growth and proliferation of cardiomyocytes and fibroblasts, however, its effects on endothelial proliferation and microvessel growth remain unclear. Microvessel growth is driven by many stimuli, like tissue hypoxia and myocardial stretch (for review see Ref. [3]). In-vitro, it has been established that the AT1 receptor exerts pro-angiogenic activity [4,5], mainly by upregulating vascular endothelial growth factor (VEGF), as shown in endothelial cells [6,4] and retinal cells [7]. In skeletal muscle studies a potential pro-angiogenic effect was clearly confirmed in-vivo, as AT1 receptor blockade inhibited microvessel growth in several studies [8–10]. In contrast, other in-vivo studies yielded opposite effects, specifically in the heart. Post-MI, studies from Schieffer et al. [11] and Sladek et al. [12] showed beneficial effects of AT1 receptor blocker on cardiac microvasculature, suggesting a rather anti-angiogenic effect of the AT1 receptor. However, some other studies failed to show a pro-angiogenic effect in the heart of AT1 blockade [13]. Therefore, it remains unclear to date whether increased AT1 receptor signaling in-vivo promotes or rather inhibits microvessel growth in the heart post-MI. Likewise, it is unknown if AT1 receptor blockade when started directly after MI confers pro- or anti-angiogenic effects on the heart. To address the effects of the AT1 receptor on myocardial microvessel growth after MI, we inflicted MI in transgenic rats with myocardial AT1 receptor over-expression and in control (non-transgenic) rats, and assessed microvessel density in hearts after MI. Further, we treated some of the transgenic and control rats with a selective AT1 receptor antagonist, in order to create a maximum window of AT1 receptor signaling.

2. Methods

2.1. Animals

We used transgenic rats (TGR[αMHC-hAT1], or TGR), which overexpress the human AT1 receptor by about 10-fold, as described in detail [14]. Transgene-negative Sprague–Dawley (SD) rats from the same breeding colony (Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany) were used as controls. Males, 7 weeks old, weighing around 250 g, were used. Animals were fed ad libitum, with free access to tap water, and housed according to institution rules (with 12:12 h light–dark cycles, and ventilation, temperature (22 °C), and humidity (50%) control present). The experiments described conform with the Guide for the Care and Use of of Laboratory Animals (Published by the US National Institutes of Health, NIH publication No. 85-23, revised 1996), and were approved by an institutional animal studies review committee (University of Groningen, the Netherlands).

2.2. Design of the study

Rats were either sham-operated or infarcted and treated with losartan or placebo, yielding six groups: SD and TGR rats with sham operation (n = 8 and n = 6, respectively), SD with MI (SD MI; n = 11), SD with MI and losartan (SD MI Los; n = 9), TGR with MI (TGR MI; n = 9), and TGR with MI with losartan (TGR MI Los; n = 7). Losartan, a selective AT1 receptor blocker [15] (Merck-Sharp-Dohme, Haarlem, Netherlands) was dissolved in drinking water (400 mg/l). Treatment started 24 h prior to surgery. We aimed to administer a daily dose of 40 mg per kg per day, according to previous studies [16,17]. Drinking bottles were weighed daily and from this the losartan intake was calculated (range: 35–42 mg/kg/day).

2.3. Myocardial infarction model

This model has been described previously [18]. Rats were anaesthetized with 2–4% fluothane in a gas mixture of N2O/O2 (2/1). A left-sided thoracotomy was performed and MI was created by ligating the left coronary artery. In sham-operated rats, the same operation was performed, without ligating the suture.

2.4. Determination of left ventricular (LV) function

After 3 weeks rats were anaesthetized as described above. At this time point, AT1 receptor density was anticipated to be still increased at least 2-fold [14]. The right carotid artery was cannulated with a pressure transducer catheter (Micro-Tip 3French, Millar Instruments, Germany) connected to a 486 PC equipped with an analog-to-digital converter and appropriate software (Millar Instruments, Germany). The catheter was advanced into the aorta and the LV. The zero-pressure baseline was obtained by placing the pressure sensor in 38°C saline before measurements. After a 3-min period of stabilization, maximal LV pressure (LVP), LV end-diastolic pressure (LVEDP), and heart rate were recorded. Hereafter, the catheter was withdrawn to measure systolic blood pressure in the aortic root. As indices of global contractility and relaxation, we determined the maximal rates of increase and decrease in LVP (systolic dP/dt and diastolic dP/dt), that were corrected for peak systolic LVP.

2.5. Plasma renin and N-terminal ANP

Both assays have previously been described [19]. Arterial blood was anti-coagulated with EDTA, and plasma was stored at −80°C until assayed. Active plasma renin concentration (APRC) was measured by determining the amount of angiotensin I generated from angiotensinogen.
with radioimmunoassay. Plasma N-terminal atrial natriuretic peptide (N-ANP) was measured by a commercially available radioimmunoassay (Biotop, Oulu, Finland).

2.6. Tissue procurement

Hearts were excised and arrested in diastole in ice-cold KCl (2 M). The LV was dissected free and weighed. From the LV, a midpapillary slice was prepared for immunohistochemistry. The infarcted parts were carefully dissected from the spared myocardium, and these parts were separately snap-frozen in liquid nitrogen. The LV slice was fixed in 2% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 3 h and maintained overnight in 6% sucrose in PBS, rinsed with acetone, and embedded in Technovit 8100 (Heraeus Kulzer, Wehrheim, Germany). Slides were stained with hematoxylin–eosin for histological analysis. Endocardial and epicardial circumferences of the whole left ventricle and of the scar tissue were determined by computerized surface measurement (Quantimet 520, Cambridge Instruments, Cambridge, UK). Infarct sizes were calculated by dividing the sum of scar lengths by the sum of the total circumference, as described before [18]. To visualize microvessels we employed the well-characterized Rat Endothelial Cell Antigens antibody (RECA-1; Abcam Ltd., Cambridge, UK; dilution: 1:10), that recognizes an antigen appearing on the cell-surface of rat endothelium [20].

2.7. Morphometry

Microvessel density (microvessels/mm²) was measured in the spared myocardium (opposing the infarction, usually ventricular septum or posterior wall). From each rat, seven to ten microscopic high power fields (×400; total area ranging from 257,802 to 368,288 μm²) with transversely sectioned myocytes were digitally recorded with appropriate software (Image Analysis, Leica, Germany). Microvessels were counted blinded by two investigators (RADB and SP). The microcirculation was defined as vessels beyond the third order arteriolas, with a diameter of 150 μm or less, supplying tissue between arterioles and venules [21]. To correct for differences in LV hypertrophy, we divided microvessel density by LV weights (corrected for body weight). Values of sham-operated SD rats are presented as 100%, scores of other groups are given as a percentage of this score.

2.8. Protein isolation and western blotting

We have described this methodology previously [22]. In short, whole-cell protein extracts were obtained from tissue specimens after homogenization (using rotor/stator device) in cold RIPA buffer. Protein concentrations were determined by Bradford reagent (Sigma Co.). Western blotting was performed by loading 20 μg of extract per lane onto sodium dodecyl sulphate (SDS) polyacrylamide gels (10%). Gels were separated by electrophoresis, and electrophoblotted onto a nitrocellulose membrane (Bio-Rad, Netherlands). After blocking, membranes were incubated with primary antibodies: polyclonal anti-goat VEGF antibody (Santa Cruz Biotechnology, Netherlands; 1:200) and monoclonal anti-rabbit glyceraldehyde phosphate dehydrogenase (GAPDH, Affinity Bioreagents, Golden, CO, USA; 1:5000). Hereafter, peroxidase-conjugated secondary antibody anti-goat and anti-rabbit IgG (Santa Cruz; 1:5000) were applied, and detected with an enhanced chemiluminescence detection system (ECL-detection reagent, Amersham, Netherlands). Antigen–antibody complexes were visualized on film (Kodak, Netherlands), and quantified by densitometry. Data are given in arbitrary units, after normalization for GAPDH.

2.9. Angiotensin II receptor binding and quantitative receptor autoradiography

Left ventricular apical sections (16 μm) were cut in a cryostat at −20°C, thaw-mounted on gelatin-coated glass slides, and dried overnight in a desiccator at 4°C and stored at −80°C. Receptor binding was performed as described earlier [23]. Sections were preincubated for 15 min at 22°C in a 10-mM sodium phosphate buffer, pH 7.4, containing 0.005% bacitracin, 5 mM NaEDTA, 120 mM KCl (2 M). The LV was dissected free and weighed. From the spared myocardium, and these parts were applied, and detected with an enhanced chemiluminescence detection system (ECL-detection reagent, Amersham, Netherlands). Antigen–antibody complexes were visualized on film (Kodak, Netherlands), and quantified by densitometry. Data are given in arbitrary units, after normalization for GAPDH.

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were transformed to corresponding values of fmol/mg protein [24]. At least two sections were analyzed, and results were averaged per animal.

2.10. Statistical analysis

Data are presented as means ± S.E.M. To compare multiple group differences, statistical significance was determined with two-way ANOVA. When a statistically significant difference was detected, a protected t-test was performed to determine differences between groups. Correlation analysis was performed with Pearson’s correlation tests. P-values of <0.05 were considered statistically significant.

3. Results

3.1. Mortality, body weights, LV weights, and infarct sizes (Table 1)

Mortality occurred only in the first 48 h after MI (SD: 44%; TGR: 68%; P<0.05). AT₁ receptor blockade reduced mortality to 31 and 30%, respectively. No mortality was observed in sham-operated animals. Body weight was comparable between groups. In sham-operated rats, LV weight (corrected for body weight) was lower in TGR than in SD (P<0.05). LV hypertrophy was apparent in both SD MI and TGR MI, and was prevented by AT₁ receptor blockade in TGR MI. Infarct sizes were comparable between groups.

3.2. LV function (Table 2)

SD and TGR with MI had increased LVEDP and decreased systolic and diastolic dp/dt (P<0.05 vs. sham), and this was prevented by AT₁ receptor blockade. AT₁ receptor blockade decreased blood pressure in TGR with MI (P<0.01 vs. other groups).

3.3. Neurohormones (Table 2)

Plasma N-ANP was increased in SD and TGR with MI (P<0.05 vs. sham-operated animals), but with AT₁ receptor blockade, plasma N-ANP levels did not rise (SD MI Los vs. SD MI: P=0.05, and TGR MI Los vs. TGR MI: P<0.05). APRC was not increased after MI. AT₁ receptor blockade was associated with significant APRC increases in both SD and TGR with MI (P<0.05 vs. untreated SD and TGR with MI).

3.4. Morphometric analyses (Fig. 1)

Microvessels stained with the RECA-antibody were clearly discernable in the myocardium (Fig. 1A). No baseline differences in microvessel densities were found between sham-operated SD and TGR rats (2167±87/mm² vs. 2041±43/mm², P NS). MI reduced microvessel density in both SD MI (1653±37) and TGR (1298±33) with MI (both: P<0.01 vs. sham-operated). In the TGR with MI, microvessel density was more severely decreased than in the SD MI (P<0.01). AT₁ receptor blockade

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Table 1: Body weight, LV weight, and infarct sizes

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>LVW-BW⁻¹ (mg·g⁻¹)</th>
<th>Infarct size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD Sham</td>
<td>343±5</td>
<td>2.51±0.05</td>
<td>0±0</td>
</tr>
<tr>
<td>TGR Sham</td>
<td>345±10</td>
<td>2.15±0.03*</td>
<td>0±0</td>
</tr>
<tr>
<td>SD MI</td>
<td>347±6</td>
<td>2.76±0.07*</td>
<td>26±4*</td>
</tr>
<tr>
<td>SD MI Los</td>
<td>344±6</td>
<td>2.81±0.09*</td>
<td>29±5*</td>
</tr>
<tr>
<td>TGR MI</td>
<td>336±11</td>
<td>2.75±0.08*</td>
<td>26±2*</td>
</tr>
<tr>
<td>TGR MI Los</td>
<td>312±10</td>
<td>2.21±0.06*</td>
<td>29±2*</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.; 7–12 rats per group.

BW, body weight; LVW-BW⁻¹, normalized LV weight. *, P<0.05 vs. SD sham; †, P<0.05 vs. TGR sham; ‡, P<0.05 vs. SD MI; §, P<0.05 vs. SD MI Los; ‖, P<0.05 vs. TGR MI.
prevented the decrease in microvessel density (SD MI Los: 2046 ± 195; P NS vs. sham-operated SD). Microvessel density was restored by AT$_1$ receptor blockade in TGR (1742 ± 47; P < 0.01 vs. TGR MI), but was still lower in TGR MI Los when compared to TGR sham (P < 0.05). This is plotted in Fig. 1B. When corrected for LV weights (corrected for body weight), outcomes for microvessel density were similar. After MI, microvessel density decreased in SD and TGR rats, however more profoundly in TGR. Losartan restored this to normal (SD) or near-normal (TGR) values (Fig. 1C).

3.5. VEGF protein levels (Fig. 2)

VEGF protein level was strongly increased in the infarcted parts of the hearts of both SD MI and TGR MI (SD MI: 12.3-fold, TGR MI: 8.1-fold; both P < 0.01 vs. sham-operated controls). Similarly, VEGF protein level was increased in the spared myocardium of both the SD MI (1.43 ± 0.18) and the TGR MI (0.66 ± 0.06) compared with SD sham (0.21 ± 0.03) TGR sham (0.19 ± 0.02; both P < 0.05). However, VEGF protein level was significantly higher in SD MI than TGR MI (P < 0.05). With AT$_1$ receptor blockade, VEGF protein levels decreased (SD MI Los: 1.13 ± 0.08; and TGR MI Los: 0.20 ± 0.08).

3.6. AT$_1$ and AT$_2$ receptor densities (Fig. 3)

AT$_1$ receptor density was increased in TGR sham (0.55 ± 0.07, fmol/mg protein) when compared to SD sham (0.22 ± 0.14, P < 0.05). AT$_1$ receptor density remained elevated in TGR with MI (0.49 ± 0.09) when comparing SD with MI (0.18 ± 0.05; P < 0.05). AT$_1$ receptor blockade diminished AT$_1$ receptor densities, which however remained higher in TGR MI (0.34 ± 0.10) compared with SD MI (0.06 ± 0.03, P < 0.05). AT$_2$ receptor densities generally tended to be lower in TGR rats (0.07 ± 0.03, fmol/mg protein) than in SD sham (0.32 ± 0.17, P = 0.07). After MI, AT$_2$ receptor density was similar (SD MI: 0.20 ± 0.04 vs. TGR MI: 0.09 ± 0.04). AT$_1$ receptor blockade did not significantly affect AT$_2$ receptor density, although there seemed to be a differential effect, with a decrease in SD (0.07 ± 0.03) and an increase in TGR (0.27 ± 0.13).
myocardial microvessel response remains unclear. We report that an increased AT₁ receptor expression is related to a decreased microvessel density after MI. Conversely, AT₁ receptor blockade prevents the decrease in microvessel density. Since the AT₁ receptor is upregulated after MI, AT₁ receptor blockade initiated early after MI may have a beneficial effect on myocardial function by preserving the microvasculature.

The TGR model of cardiac specific AT₁ receptor overexpression was described previously [14]. AT₁ receptor densities are increased 10-fold at 7 weeks, and remain increased up to 2-fold at the age of 12 weeks [14]. Importantly, under basal conditions the TGR does not have an altered phenotype. The TGR model responds, however, more readily to RAS activation upon loading of the heart [14]. Now, we report a larger decrease in microvessel density after MI. The AT₁ receptor overexpression did not adversely affect hemodynamics after MI, in accordance with preliminary data from Willenbrock et al. [25]. TGR rats had decreased LV weights at baseline. The reason for this is not clear, but it is in line with a study from Krege et al. [26], who observed a decreased heart weight (in mice) in the presence of extra copies of the angiotensin-converting enzyme (ACE) gene. Possibly, the reciprocal decrease of heart weight in response to overexpression of RAS components, should be regarded as a homeostatic compensation, that eliminates the effect of the transgene. More TGR died following MI, and we recently showed that this is due to enhanced ventricular arrhythmias [27]. AT₁ receptor signaling further seems to depend on local RAS activation, since plasma renin did not increase after MI (Table 2), as described earlier by Pinto et al. [18].

There is an ongoing dispute in the literature on the effect of the renin–angiotensin system on microvessel growth. The impact of expression levels of AT₁ receptors, however, is unknown. From our results, we conclude that increased levels of AT₁ receptors decrease myocardial microvessel density. Furthermore, the results confirm findings of previous studies with AT₁ receptor blockade after MI. Schieffer et al. [11] found that both ACE-inhibition and AT₁ receptor blockade (partially) restored myocardial capillary density. Sladek et al. [12] also reported that AT₁ receptor blockade restored capillary density post-MI. This conclusion is in line with the finding that infusion of angiotensin II decreases myocardial microvessels in-vivo [28]. Since microvessel density could be restored with AT₁ receptor blockade, our findings support these former studies of early initiation of AT₁ receptor blockade after a myocardial ischemic event (such as MI or acute coronary syndromes) in order to promote angiogenesis.

These findings are in sharp contrast with in-vitro studies, that provide evidence for angiotensin II-induced angiogenesis, specifically by upregulation of VEGF [4–6]. This signal is conferred by the AT₁ receptor, since selective AT₁ blockade abolishes these effects [5,4,6].
L V function and neurohormones

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>dP/dt systolic (kPa/s)</th>
<th>dP/dt diastolic (kPa/s)</th>
<th>LVEDP (mmHg)</th>
<th>SBP (mmHg)</th>
<th>N-ANP (nmol/l)</th>
<th>Renin (ng AngI/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD Sham</td>
<td>359±11</td>
<td>−85.3±1.3</td>
<td>8.3±1.3</td>
<td>118±4</td>
<td>2.1±0.2</td>
<td>24±5</td>
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</tr>
<tr>
<td>TGR sham</td>
<td>332±7</td>
<td>84.3±1.8</td>
<td>−86.2±2.6</td>
<td>9.4±0.6</td>
<td>115±3</td>
<td>1.8±0.1</td>
<td>24±5</td>
</tr>
<tr>
<td>SD MI</td>
<td>372±23</td>
<td>−72.3±4.9*</td>
<td>20.3±2.7*</td>
<td>103±4*</td>
<td>3.2±0.2*</td>
<td>29±4</td>
<td></td>
</tr>
<tr>
<td>SD MI Los</td>
<td>373±13</td>
<td>−82.3±3.5†</td>
<td>13.0±2.6†</td>
<td>107±5</td>
<td>2.5±0.4</td>
<td>108±25*</td>
<td></td>
</tr>
<tr>
<td>TGR MI</td>
<td>369±6</td>
<td>−65.2±3.8*</td>
<td>15.2±1.4*</td>
<td>113±4†</td>
<td>3.0±0.3*</td>
<td>26±5†</td>
<td></td>
</tr>
<tr>
<td>TGR MI Los</td>
<td>322±12</td>
<td>−74.2±3.7†</td>
<td>12.2±1.9†</td>
<td>76±2*</td>
<td>2.0±0.2†</td>
<td>543±105*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±S.E.M.; 7–12 rats per group. HR (bpm), heart rate (beats per min). dP/dt is corrected for peak systolic LVP. SBP, systolic blood pressure.

Remarkably, they also contrast with findings in non-cardiac models, where the AT1 receptor seems to confer pro-angiogenic effects, and AT1 receptor blockade hampers angiogenesis. In cremaster muscle, AT1 receptor blockade inhibited angiotensin II-induced microvessel growth [8]. Also, in the well-described ischemic hindlimb model, AT1 receptor blockade impairs reparative angiogenesis [9,10]. This is an important issue, as Sasaki et al. [10] speculate that the use of AT1 receptor blockade early after ischemia may suppress angiogenesis, resulting in an exacerbation of ischemia. They conclude from their findings (in a model of ischemic hindlimb) that AT1 receptor blockers after ischemic events may be contra-indicated. However, our findings argue rather in favor of an early start of AT1 receptor blockade after a myocardial ischemic event. One obvious hypothesis to explain the discrepant effect of the AT1 receptor in differing tissues is that the role of the AT1 receptor in angiogenesis differs between different organs (muscles) due to differences in AT1 receptor and AT2 receptor related signaling. Other factors, like choice of model, timing and ischemic trigger may also play a role.

The role of the endothelium may also be important in this aspect. There are extensive data on the role of the endothelium in angiotensin II-related effects on angiogenesis. Chua et al. [6] showed that angiotensin II induced VEGF mRNA production in rat heart endothelial cells. Similarly, Fujiyama et al. [4] showed that in cardiac microvascular endothelial cells the binding of angiotensin II to the AT1 receptor induces angiopoietin-2 and VEGF release. AT2 receptor stimulation inhibited AT1-mediated angiopoietin-2 expression. Again, these in-vitro data suggest a pro-angiogenic effect of angiotensin II via binding to its AT1 receptor. There are several other components of the angiogenic cascade, expressed by endothelium, that are also regulated by angiotensin II, such as endoglin [29], TGF-β [30], and basic fibroblast growth factor [31]. Since we employed a model of myocytic AT1 receptor overexpression, it remains speculative what the contribution of the endothelium was in our experimental setting.

ACE-inhibition differs fundamentally from AT1 receptor blockade by its capacity to activate kinins, and the pro-angiogenic capacity of ACE-inhibition have been linked to kinin production [32]. The use of ACE-inhibition to enhance microvessel growth has also rendered equivocal results. Post-MI, Nelissen-Vrancken et al. [33] showed that captopril inhibits endothelial DNA synthesis in hearts post-MI, which suggests an anti-angiogenic effect of ACE-inhibition. Kalkman et al. [34] contested these findings and showed a rather neutral effect, whereas Schieffer et al. even showed an increase of capillary density [11]. On the other hand, AT1 receptor blockade with losartan may also exert beneficial effects that go beyond AT1 receptor antagonism, by means of its active metabolites, as repetitively reported [35,36]. This study focussed on the importance of AT1 receptor signaling, but clearly some mechanisms that underlie the discrepancies as seen with ACE-inhibitors, are similar to the mechanisms that may clarify the discrepant effects that AT1 receptor blockade have yielded.

The impact of the level of AT1 receptor expression has not been studied in this respect before. It is well known that the expression of AT1 receptors is upregulated following MI [2,1]. In our study, we did not observe an increased AT1 receptor expression following MI. This may be explained by the fact that we assessed the AT1 receptor density in the non-infarcted area, whereas it is thought that AT1 receptor density is specifically upregulated in the scar tissue. We postulate from our data that high levels of AT1 receptors in the TGR adversely affect microvessel density in the heart post-MI. The increased AT1 receptor signaling exerts detrimental effects, like vasoconstriction (oxygen depletion), toxicity, and apoptosis [37]. These effects may account for the observed decline in microvessel density. Furthermore, in losartan-treated rats, the activation of the renin–angiotensin system (reflected by dramatic increase in APRC, Table 2) may have caused signaling of the (unblocked) AT2 receptor. Recently it was shown that AT2 receptor signaling causes vasodilation, NO release and Bradykinin production [38], and this may have caused microvessel dilatation, stretch on the vessel wall and increased flow, important inducers of angiogenesis [3]. This may explain (at least in part) the vasotrophic response of angiotensin II.
We found that LVEDP negatively correlated with microvessel density, in accordance with Sladek et al. [12]. In vitro, it was shown that myocardial stretch induces VEGF production [39]. Kim et al. [40] showed that VEGF expression is also upregulated after short-term in vivo stretch and ischemia. In this in vivo study, MI clearly created hemodynamic abnormalities that are related to wall stress (increased LVEDP and plasma N-ANP), however, this apparently did not translate into angiogenesis. From this we conclude that additional factors may be operative during in vivo LV remodeling.

VEGF protein levels increased after MI, but did not prevent microvessel density from declining. AT1 receptor blockade restored microvessel density, whereas VEGF protein levels decreased (Fig. 3). Therefore, in this model of LV remodeling, microvessel density was related more to hemodynamics than VEGF protein levels. This suggests that in vivo VEGF protein level may be under the control of myocardial stretch (as it is in vitro), and less governed by the AT1 receptor.

We conclude that myocardial AT1 receptor overexpression decreases microvessel density in the spared myocardium post-MI and this is prevented by AT1 receptor blockade. Hemodynamic status, reflected by LVEDP and plasma N-ANP were related to microvessel density rather than to VEGF protein levels. This study clearly underlines the differences between in vitro findings and in vivo studies, and suggests that with respect to the role of the AT1 receptor in angiogenesis, there might be even differences in vivo between tissues. We describe an early beneficial effect of AT1 receptor blockade on microvessel density. AT1 receptor levels rise quickly after MI [2], whereas ACE-activity rises over time [18]. Therefore, AT1 receptors may represent an important target for early intervention to preserve microvessel density. These findings support the early start of AT1 receptor blockade after MI. In this view, we await the results of large-scale clinical trials, that will evaluate the use of AT1 receptor blockers immediately after MI [41,42].

Acknowledgements

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References

[8] Sladek T, Sladkova J, Kolar F et al. The effect of AT1 receptor signal transduction on the repair of rat myocardial infarction decreases microvessel density in the spared myocardium. J Mol Cell Cardiol 1996;28:429–440. MI. In this view, we await the results of large-scale clinical trials, that will evaluate the use of AT1 receptor blockers immediately after MI [41,42].


