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RESEARCH PAPER

Hypercholesterolaemia exacerbates ventricular remodelling after myocardial infarction in the rat: role of angiotensin II type 1 receptors

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Background and purpose: Diet-induced hypercholesterolaemia exacerbates post-myocardial infarction (MI) ventricular remodelling and heart failure, but the mechanism of this phenomenon remains unknown. This study examined whether worsening of post-MI ventricular remodelling induced by dietary hypercholesterolaemia was related to upregulation of angiotensin II type 1 (AT_1) receptor in the rat heart.
Experimental approach: MI was induced surgically in rats fed normal or high cholesterol diet. Both groups of rats were then assigned to control, atorvastatin, losartan or atorvastatin + losartan-treated subgroups and followed for 8 weeks. Left ventricular (LV) function was assessed with echocardiography. In isolated hearts, LV pressures were measured with a latex balloon and a tip catheter. AT_1-receptor density was assessed in LV membranes with radioligand-binding assays.
Key results: High cholesterol diet exacerbated LV dilation and dysfunction in post-MI hearts. Atorvastatin or losartan prevented these hypercholesterolaemia-induced effects, whereas their combination was not more effective than each drug alone. AT_1 receptors were upregulated 8 weeks after MI, this was further increased by hypercholesterolaemia and restored to baseline levels by atorvastatin.
Conclusions and implications: Hypercholesterolaemia exacerbated LV remodelling and dysfunction in post-MI rat hearts and upregulated cardiac AT_1 receptors. All these effects were effectively prevented by atorvastatin. Thus, the pleiotropic statin effects may include interference with the renin-angiotensin system through downregulation of AT_1 receptors.
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Keywords: myocardial infarction; hypercholesterolaemia; statin; sartan; heart failure; remodelling; rat

Abbreviations: AT_1 receptor, angiotensin II type 1 receptor; H, hypercholesterolemic; HDL, high density lipoprotein; LDL, low density lipoprotein; LV, left ventricle; LVDd, LV end-diastolic diameter; LVSd, LV end-systolic diameter; MI, myocardial infarction; N, normocholesterolemic; PMSF, phenylmethylsulphonyl fluoride; RAS, renin-angiotensin system; WMI, wall motion index

Introduction

Left ventricular (LV) remodelling after acute myocardial infarction (MI) is a process of progressive changes in LV chamber size, shape, composition and resulting function (Pfeffer and Braunwald, 1990). Highly deleterious LV dilation, significantly related to poor outcome (White *et al.*, 1987), is its most characteristic feature. The intensity of ventricular remodelling depends mainly on ventricular wall stress and neurohumoral factors, such as the renin-angiotensin system (RAS) and the sympathetic nervous system (Sutton and Sharpe, 2000).

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We have recently shown that diet-induced hypercholesterolaemia exacerbates post-MI remodelling in the rat heart (Maczewski and Maczewska, 2006). This phenomenon was unrelated to the effects of high cholesterol feeding on infarct size, which supported data obtained in humans after MI, linking hypercholesterolaemia with more aggressive remodelling after MI (Wang *et al.*, 1998). However, the mechanism of this effect remains unknown.

Animal and human studies indicate that angiotensin II type 1 (AT_1) receptors play a major role in the process of post-MI remodelling: AT_1-receptor knockout mice displayed less ventricular remodelling and improved survival after MI (Harada *et al.*, 1999), whereas cardiac-specific overexpression of AT_1 receptors was associated with impaired post-MI angiogenesis and exacerbated LV remodelling (de Boer *et al.*, 2003). Human studies indicate that AT_1-receptor

antagonists and angiotensin converting enzyme inhibitors effectively prevent post-MI remodelling and heart failure (Solomon *et al*., 2005). Furthermore, we have recently shown that AT_1-receptor density on blood platelets is a good predictor of LV remodelling post-MI in humans (Maczewski *et al*., 2006).

There is growing evidence that AT_1 receptors are heterotropically regulated and hypercholesterolaemia has been shown to upregulate AT_1 receptors in aortic smooth muscle cells in the rat (Nickenig *et al*., 1997) and rabbit (Yang *et al*., 1998) and human blood platelets (Nickenig *et al*., 1999). It is unknown whether hypercholesterolaemia has any effect on cardiac AT_1 receptors, either at baseline or post-MI.

Thus, we hypothesized that high cholesterol diet would upregulate cardiac AT_1 receptors and exacerbate ventricular remodelling and worsen LV function in the rat model of MI. We further hypothesized that these detrimental events could be prevented by anti- AT_1-receptor interventions, such as losartan, a AT_1-receptor antagonist and atorvastatin, which was shown to downregulate AT_1 receptors independently of its effect on plasma lipids (Wassmann *et al*., 2001).

Methods

Study design and induction of myocardial infarction

The study was conducted in accordance with the Guide for the care and use of laboratory animals (US National Institutes of Health Publication No. 85-23, revised 1985) and was approved by the Ethics Committee of our institution.

The 6-week-old Wistar–Kyoto male rats were randomly assigned to normal chow (normocholesterolemic, N) or high cholesterol chow (hypercholesterolemic, H; $n = 115$ per arm) and were maintained on such diets for 8 weeks before the induction of MI and until the end of the experiment. Normal pelleted rat chow contained: 23.5% protein, 4.0% fat, 3.9% fibre (crude), 49.0% nitrogen-free extract, 6.9% ash (Wytwórnia Pasz Andrzej Morawski, Kcynia, Poland). High cholesterol chow was provided by the same supplier and consisted of normal chow, supplemented with cholesterol (2% by weight). Blood (200–300 μL) was taken from the tail vein to measure plasma lipids. Plasma lipids were determined by the use of commercially available enzyme kits at the time of surgery.

To induce MI, rats were anaesthetized with ketamine HCl and xylazine (100 mg and 5 mg kg^{-1} body weight, i.p.). Left thoracotomy was performed, the heart was externalized through an incision in the fifth intercostal space and a suture (5–0 silk) was placed around the proximal left coronary artery and tightly tied. Twenty-five rats from each arm were subjected to the same protocol except that the snare was not tied, and these rats served as the sham group. The heart was immediately internalized, the chest was closed and pneumothorax was reduced with negative pressure.

Twenty-four hours after MI induction, each of these two arms was further subdivided into four groups based on the results of echocardiographic examination (see Results section): (1) control (2) receiving losartan 3 mg kg^{-1} d^{-1} for 7 days, then 10 mg kg^{-1} d^{-1} for 14 days and then 30 mg kg^{-1} d^{-1} until the end of the experiment; (3) atorvastatin 10 mg kg^{-1} d^{-1}; (4) losartan plus atorvastatin; giving a total of eight experimental groups plus eight corresponding sham-operated groups. Both drugs were added to drinking water. The dose of losartan was based on previous rat studies (Pourdjabbar *et al*., 2005) and adjusted to mimic clinical practice—slow increase of dose to the final level of 30 mg kg^{-1} d^{-1} to avoid excessive mortality due to hypotension early after MI (Pourdjabbar *et al*., 2005). Atorvastatin (10 mg kg^{-1}) was shown to reduce infarct size in the rat (Atar *et al*., 2006). To avoid possible interference of losartan in the AT_1-receptor-binding assay and its immediate haemodynamic effects, losartan was withdrawn 24 h before the final assessment at week 8 in all groups.

Echocardiography

Left ventricular function was evaluated by echocardiography using Aloka SSD-900 (Aloka, Tokyo, Japan) with 10 MHz linear array transducer. Each rat was examined at baseline, 24 h, 7 days and 8 weeks after MI induction. The rats were anaesthetized with ketamine HCl and xylazine (75 mg and 3.5 mg kg^{-1}, i.p.), the chest was shaved and the animal was placed in the supine position. M-mode and two-dimensional studies were performed from a parasternal window.

Left ventricular end-diastolic (LVDd) and end-systolic (LVSd) diameters were determined from the short-axis view at the midpapillary level and LV fractional shortening was calculated. Regional LV wall motion abnormalities were quantitiated according to modified method by Morgan *et al*. (2004), as described previously (Maczewski and Maczewska, 2006) using a 23-segment model. Briefly, contractility of each of 12 wall segments visualized in the midpapillary short-axis view and 11 wall segments visualized in the long-axis view was graded as 1 (normal) or 0 (abnormal: hypokinesis, akinesis or dyskinesis) and the total score was calculated. Normal hearts had wall motion index (WMI) = 23, our previous results revealed that WMI closely correlated with infarct size and WMI = 15 corresponded to an infarct size of about 40% (Maczewski and Maczewska, 2006).

Echocardiographic examination was performed by an echocardiographer without the knowledge of the study group. A total of 60 examinations were performed by two independent echocardiographers. Interobserver variability for LVDd and LVSd was $<8\%$. Difference in WMI between echocardiographers never exceeded two points on the 23-point scale.

Haemodynamic measurements

Eight weeks after induction of MI, rats were anaesthetized with ketamine HCl and xylazine (100 mg and 5 mg kg^{-1}, i.p.) and 2.0-Fr microtipped pressure transducer catheter (SPC-320, Millar Instruments Inc. Houston, TX, USA) was introduced into the LV through the right carotid artery. Heart rate, LV systolic pressure, LV end-diastolic pressure, peak rate of rise in LVP ($dP\,dt^{-1}$) and blood pressure were recorded using Hugo Sachs isolated heart software.

Then, after heparinization (1000 IU kg^{-1}, i.p.) and pentobarbital anaesthesia (50 mg kg^{-1}, i.p.), the heart was excised and Langendorff perfused with Krebs-Henseleit buffer. A saline-filled latex balloon, connected to a pressure transducer for the measurement of LV pressure was introduced to LV through the left atrium and was gradually inflated, enabling the evaluation of LV diastolic and systolic pressure–volume relationships.

Determination of infarct size and tissue processing for AT_1-receptor determination

After haemodynamic measurements, the cardiac left ventricle was divided into fibrotic scar tissue and healthy myocardial tissue. Infarct size was determined by planimetric measurement and calculated as fibrotic area divided by the area of fibrotic and healthy tissue. The healthy tissue was immediately frozen in liquid nitrogen to undergo AT_1-receptor density determination.

Determination of AT_1-receptor density

The density of AT_1 receptors in cardiac membranes was quantified by radioligand-binding assays, as described by Lopez *et al*. (1994). Briefly, the left ventricle was homogenized (1:10) in ice-cold solution containing 0.25 M sucrose, 0.03 M histidine, 1 mM EDTA and 0.1 mM phenylmethylsulphonyl fluoride. The homogenate was centrifuged at 8000 *g* for 20 min and the pellet was discarded. The supernatant was centrifuged at 35 000 *g* for 20 min and the resulting pellet was saved and resuspended in 50 mM Tris.HCl (pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA and 0.1 M phenylmethylsulphonyl fluoride to give the myocardial membrane fraction with a protein concentration of 1–2 mg mL^{-1}. The pellet was stored at −80 °C until use. To assay AT_1-receptor density, the membranes were incubated with increasing concentrations of ^{125}I-angiotensin II (0.2–2 nmol L^{-1}, Amersham, UK). The incubation volume was 200 μL. Non-specific binding was assessed in the presence of 1 μmol L^{-1} losartan (Merck, Whitehouse Station, NJ, USA) and was subtracted from the total binding, giving AT_1-receptor-specific binding. Incubation was performed at room temperature for 120 min. Reactions were terminated by the addition of ice-cold buffer containing 0.5% bovine serum albumin and 10 mmol L^{-1} Tris-HCl and subsequent vacuum filtering. The filters were cut out and bound radioactivity was counted with a Beckman γ-counter.

Statistical analysis

Values shown are means ± s.e.mean. Statistical significance was determined using two-way ANOVA. If ANOVA indicated a significant difference, a two-tailed Student's *t*-test for paired observation with Bonferroni's correction was used to test the differences between individual groups. Categorical variables were compared with the χ^2 test. Values of $P<0.05$ were assumed to be significant. All statistical procedures were performed using the Statistica statistical software package (version 6.0, StatSoft Polska, Poland).

Materials

Atorvastatin was from Lek Pharmaceuticals (Ljubljana, Slovenia) and losartan from Merck (Whitehouse Station, NJ, USA). Other reagents, including cholesterol, were from Sigma-Aldrich, Poland.

Results

Baseline data and outcome of surgical induction of myocardial infarction

Baseline plasma lipids were higher in H (hypercholesterolemic diet) vs N (normocholesterolemic diet) rats, whereas body weights did not differ (Table 1). Mortality within the first 24 h after MI tended to be higher in H vs N rats (Table 1). Echocardiography at 24 h post-MI revealed that the number of rats with a large MI was approximately equal in the two diet groups (N or H; Table 1).

To obtain homogenous subgroups with respect to infarct size at 24 h, rats with a large MI from each group were further subdivided into control, atorvastatin-, losartan- and atorvastatin and losartan-treated subgroups and matched for infarct size, so that infarct sizes were almost identical across all eight experimental groups (each group $n=13$, mean WMI 13.3–13.4). Figure 1 shows that at 24 h LVDd, LVSd and fractional shortening were also not different across all experimental groups.

Echocardiography

During further follow-up, a total of 23 rats died in the study. All these rats had large MI, massive pleural effusions and ascites, suggesting that they died of heart failure.

Figure 1 shows that H animals demonstrated more extensive LV dilation and more rapid decline of fractional shortening than N rats over 8 weeks of echocardiographic follow-up after MI. LV remodelling was partially prevented by atorvastatin and losartan in both N and H rats. Combination of losartan with atorvastatin had no additional

Table 1 Body weights, plasma lipids and outcome of myocardial infarction in normo- and hypercholesterolemic rats

	Normocholesterolemic n = *90*	*Hypercholesterolemic* n = *90*
Body weight (g)	285 ± 4	292 ± 6
Plasma total cholesterol (mg L^{-1})	480 ± 50	1400 ± 100*
Plasma LDL cholesterol (mg L^{-1})	120 ± 30	780 ± 70*
Plasma HDL cholesterol (mg L^{-1})	280 ± 20	510 ± 50*
Plasma triglyceride (mg L^{-1})	400 ± 40	680 ± 80*
Mortality within 24 h after MI (*n*)	19	28
Large MI (WMI ⩽15) (*n*)	58	52

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction; WMI, wall motion index.
Values shown in the Table are means ± s.e.mean.*$P<0.05$ vs normocholesterolemic.

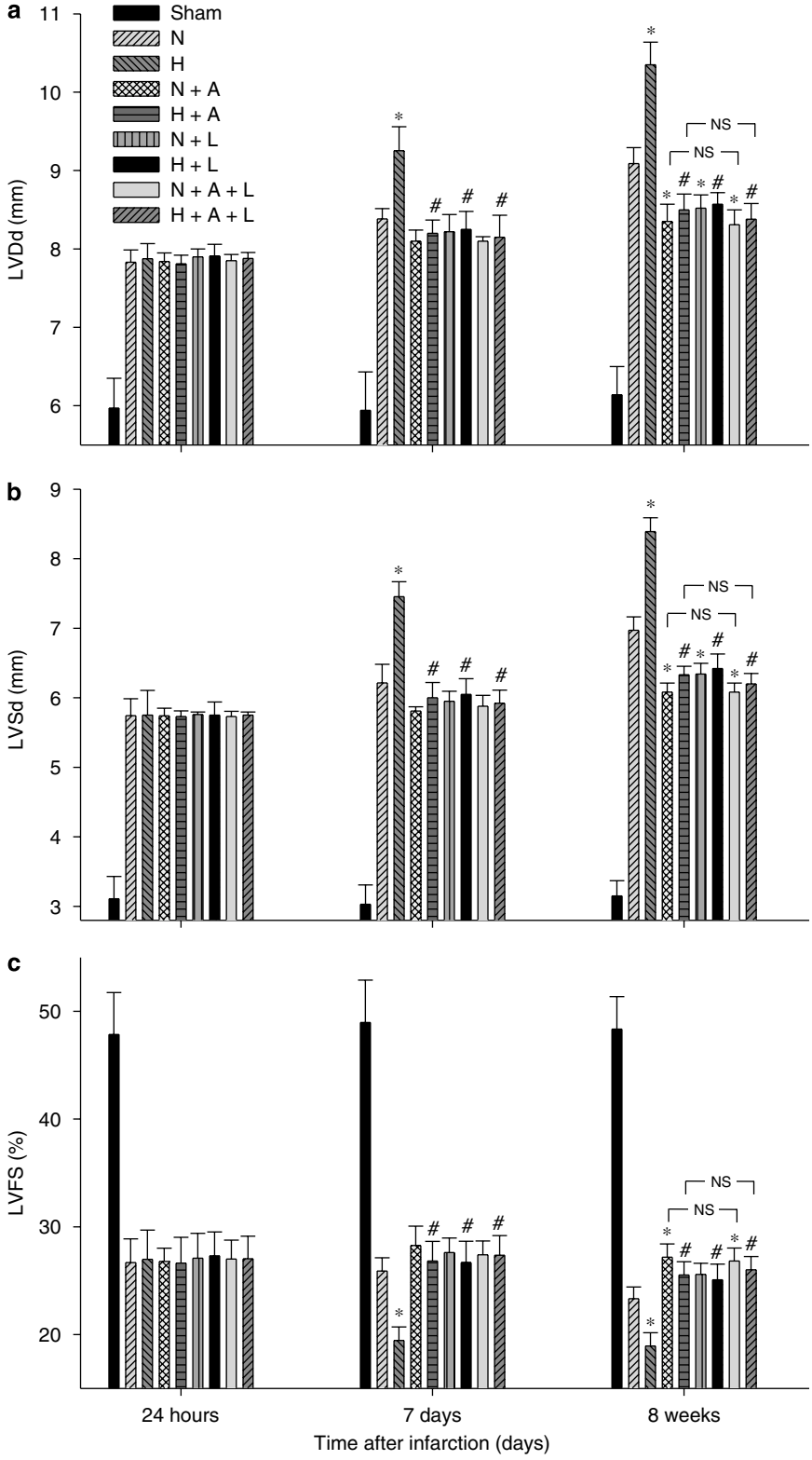

Figure 1 Echocardiographic indices of left ventricular remodelling after myocardial infarction in normo- and hypercholesterolemic and sham-operated rats. (**a**) Left ventricular diastolic diameter (LVDd); (**b**) left ventricular systolic diameter (LVSd); (**c**) left ventricular fractional shortening (LVFS). Sham, sham-operated animals; N, normocholesterolemic; H, hypercholesterolemic; A, atorvastatin treated; L, losartan treated; A + L, atorvastatin and losartan treated. For the clarity of presentation all sham groups were pooled ($n = 14$). All myocardial infarction groups were significantly different from sham animals (not shown). For the number of animals per group, see Table 2. $^{*}P < 0.05$ vs N. $^{\#}P < 0.05$ vs H.

effect over that achieved with either drug alone. In the H group of rats, both atorvastatin and losartan prevented hypercholesterolaemia-induced aggravation of post-MI LV remodelling to a value comparable with that achieved in N rats (Figure 1).

Haemodynamics in vitro *and* in vivo

Myocardial infarction resulted in a rightward shift of the LV diastolic pressure–volume curve in N rats, indicating an increase of LV volume as compared to sham-operated animals (Figure 2a). H rats demonstrated clearly a further rightward shift of the pressure–volume curve (Figure 2b). Atorvastatin, losartan and both drugs given in combination partially prevented this rightward displacement both in N and H animals to a final value that was not different between these six groups.

There were no differences between respective sham-operated animals with respect to morphological and haemodynamic parameters (Table 2). Haemodynamic examinations conducted 8 weeks after the infarction revealed lower LV-developed pressure and higher LV end-diastolic pressure in H animals as compared to N rats. Atorvastatin, losartan and combination of both drugs restored haemodynamic parameters to comparable values in both H and N rats (Table 2).

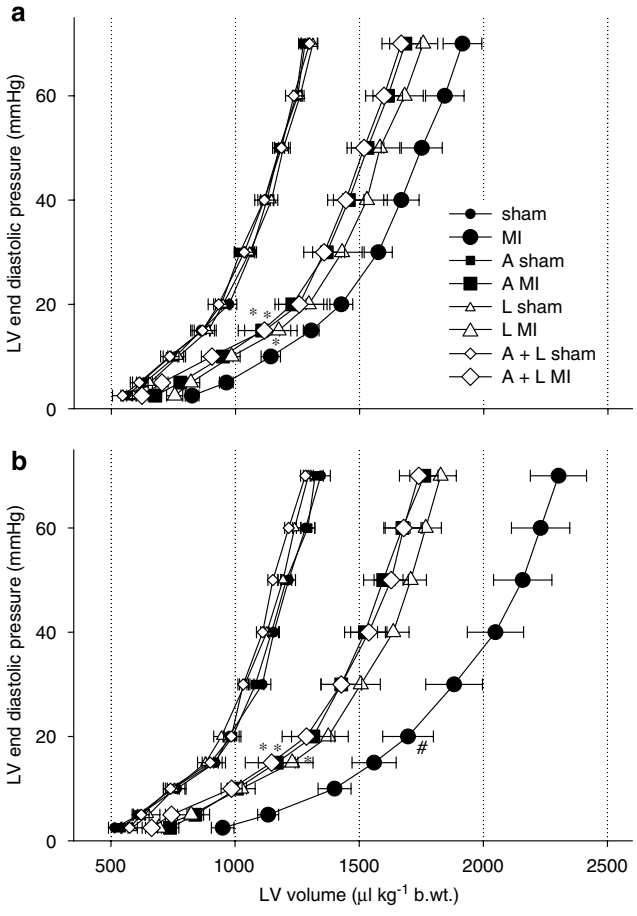

Figure 2 Left ventricular end diastolic pressure–volume relationships in normo- (**a**) and hypercholesterolemic (**b**) rats 8 weeks after myocardial infarction and respective sham operation. LV, left ventricular; Sham, sham-operated animals; MI, animals with myocardial infarction; A sham, atorvastatin-treated shams; A MI, atorvastatin-treated MI; L sham, losartan-treated shams; L MI, losartan-treated MI; A + L sham, atorvastatin + losartan-treated shams; A + L MI, atorvastatin + losartan-treated MI; $^{*}P<0.05$ vs MI at 20 mm Hg. $^{\#}P<0.05$ for H group vs respective N group. All MI groups were significantly different from respective sham groups (not shown).

Plasma lipids and angiotensin AT_1-receptor density

Neither MI nor any of the drugs had any effect on plasma lipids (total cholesterol shown in Table 2) 8 weeks after the infarction; in particular, atorvastatin did not lower plasma cholesterol. Sham-operated animals on the hypercholesterolemic diet (H) had AT_1-receptor density (measured as maximal binding of radioligand to AT_1 receptors) on LV membranes increased almost fourfold as compared to N animals (Figure 3). Losartan had no effect on AT_1-receptor density either in N or H sham-operated animals, whereas atorvastatin, given alone or in combination with losartan, did not change AT_1-receptor density in N animals and completely prevented AT_1-receptor upregulation associated with hypercholesterolaemia.

Eight weeks after MI, AT_1-receptor density on LV membranes in N rats increased almost twofold, which was partially prevented by losartan and completely prevented by atorvastatin, alone or in combination with losartan. AT_1-receptor density in H animals after MI was increased fivefold compared to N sham-operated animals. This effect was reduced by 50% by losartan and was prevented completely by atorvastatin, alone or in combination with losartan.

The apparent affinity of AT_1 receptors was not different between all 16 experimental groups ($P>0.05$ by one-way ANOVA) and ranged from 0.28 to 0.48 nM.

Discussion

In this study, we show that (1) diet-induced hypercholesterolaemia exacerbated LV remodelling and heart failure in the rat model of non-reperfused myocardial infarction; (2) both atorvastatin and losartan prevented exacerbation of remodelling in hypercholesterolemic rats to a level comparable to that achieved in normocholesterolemic rats; combination of these two drugs offered no additional benefit over that achieved with either drug alone; (3) MI was associated with upregulation of AT_1 receptors in LV cardiac membranes, which was further enhanced by hypercholesterolaemia and prevented by atorvastatin, whereas losartan prevented only MI-induced AT_1-receptor upregulation without affecting hypercholesterolaemia-induced changes. Taken together, these results suggested that the detrimental effects of hypercholesterolaemia on post-MI remodelling are at least partially, related to AT_1-receptor upregulation.

Hypercholesterolaemia and post-MI remodelling: effect of atorvastatin and losartan

In our study, most of the difference between the N (normocholesterolemic diet) and H (hypercholesterolemic

Table 2 Hemodynamic and morphological characteristics of sham-operated and MI rats

	N sham (n = *6*)	*N MI* (n = *11*)	*H sham* (n = *6*)	*H MI* (n = *9*)	*N + A sham* (n = *6*)	*N + A MI* (n = *11*)	*H + A sham* (n = *6*)	*H + A MI* (n = *10*)
Body weight, g	323 ± 10	325 ± 8	330 ± 5	335 ± 8	325 ± 10	316 ± 4	327 ± 11	337 ± 8
Total cholesterol, mg L^{-1}	470 ± 80	460 ± 40	1400 ± 150	1440 ± 100	490 ± 50	480 ± 50	1380 ± 160	1350 ± 100
LV weight, mg g^{-1}	2.42 ± 0.06	3.38 ± 0.07*	2.22 ± 0.07	3.41 ± 0.18*	2.29 ± 0.08	3.06 ± 0.20*	2.40 ± 0.06	3.07 ± 0.15*
Lung weight, mg g^{-1}	3.62 ± 0.22	6.11 ± 0.35*	3.62 ± 0.22	6.92 ± 0.36*	3.52 ± 0.30	4.80 ± 0.37*#	3.45 ± 0.32	4.95 ± 0.45*#
Heart rate, beats min^{-1}	232 ± 9	233 ± 9	229 ± 6	230 ± 10	225 ± 8	221 ± 5	220 ± 5	223 ± 7
Infarct size (%)	—	43.2 ± 3.3*	—	42.1 ± 3.2*	—	38.1 ± 2.1*#	—	38.0 ± 2.0*
Peak LVP, mm Hg	121 ± 4	95 ± 5*	118 ± 4	92 ± 4*	123 ± 4	108 ± 6*	120 ± 3	105 ± 5*#
Peak LVEDP, mm Hg	4.1 ± 0.6	19.1 ± 1.1*	4.1 ± 0.5	22.3 ± 1.1*#	4.0 ± 0.4	11.6 ± 2.7*#	4.3 ± 0.3	12.0 ± 2.6*#
Developed LVP, mm Hg	117 ± 6	77 ± 4*	114 ± 9	70 ± 4*	119 ± 6	96 ± 4*#	116 ± 4	93 ± 7*#
Peak LV dp dt^{-1}, mm Hg s^{-1}	5068 ± 195	2537 ± 198*	4983 ± 145	2336 ± 165*	5150 ± 122	4014 ± 237*#	5051 ± 82	3726 ± 450*#
	N + L sham (n = *6*)	*N + L MI* (n = *9*)	*H + L sham* (n = *6*)	*H + L MI* (n = *10*)	*N + A + L sham* (n = *6*)	*N + A + L MI* (n = *10*)	*H + A + L sham* (n = *6*)	*H + A + L MI* (n = *10*)
Body weight, g	325 ± 18	325 ± 17	333 ± 10	330 ± 9	326 ± 12	332 ± 11	341 ± 9	325 ± 8
Total cholesterol, mg L^{-1}	460 ± 60	430 ± 70	1360 ± 210	1380 ± 120	470 ± 70	450 ± 50	1410 ± 180	1380 ± 100
LV weight, mg g^{-1}	2.27 ± 0.10	3.00 ± 0.15*	2.22 ± 17	3.03 ± 0.18*	2.27 ± 0.19	3.25 ± 0.15*	2.35 ± 0.23	3.09 ± 0.16*
Lung weight, mg g^{-1}	3.57 ± 0.21	4.92 ± 0.31*#	3.70 ± 0.17	5.01 ± 0.27*#	3.60 ± 0.40	4.86 ± 0.32*#	3.67 ± 0.28	4.90 ± 0.25*#
Heart rate, beats min^{-1}	223 ± 5	215 ± 11	221 ± 15	216 ± 10	224 ± 12	213 ± 5	219 ± 8	220 ± 12
Infarct size (%)	—	37.2 ± 3.3*#	—	36.9 ± 2.5*#	—	36.5 ± 2.5*#	—	37.2 ± 1.8*#
Peak LVP, mm Hg	121 ± 4	103 ± 5*	116 ± 4	102 ± 6*	122 ± 5	106 ± 4*	118 ± 7	106 ± 6*#
Peak LVEDP, mm Hg	4.3 ± 0.4	13.8 ± 2.8*#	4.2 ± 0.5	12.5 ± 3.0*#	4.1 ± 0.2	12.9 ± 2.9*#	4.1 ± 0.8	11.2 ± 2.2*#
Developed LVP, mm Hg	117 ± 5	90 ± 5*#	112 ± 5	90 ± 6*#	118 ± 5	93 ± 5*#	114 ± 5	94 ± 4*#
Peak LV dp dt^{-1}, mm Hg s^{-1}	5045 ± 193	3428 ± 120*#	4898 ± 188	3155 ± 145*#	5089 ± 122	3823 ± 127*#	4911 ± 128	3773 ± 201*#

Abbreviations: A, atorvastatin treated; A + L, atorvastatin and losartan treated; H, hypercholesterolemic; L, losartan treated; LV, left ventricle; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; MI, rats with myocardial infarction; N, normocholesterolemic; sham, sham-operated rats.
Values are mean ± s.e.mean *$P<0.05$ vs respective sham, #$P<0.05$ vs N MI for normocholesterolemic or vs H MI for hypercholesterolemic rats.

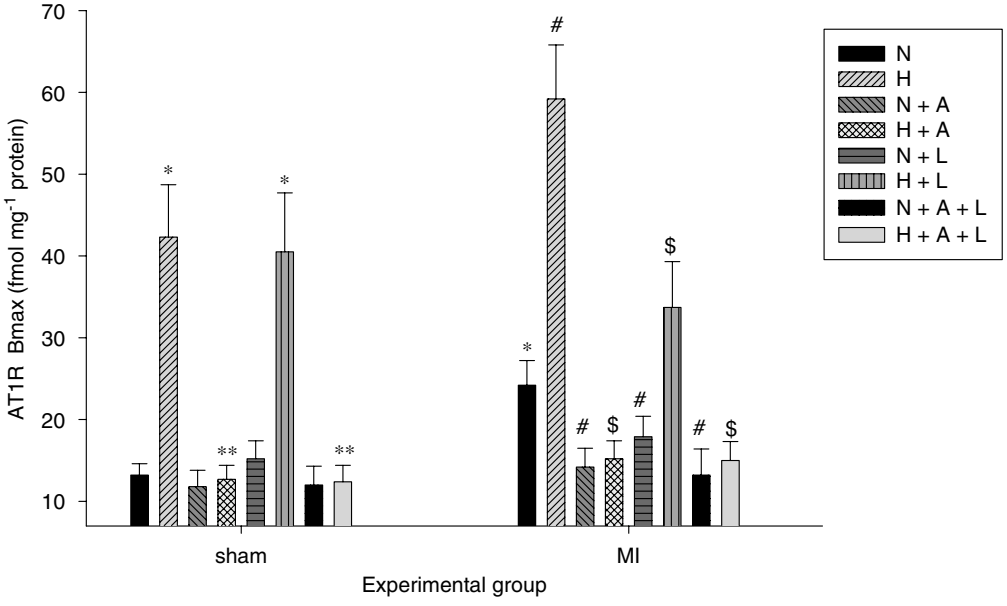

Figure 3 Angiotensin II type 1-receptor density (AT1Bmax) on left ventricular membranes measured with radioligand-binding assay in the sham-operated rats (sham) and rats with myocardial infarction (MI) 8 weeks after the intervention. Sham, sham-operated animals; N, normocholesterolemic; H, hypercholesterolemic; A, atorvastatin treated; L, losartan treated; A + L, atorvastatin and losartan treated. *$P<0.05$ vs N sham; **$P<0.05$ vs H sham; #$P<0.05$ vs N MI; $$P<0.05$ vs H MI.

diet) arms with respect to LV diameters was evident already 7 days after the infarction and continued to increase throughout the study period. This suggests that hypercholesterolaemia exacerbated mainly an early phase of post-infarction remodelling. Whereas the final infarct size in H rats was not different from that in N animals, losartan and atorvastatin led to reduced infarct size at final examination in both arms. As, at randomization, infarct sizes were identical in all experimental groups, both losartan and atorvastatin presumably exerted their effects on the process of scar formation and prevented early infarct expansion, whereas hypercholesterolaemia exacerbated both early infarct expansion and dilation of non-infarct segment. Indeed, in our model, significant effects of both atorvastatin and losartan on post-MI ventricular dilation were noted as early as 7 days after the infarction.

Atorvastatin partially prevented post-MI LV dilation and significantly improved haemodynamic parameters in post-MI hearts from both N and H groups, so that parameters of LV remodelling and function did not differ between N and H hearts in rats treated with atorvastatin. Concurrently, atorvastatin did not affect plasma cholesterol concentration, which was consistent with earlier observations that statins had no effect on plasma lipids in the rat (Endo *et al*., 1979). Induction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the liver but not in other tissues was observed as early as a few hours after the introduction of a statin in the rat (Fujioka and Tsujita, 1997) that compensated for its inhibition and some authors even observed increased hepatic cholesterol production (Fujioka *et al*., 1995). Thus, statin administration in the rat does not result in low density lipoprotein (LDL)-receptor upregulation on liver cells (Fujioka and Tsujita, 1997), so there is no decrease of plasma cholesterol, despite effective blockade of cholesterol
production in extrahepatic tissues (Wassmann *et al*., 2001). The rat model offers a unique opportunity to dissociate statin effects on plasma cholesterol from statin-dependent inhibition of cellular cholesterol synthesis in extrahepatic tissues, including myocardium. In this regard, our study demonstrates that the beneficial effects of atorvastatin on hypercholesterolaemia-induced exacerbation of post-MI remodelling is not dependent on plasma cholesterol lowering (which by affecting for example, fluidity of plasma membranes could modify cardiomyocyte contractility (Huang *et al*., 2004)) but rather on blockade of intracellular cholesterol production and related effects, such as decreased mevalonate levels.

Losartan offered similar protection with regard to the measures of post-MI LV dilation and function, which was not different from that provided by atorvastatin, although a clear trend towards a lower efficacy of losartan was evident. This might be caused by the study design: it is widely known that larger doses of anti-RAS interventions, early after MI, offer more complete protection with respect to remodelling process, but at a cost of excessive mortality, probably related to excessive hypotension, both in clinical (Swedberg *et al*., 1992; Pfeffer *et al*., 1997) and experimental settings (Pourdjabbar *et al*., 2005). To avoid excessive hypotension accompanying the administration of large doses of losartan early post-MI (Pourdjabbar *et al*., 2005), in our experiments, the dose of losartan was slowly increased over 3 weeks (see Methods).

The RAS in the post-MI heart: role of AT_1 receptors

Myocardial infarction in the rat is accompanied by both increased production of local angiotensin II (Leenen *et al*., 1999) and increased density of AT_1 receptors, both in infarct (Sun and Weber, 1994) and non-infarct area (Meggs *et al*., 1993), on cardiac myocytes (Meggs *et al*., 1993), fibroblasts (Sun and Weber, 1994) and macrophages (Gurantz *et al*., 2005), as early as after 25 min of ischaemia and 30 min of reprefusion in the isolated rat heart (Yang *et al*., 1997), which remains elevated for at least 8 weeks after experimental MI (Sun and Weber, 1994). Moreover, increased AT_1-receptor density leads to increased cellular response to angiotensin II (Meggs *et al*., 1993) (Sola *et al*., 2006). This indicates that, early after MI, intracardiac RAS activity increases and AT_1-receptor upregulation is a significant component of this increased activity.

We show that hypercholesterolaemia and MI upregulated AT_1 receptors in the rat heart and that these effects are additive. Atorvastatin prevents both hypercholesterolaemia and MI-induced changes, whereas losartan prevents only the AT_1-receptor upregulation following MI.

AT_1-receptor density is regulated on multiple levels. Hypercholesterolaemia has been shown to upregulate, whereas atorvastatin downregulate AT_1 receptors through stabilization and destabilization of AT_1-receptor mRNA, respectively (Nickenig *et al*., 1997; Wassmann *et al*., 2001). Both these effects occur without affecting the rate of AT_1-receptor gene transcription. On the other hand, MI has been shown to induce AT_1-receptor gene transcription, which was prevented by AT_1-receptor antagonists (Nio *et al*., 1995). These findings indicate that hypercholesterolaemia and statin act downstream of AT_1-antagonists in the AT_1-receptor regulation cascade, which is consistent with our results demonstrating that losartan did not prevent AT_1-receptor upregulation induced by hypercholesterolaemia.

Hypercholesterolaemia-induced exacerbation of post-MI remodelling is at least partially dependent on AT_1 receptors

Thus, three lines of evidence suggest that hypercholesterolaemia-induced exacerbation of post-MI remodelling is at least partially dependent on AT_1-receptor upregulation. (1) Hypercholesterolaemia potentiates AT_1-receptor upregulation induced by MI, whereas atorvastatin restores baseline AT_1-receptor density. (2) Both losartan and atorvastatin prevent hypercholesterolaemia-induced exacerbation of LV remodelling, restoring parameters of LV remodelling to values comparable with those achieved in N rats. (3) Losartan combined with atorvastatin offers no additional protection with respect to LV remodelling over that already achieved with atorvastatin in H hearts, which suggests that both drugs acts through a common pathway, presumably by antagonizing or downregulating AT_1 receptors.

Limitations of the study

We assessed AT_1-receptor binding in LV membrane preparations, which were obtained from ventricular tissue containing a range of cell types—cardiac myocytes, fibroblasts and vascular tissue. Functional AT_1 receptors have been found in all of these cells (Meggs *et al*., 1993; Villarreal *et al*., 1993; Strehlow *et al*., 1999; Wassmann *et al*., 2001). Thus, we are unable to differentiate between possible changes of AT_1-receptor density on each of these cells and dissect a role that each of these cells plays in the post-MI remodelling.

Other cardiac effects of statins have been found, with a potential to affect the post-MI remodelling, potentially unrelated to AT_1-receptor downregulation. They include the reduction of fibrosis, upregulation of eNOS expression (Bauersachs *et al*., 2001), increased mobilization of progenitor cells (Landmesser *et al*., 2004) and downregulation of matrix metalloproteinases (Hayashidani *et al*., 2002). Any or all of these could contribute to the beneficial effects of atorvastatin on post-MI remodelling.

Clinical implications

Our study suggests that hypercholesterolaemia worsens ventricular remodelling and heart failure and that this phenomenon is related to the upregulation of cardiac AT_1 receptors, whereas atorvastatin effectively prevents these effects through mechanisms unrelated to plasma cholesterol. This anti-AT_1-receptor effect of statins may prove valuable in the setting of heart failure treatment and prevention, where hypotension frequently limits the doses of drugs directly acting on the RAS.

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Conflicts of interest

The authors state no conflict of interest.

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