ELSEVIER

European Journal of Pharmacology 385 (1999) 171–179

ejp

www.elsevier.nl/locate/ejphar

Effects of a novel angiotensin AT_1 receptor antagonist, HR720, on rats with myocardial infarction

Qin-Gui Xia, Oliver Chung, Heidi Spitznagel, Steffen Sandmann, Sascha Illner, Birthe Rossius, Gunnar Jähnichen, Alexander Reinecke, Peter Gohlke, Thomas Unger *

Institute of Pharmacology, Christian-Albrechts-University of Kiel, Hospitalstr. 4, 24105 Kiel, Germany
German Institute for High Blood Pressure Research, 69120 Heidelberg, Germany

Received 3 August 1999; received in revised form 15 October 1999; accepted 19 October 1999

Abstract

Cardiac remodeling after myocardial infarction is associated with impaired ventricular function and heart failure and has important implications for survival. The purpose of the present study was to assess the effects of chronic treatment with a novel angiotensin AT_1 receptor antagonist 2-butyl-4-(methylthio-)-1-[[2′[[[(propylamino)carbonyl]amino]sulfonyl](1,1′-biphenyl)-4-yl]methyl]-1*H*-imidazole-5-carboxylate (HR720), on cardiac remodeling and left ventricular dysfunction in a rat model of large myocardial infarction. Rats were subjected to permanent ligation of the left coronary artery and were treated for six weeks with placebo or HR720 (3 mg/kg/day) initiated 24 h after surgery. Sham-operated rats served as normal controls. Mean arterial blood pressure, the maximum rate of rise of the left ventricular systolic pressure (dP/dt_{max}), left ventricular end-diastolic pressure, left ventricular inner diameter and circumference, septal thickness, left ventricular collagen content and heart weight were measured at the end of the treatment. HR720 treatment versus placebo attenuated the cardiac hypertrophy (heart weight/body weight: 2.88 ± 0.08 mg/g vs. 3.16 ± 0.09 mg/g, $P < 0.05$), reduced interstitial collagen content ($3.47 \pm 0.28\%$ vs. $5.25 \pm 0.45\%$, $P < 0.01$), limited infarct size ($33.0 \pm 3.0\%$ vs. $41.5 \pm 2.3\%$, $P < 0.05$), decreased left ventricular end-diastolic pressure (13.7 ± 2.2 vs. 21.4 ± 1.6 mm Hg, $P < 0.01$) and improved dP/dt_{max} (9000 ± 430 vs. 6000 ± 840 mm Hg/s, $P < 0.05$). The present results demonstrate that chronic treatment with the angiotensin AT_1 receptor antagonist HR720 can limit infarct size, partially prevent cardiac hypertrophic remodeling and improve left ventricular function in rats with myocardial infarction.

Keywords: Angiotensin AT_1 receptor antagonist; HR720; Myocardial infarction; Cardiac remodeling; Ventricular function, left; (Rat)

1. Introduction

Myocardial infarction, particularly large transmural infarction, can produce progressive alterations in the topography of both the infarcted and noninfarcted regions of the ventricle. This structural remodeling can profoundly affect the function of the ventricle and the prognosis for survival. (Pfeffer and Braunwald, 1990). Accumulating evidence suggests that the cardiac renin–angiotensin system plays an important role in the structural and functional remodeling process after myocardial infarction (Baker et al., 1992). Ventricular remodeling in post-myocardial infarction has been associated with increased angiotensin-converting enzyme activity (Hirsch et al., 1991) and angiotensin II receptor density (Meggs et al., 1993) in the non-infarcted myocardium and with increased concentration of angiotensin II in the infarct zone (Yamagishi et al., 1993). Many studies have demonstrated that angiotensin II is a potent growth promoter of both cardiac myocytes and fibroblasts during the cardiac remodeling post myocardial infarction (Booz and Baker, 1996). Angiotensin II causes hypertrophy of cardiac myocytes, and stimulates the proliferation and collagen synthesis of cardiac fibroblasts (Sadoshima and Izumo, 1993). However, angiotensin II exerts its effects by interaction with specific receptors. Two subtypes of angiotensin receptors, AT_1 and AT_2, have been cloned and pharmacologically characterised. The angiotensin AT_1 receptor mediates virtually all the known actions of angiotensin II including those on cellular growth and proliferation (Unger et al., 1996). Angiotensin II, through its interaction with the angiotensin AT_1 receptor, has been implicated as a major determinant in the develop-

* Corresponding author. Tel.: +49-431-597-3500; fax: +49-431-597-3522.

E-mail address: th.unger@pharmakologie.uni-kiel.de (T. Unger)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00731-1

ment of cardiac remodeling after myocardial infarction. Moreover, Angiotensin AT_1 receptor blockade with losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl] imidazol) or irbesartan (2-*n*-butyl-3-[(2′-(1*H*-tetrazol-5-yl)-biphenyl-4-yl)methyl]-1,3-diaza-spiro [4,4]non), has been shown to prevent myocardial hypertrophy, attenuate cardiac fibrosis, improve systemic and coronary hemodynamics, and increase survival (Unger et al., 1998).

2-Butyl-4-(methylthio-)-1-[[2′-[[[(propylamino)carbonyl]amino]sulfonyl](1,1′-biphenyl)-4-yl]methyl]-1*H*-imidazole-5-carboxylate (HR720) is a new and potent non-peptide angiotensin AT_1-selective receptor antagonist (Jin et al., 1997). The purpose of this study was to assess whether chronic treatment with HR720 affects cardiac structural remodeling and left ventricular function in a rat model of myocardial infarction and to determine whether the cardioprotective effects of losartan and irbesartan can be shared by the new angiotensin AT_1 receptor antagonist HR720.

2. Materials and methods

2.1. Animals

The experiments were performed in male Wistar rats (Charles River Viga, Sulzfeld, Germany) initially weighing 250–300 g. The animals were housed in individual cages under controlled conditions of constant temperature and humidity and exposed to a 12-h dark/light cycle. The rats had free access to a standard diet and to drinking water. All of the experiments were performed in accordance with the national animal protection law.

2.2. Animal model of myocardial infarction

Myocardial infarction was induced by permanent ligation of the left descending coronary artery as previously described (Stauss et al., 1994). Briefly, under ether anaesthesia, rats were intubated, and artificially ventilated. Anaesthesia was maintained by adding ether to the inspiration gas. Skin and muscles were cut along the left side of the sternum, and the third and fourth ribs were cut. A rib-spreading chest retractor was inserted and the left descending coronary artery was ligated with sterile 6.0 suture material (Ethibond, Ethicon, Norderstedt, Germany) under a stereomicroscope. In sham operated rats, the ligation was placed beside the coronary artery. The muscle and skin layers were sutured and the thoracic cavity was closed. The acute mortality within 24 h was about 40%.

2.3. Drug

HR720 was kindly provided by Hoechst Marion Roussel (Frankfurt/Main, Germany). HR720 was dissolved in isotonic saline and loaded into osmotic minipumps (Alzet Model 2001; Alza, Palo Alto, CA, USA). In pilot experiments, we chose a dose of 3 mg/kg body weight per day, that did not change blood pressure on healthy rats and had a sufficient efficacy over 24 h (e.g., 70–80% cardiovascular effects of Ang II 100 ng after intravenous dosing was blocked by HR720 over 24 h).

2.4. Experimental protocol

Rats were divided randomly into three groups and treated according to the following protocol:

group 1 (sham-operated group, $n = 12$);
group 2 (placebo-treated infarct group, $n = 14$): water, 1 ml/kg/day via gavage;
group 3 (HR720-treated infarct group, $n = 12$): HR720, 3 mg/kg/day, subcutaneous infusion via osmotic minipumps, the treatment was started 24 h after myocardial infarction.

The osmotic minipump filled with HR720 was implanted subcutaneously between the shoulder blades, all minipumps were replaced under ether anaesthesia at intervals of two weeks, and the treatment was continued until 6 weeks after myocardial infarction. At the end of the treatment period, arterial, venous and left ventricular catheters were chronically implanted. 24 h later, hemodynamic signals were recorded in conscious rats. At the end of the recording, rats were sacrificed and hearts were taken out for morphological examination.

2.5. Arterial, venous and left ventricular catheterization

Under chloralhydrate (400 mg/kg, i.p.) anaesthesia, polypropylene tubes (Portex, London, UK) were inserted into the right femoral artery and vein and exteriorized at the nape of the neck. Then, the right carotid artery was cannulated with a specially constructed pig-tail catheter consisting of a PP 10 and a PP 50 polypropylene tube. During catheterization, the PP 50-end of the catheter was connected to a transducer and pressure monitor. As soon as the tip of the pig-tail entered the left ventricle, the pressure signals on the monitor began to oscillate between zero and the systolic ventricular pressure. The catheter was exteriorized and anchored at the posterior neck region.

2.6. Hemodynamic measurements

Blood pressure, heart rate and left ventricular pressure were recorded 24 h after catheterization in conscious rats. The arterial and left ventricular catheters were connected to pressure transducers (DTX/Plus, Spectramed, Oxnard, CA, USA) and all hemodynamic signals were processed by two pressure processors (Gould, Valley View, OH, USA). The output signals (mean arterial blood pressure, heart rate and left ventricular pressure) from these pressure proces-

sors were recorded on a pen recorder (Gould Series 2000, Gould) and analysed by a computer-based recording and analyzing system (MEGA) (Stauss et al., 1990). Left ventricular end-diastolic pressure and maximum rate of rise of left ventricular pressure (dP/dt_{max}) were calculated offline from the left ventricular pressure signal by the MEGA program. Left ventricular end-diastolic pressure was measured at the point where the slope of the ventricular pressure signal changed from the slowly to the rapidly increasing portion. This point has been shown to be closely linked to the R-wave of the electrocardiogram and, therefore, represents the end-diastolic pressure in rats (Stauss et al., 1994). Rats were allowed to get acquainted with the recording circumstances for 30 min before the hemodynamic measurement.

2.7. Tissue processing

After recording of the hemodynamic signals, under ether anaesthesia, the heart was arrested in diastole by an intravenous injection of KCl solution. The heart was excised and the atria and large vessels removed. The heart was cleaned and weighed, then placed in 4% phosphate-buffered formalin in 0.15 M NaCl for at least 24 h, and cut transversely into five sections from the apex to the base. These sections were transferred into 10% phosphate-buffered formalin and were kept overnight. After dehydration, the sections were embedded in paraffin, and cut in serial 4-μm-thick slices. The slices were mounted onto glass slides and were stained with haematoxylin–eosin.

2.8. Morphological study

Measured morphological parameters were total heart weight, infarct size, septal thickness, left ventricular circumference and left ventricular inner diameter. For measurements of infarct size, left ventricular circumference and left ventricular inner diameter, a computerized surface determination method which employed onscreen visualisation of the cardiac transsections [Quantimet 570 morphometer including morphometry software (Leica, Cambridge Instruments, Cambridge, UK) connected to a video camera] was used (Sandmann et al., 1998). The endocardial and epicardial circumferences of the infarcted portion and the entire left ventricle were determined by the computerized morphometry. The inner diameters and circumferences (entire left ventricle and infarcted portion) from all five slices were added together and averaged, respectively. The infarct size of the left ventricle was calculated from these measurements and expressed as a percentage of the total circumference of the left ventricle.

2.9. Measurement of interstitial collagen

After deparaffinization (2×5 min in xylol, 2×5 min in 100% ethanol, 2×5 min in 70% ethanol, and 2×5 min in 50% ethanol), the sections were washed in tap water for 10 min and in distilled water for 5 min and treated for 5 min with 0.2% aqueous phosphomolybdic acid, and then stained for 90 min with 0.1% Sirius red F3BA (C.I 35780, Polysciences, Warrington, PA, USA) in saturated aqueous picric acid, and washed for 2 min with 0.01M HCI. They were then dehydrated (45 s in 70% ethanol, 2×3 min in 100% ethanol, 2×3 min in xylol), and mounted with coverslips (Junqueira et al., 1979). The interstitial and perivascular collagen regions were clearly detectable within the collagen accumulating tissue showing a red color and the remaining tissue a yellow color. The collagen content was determined under a microscope coupled to a computerized morphometric system (Quantimet 570, Leica, Cambridge, UK). Forty fields (Magnification, $400 \times$) in two tissue sections were analyzed in the midwall area of the interventricular spetum. Collagen content (mean of the twenty fields determinations) was expressed as the percentage of the sum of positive Sirius red areas to the sum of measured twenty fields areas in the non-infarcted left ventricular free wall and the interventricular septum. Measurements were restricted to the interstitial collagen; perivascular and endocardial collagens were excluded from the measurements.

2.10. Statistical analysis

All data are expressed as mean ± SEM Comparisons among three groups were performed by one-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Body weight and cardiac morphological parameters

After induction of myocardial infarction, food and water intake was reduced in all animals and a decrease of body weight (23.9 ± 2.3 g) was observed. One week after coronary ligation the food and water intake was normalized and was not different from sham-operated animals (data not shown). At the end of the study, the body weights were similar in the three groups, although infarcted rats have a small, nonsignificant decrease in body weight.

Cardiac morphometric measurements are summarized in Table 1.

Infarct size as a percentage of left ventricular circumference is expressed. None of the sham-operated rats had evidence of infarction by gross inspection. The coronary ligation animals had microscopic evidence of transmural infarction. HR720 treatment decreased infarct size when compared to placebo-treated infarct group ($P < 0.05$).

Table 1
Effect of HR720 treatment on cardiac morphological parameters
Values represent the mean ± SEM; LV, left ventricular; HW/BW, heart weight to body weight ratio.

	Sham-operated rats ($n = 12$)	Infarcted rats treated with	
		Placebo ($n = 14$)	HR720 ($n = 12$)
Body weight (g)	459 ± 8	449 ± 9	445 ± 13
HW/BW (mg/g)	2.77 ± 0.05	3.16 ± 0.09[a]	2.88 ± 0.08[b]
Infarct size (%)	0	41.5 ± 2.3	33.0 ± 3.0[b]
LV inner diameter (mm)	5.41 ± 0.24	7.07 ± 0.24[c]	6.58 ± 0.32
LV circumference (mm)	22.3 ± 0.4	24.8 ± 0.8[d]	23.7 ± 0.73
Septal thickness (mm)	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.2
LV collagen content (%)	1.98 ± 0.24	5.25 ± 0.45[c]	3.47 ± 0.28[d,e]

[a] $P < 0.01$ as compared with sham-operated group.
[b] $P < 0.05$ as compared with placebo-treated infarct group.
[c] $P < 0.001$ as compared with sham-operated group.
[d] $P < 0.05$ as compared with sham-operated group.
[e] $P < 0.01$ as compared with placebo-treated infarct group.

Myocardial hypertrophy was evaluated by comparing the ratio of heart weight to body weight among the three groups. Although 41% of the left ventricular myocardium was replaced by a paper-thin scar tissue, the ratio of heart weight to body weight was significantly increased in placebo-treated infarct group when compared to sham-operated group ($P < 0.01$); HR720 treatment significantly decreased the ratio of heart weight to body weight when compared to placebo-treated infarct group ($P < 0.05$).

The left ventricular inner diameter and left ventricular circumference were used to determine the left ventricular expansion. In placebo-treated infarct rats left ventricular inner diameter and left ventricular circumference were significantly increased when compared to sham-operated rats ($P < 0.001$ and $P < 0.05$, respectively). Both parameters were reduced by HR720 treatment as compared to the placebo-treated infarct rats, but this difference did not reach statistical significance.

Myocardial infarction resulted in a decrease of septal thickness when compared to the sham-operated group, HR720 treatment slightly but not significantly increased the septal thickness.

The interstitial collagen content was increased in placebo-treated infarct rats as compared to sham-operated animals ($P < 0.001$), and the interstitial collagen content was decreased by HR720 treatment as compared to the placebo-treated infarct group ($P < 0.01$) but remained significantly elevated compared with sham-operated rats ($P < 0.05$).

3.2. Hemodynamic parameters

The hemodynamic parameters are summarized in Table 2.

Six weeks after induction of myocardial infarction, mean arterial blood pressure was decreased in placebo-treated infarct animals when compared to sham-operated rats, but this difference did not reach the level of statistical significance. HR720 treatment further decreased mean arterial blood pressure when compared to sham-operated rats ($P < 0.05$).

Heart rate showed little change in either the placebo-treated infarct animals or HR720-treated infarct rats, nor was there a significant difference between groups.

Table 2
Effect of HR720 treatment on hemodynamic parameters
Values represent the mean ± SEM; MAP, mean arterial blood pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, the maximum rate of rise of the left ventricular systolic pressure.

	Sham-operated rats ($n = 12$)	Infarcted rats treated with	
		Placebo ($n = 14$)	HR720 ($n = 12$)
MAP (mm Hg)	107 ± 3.9	95.9 ± 3.5	89.4 ± 2.2[a]
Heart rate (beats/min)	392 ± 9	382 ± 13	371 ± 12
LVEDP (mm Hg)	8.2 ± 1.0	21.4 ± 1.6[b]	13.7 ± 2.2[c]
dP/dt_{max} (mm Hg/s)	11,000 ± 620	6000 ± 840[b]	9000 ± 430[d]

[a] $P < 0.05$ as compared with sham-operated group.
[b] $P < 0.001$ as compared with sham-operated group.
[c] $P < 0.01$ as compared with placebo-treated infarct group.
[d] $P < 0.05$ as compared with placebo-treated infarct group.

Left ventricular end-diastolic pressure was significantly increased in placebo-treated infarct rats when compared to sham-operated rats ($P < 0.001$). In HR720-treated animals, the increase of left ventricular end-diastolic pressure was markedly and significantly attenuated when compared to the placebo-treated infarct group ($P < 0.01$).

Myocardial contractility (dP/dt_{max}) was significantly reduced in placebo-treated infarct rats when compared to sham-operated rats ($P < 0.001$). HR720 treatment improved dP/dt_{max} when compared to placebo-treated infarct rats ($P < 0.05$).

4. Discussion

The ventricular remodeling after myocardial infarction is characterized by the development of eccentric cardiac hypertrophy and ventricular dysfunction. Because angiotensin II has been demonstrated to play an important role in this process, the purpose of the present study was to assess the effects of chronic treatment with a novel angiotensin AT_1 receptor antagonist HR720 on cardiac structural remodeling and hemodynamic parameters in a rat model of myocardial infarction-induced heart failure.

4.1. Effects of HR720 on cardiac structural remodeling post-myocardial infarction

The results revealed that HR720 treatment started 24 h post-myocardial infarction can attenuate cardiac hypertrophy, decrease interstitial collagen deposition and limit infarct size. This observation provided further support for the involvement of angiotensin II and its action via angiotensin AT_1 receptors for the development of cardiac remodeling in this model of myocardial infarction.

These findings are consistent with several previous experimental studies. Schieffer et al. (1994) reported in a rat model of myocardial infarction that the angiotensin AT_1 receptor antagonist, losartan, reduced cardiac hypertrophy and attenuated the development of myocardial interstitial fibrosis in the noninfarcted left ventricle. Ju et al. (1997) observed that losartan treatment for 4 weeks prevented left and right ventricular hypertrophy and decreased total collagen concentrations, but had no effect on infarct size. Recently, Richer et al. (1999) reported that the angiotensin AT_1 receptor antagonist, irbesartan, markedly decreased myocardial hypertrophy but had almost no effect on left ventricular dilation and subendocardial fibrosis. The results of the present study indicated that HR720 possessed similar cardiaoprotective effects in preventing cardiac remodeling after myocardial infarction as shown for losartan and irbesartan.

To date, the mechanism by which angiotensin AT_1 receptor antagonists prevent cardiac remodeling is mainly attributed to the blockade of angiotensin II binding to the AT_1 receptor. However, additional mechanisms seem to be involved in this effect. Abdelrahman et al. (1993) reported that treatment with losartan led to dose-related increases in plasma renin and angiotensin II levels among patients with heart failure. Campbell et al. (1995) reported that losartan administration increased plasma renin levels 100-fold, and angiotensin II levels in plasma, adrenal, lung, heart and aorta were increased 25-, 8-, 3.5-, 2.4-, and 14-fold, respectively. Recently, our laboratory also found out that after losartan treatment, plasma angiotensin II levels were markedly increased in stroke-prone spontaneously hypertensive rats (SHR-SP) (Gohlke et al., 1998). Therefore, during conditions of angiotensin AT_1 receptor blockade, the increased angiotensin II might excessively stimulate unblocked angiotensin AT_2 receptors (Chung et al., 1996). Furthermore, after myocardial infarction, the number of angiotensin AT_2 receptors is increased (Lopez et al., 1994). Angiotensin AT_2 receptors have been demonstrated to exert anti-growth effects, and can antagonize the growth promoting effects of angiotensin AT_1 receptors (Nakajima et al., 1995; Stoll et al., 1995). Recently, Liu et al. (1997) observed that most of the cardioprotective effects of losartan were blocked during a combination treatment with the angiotensin AT_2 receptor antagonist (S)-1-[[4-(dimethylamino)-3-methyl-phenyl]methyl-5-(diphenylacetyl)-4, 5, 6, 7-tetrahydro-1*H*-imidazo [4,5-*c*] -pyridine-6-carboxylic acid (PD123319), indicating that the beneficial effect of angiotensin AT_1 receptor antagonist is mediated in part by activation of the angiotensin AT_2 receptor.

In addition, Wiemer et al. (1993) reported that angiotensin II via the angiotensin AT_1 receptor increases nitric oxide release in cultured bovine aortic endothelial cells and isolated ischemic rat hearts. Seyedi et al. (1995) observed that angiotensin II, III, and angiotensin-(1–7) via angiotensin AT_1 and AT_2 receptors and angiotensin IV via angiotensin AT_2 receptor, increased nitric oxide release in isolated microvessels or large arteries of normal dog heart. Recently, Gohlke et al. (1998) showed that the angiotensin II via angiotensin AT_2 receptor increased aortic cGMP, and the effect was abolished by bradykinin B_2 receptor blockade and nitric oxide–synthase inhibition in SHR-SP. Zou et al. (1998) found out that a small elevation of plasma angiotensin II levels increased renal medullary nitric oxide production and concentration by 150% in rats. These studies indicate that angiotensin II and its fragments are able to stimulate nitric oxide release. Numerous investigations have shown that nitric oxide inhibits vascular remodeling and prevents angiotensin II-induced cardiac hypertrophy and fibrosis (Dubey et al., 1995; Hou et al., 1995; Kato et al., 1996). Therefore, the increase of nitric oxide release induced through angiotensin AT_2 receptor stimulation might play a role in angiotensin AT_1 receptor antagonist-induced prevention of cardiac remodeling.

Another possible mechanism for the anti-remodeling effects of HR720 might be mediated by the reduction of endothelin-1 production. Recently, an increasing number of observations suggest the existence of a myocardial

endothelin system and its possible involvement in the pathophysiology of cardiovascular diseases (Fraccarollo et al., 1997). The endothelin system is activated and the peptide levels of endothelin-1 and the number of myocardial endothelin receptors were significantly increased in the left ventricle of rats after myocardial infarction (Sakai et al., 1996). Endothelin-1 not only is a powerful vasoconstrictor peptide, but also a potent hypertrophic stimulus for cultured cardiomyocytes (Shubeita et al., 1990). Several studies have demonstrated that angiotensin II stimulate endothelin-1 secretion from cultured endothelial cells (Emori et al., 1989) and from cardiomyocytes (Ito et al., 1993). A more recent study from Gray et al. (1998) reported that extravascular angiotensin II receptors in the heart reside largely on cardiac fibroblasts. Following activation of angiotensin AT_1 receptors by angiotensin II, these cells release at least two paracrine effectors, endothelin-1 and transforming growth factor-β1, which cause cardiac myocyte hypertrophy, indicating that angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of transforming growth factor-β1 and endothelin-1 from cardiac fibroblasts. Thus, the beneficial effect of angiotensin AT_1 receptor antagonists on cardiac remodeling might be partly mediated through the reduction of locally produced endothelin-1.

In the present study, mean arterial blood pressure was not significantly different between HR720-treated infarct group and the placebo-treated infarct group, suggesting that the prevention of cardiac hypertrophy by HR720 was not related to a change in afterload. In this regard, Kojima et al. (1994) reported that treatment with angiotensin AT_1 receptor antagonist (±)-1-(cyclohexyloxycarbonyloxy)-ethyl 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl]-1*H*-benzimidazole-7-carboxylic acid (TCV-116) reduced left ventricular weight, wall thickness, interstitial fibrosis and transverse diameter of myocytes, whereas treatment with hydralazine slightly prevented an increase in left wall thickness but did not exert significant reduction in other parameters despite the greater antihypertensive effect of hydralazine. In addition, a more recent study from Ambrose et al. (1999) showed that the angiotensin AT_1 receptor antagonist irbesartan effectively decreased ventricular hypertrophy after myocardial infarction independent of ventricular loading conditions. These results suggest that the regression of cardiac hypertrophy by the angiotensin AT_1 receptor antagonist HR720 might be mainly attributable to the blockade of direct effects of angiotensin II on myocardial growth, which are mediated through the angiotensin AT_1 receptor. In the present study, treatment with HR720 resulted in a decrease of left ventricular end-diastolic pressure. Therefore, we cannot exclude the contribution of reduced afterload of the right ventricle to the regression of cardiac hypertrophy. Several studies have observed that the left ventricular weight, both absolute and relative, remained unaffected, while right ventricular weight was significantly increased in untreated infarcted rats (Pfeffer et al., 1991; Sladek et al., 1996). Therefore, the decrease in heart weight to body weight ratio by HR720 treatment in present study is likely related to the decreased right ventricular afterload (i.e. the reduction of the elevated left ventricular end-diastolic pressure).

In the present study, we observed that HR720 treatment initiated 24 h after coronary ligation reduced infarct size when compared to that in placebo-treated infarct rats. This is not in agreement with most studies. The exact mechanism is not clear. However, Shimizu et al. (1998) reported that the angiotensin AT_1 receptor antagonist, candesartan, when administered locally into the ischemic myocardium of pigs at a dose without any hemodynamic effect, reduced infarct size by 50%. Frimm et al. (1997) observed that losartan treatment initiated 24 h after coronary ligation reduced infarct area, and significantly diminished angiotensin AT_1 receptor binding density within the infarct tissue in rats. These results indicate that local cardiac angiotensin II is involved in the development of myocardial injury, and this damage may be initiated by activation of angiotensin AT_1 receptor. Therefore, a possible mechanism for the limiting infarct size of HR720 may be inhibition of the direct cardiotoxic action of local angiotensin II. In addition, several studies have demonstrated that in the rat model of myocardial infarction, losartan treatment increased capillary density and improved capillary supply of the infarcted heart (Schieffer et al., 1994; Sladek et al., 1996), elicited the coronary vasodilatation (Sudhir et al., 1993) and increased coronary blood flow (Richer-Giudicelli and Fornes, 1997). Thus, it is conceivable that an improvement in the blood supply of infarcted hearts might contribute to limit the infarct size.

4.2. Effects of HR720 on left ventricular dysfunction after myocardial infarction

The present study shows that HR720 treatment significantly reduces left ventricular end-diastolic pressure and increases ventricular contractility, indicating an improvement of left ventricular dysfunction after myocardial infarction. These findings are in agreement with previous studies. Ju et al. (1997) reported that the treatment with angiotensin AT_1 receptor antagonist losartan in the same experimental model significantly decreased left ventricular end-diastolic pressure and increased dP/dt_{max}. Richer et al. (1999) reported that irbesartan dose-dependently decreased left ventricular end-diastolic pressure and normalized left ventricular dP/dt_{max} and cardiac index values after myocardial infarction in rats.

After myocardial infarction the cardiac renin-angiotensin system is activated, local angiotensin II is increased. The effect of angiotensin II on normal left ventricular contractile function is controversial. Angiotensin II was reported to be an agent with negative or positive inotropic effect (Moraves et al., 1990), or biphasic inotropic effects

(Li et al., 1994) and without inotropic effect (Baker and Singer, 1988). After heart failure, angiotensin II has been demonstrated to produce negative inotropic effect and the effect was reversed by angiotensin AT_1 receptor antagonist losartan, indicating that the negative inotropic effect is mediated by the activation of angiotensin AT_1 receptors (Cheng et al., 1996). Therefore, we speculate that HR720 improved myocardial contractility through the blockade of the direct negative inotropic effect of cardiac angiotensin II.

In addition, the present study shows that HR720 treatment significantly decreased left ventricular end-diastolic pressure. An increase of myocardial contractility and a decrease of afterload may contribute to reduce left ventricular end-diastolic pressure. However, Raya et al. (1989, 1991) reported that the change of left ventricular end-diastolic pressure is mediated by venodilation, and angiotensin II via angiotensin AT_1 receptors causes venoconstriction in heart failure. Thus, the reduction of left ventricular end-diastolic pressure by HR720 might partially be mediated by blockade of the venoconstriction effect of angiotensin II.

4.3. Comparative effects of angiotensin AT_1 receptor antagonists and angiotensin-converting enzyme inhibitors on cardiac remodeling and left ventricular dysfunction post myocardial infarction

Although our experimental design did not include an angiotensin-converting enzyme inhibitor-treated group, our results can be compared with previous studies (Pfeffer et al., 1985; Capasso and Anversa, 1992). These studies have evaluated the influence of angiotensin-converting enzyme inhibitor, captopril, on cardiac remodeling and left ventricular dysfunction in infarcted rats, and have shown that chronic treatment with captopril prevented cardiac hypertrophy and decreased left ventricular end-diastolic pressure and left ventricular volume, whereas myocardial contractility (dP/dt_{max}) was not restored. In the present study, we observed that the angiotensin AT_1 receptor antagonist HR720 can increase myocardial contractility, as reported previously (Capasso et al., 1994; Makino et al., 1996; Ju et al., 1997; Richer et al., 1999), indicating that angiotensin AT_1 receptor antagonists appear to offer additional benefit over angiotensin-converting enzyme inhibitors in the treatment of heart failure. Our results were supported by the ELITE study (Pitt et al., 1997), which compared the effects of the angiotensin-converting enzyme inhibitor captopril and the angiotensin AT_1 receptor antagonist losartan in elder patients with heart failure. Although no significant difference was found for the primary or secondary endpoint, the study suggested that all-cause mortality was decreased by 46% in patients treated with losartan compared with those treated with captopril and the apparent mortality advantage for losartan seems to be due primarily to a reduction in sudden cardiac death.

Angiotensin-converting enzyme inhibitor therapy is consistently effective in preventing cardiac remodeling and improving left ventricular function after myocardial infarction. Recently, angiotensin AT_1 receptor antagonists have proven to be beneficial in the treatment of infarct-induced heart failure. Although angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists have different mechanisms of action, they both diminish the effects of angiotensin II by differential blockade of the renin–angiotensin system. The angiotensin-converting enzyme inhibitors exert a cardioprotective effect by inhibiting the formation of angiotensin II and/or decreasing the degradation of bradykinin. However, since angiotensin II can also be generated by angiotensin converting enzyme-independent enzymatic pathways, such as chymase, the functional renin–angiotensin system blockade with angiotensin-converting enzyme inhibitors may not be complete. Angiotensin AT_1 receptor antagonists specifically block angiotensin AT_1 receptors and potentially enhance the beneficial opposing effects of angiotensin AT_2 receptor on endothelial proliferation, vasoconstriction, and tissue repair (Unger et al., 1998). Therefore, angiotensin AT_1 receptor antagonists may offer advantages over angiotensin-converting enzyme inhibitors in therapy of myocardial infarction-induced heart failure.

In summary, this study demonstrates that chronic treatment with a novel angiotensin AT_1 receptor antagonist HR720 can attenuate cardiac hypertrophic remodeling, limit infarct size and improve left ventricular function after myocardial infarction in rats. Further investigations will be required to determine the contribution of nitric oxide and endothelin-1 to the angiotensin AT_1 receptor antagonist-induced improvement of left ventricular remodeling and dysfunction in this experimental model.

References

Abdelrahman, A.M., Burrell, L.M., Johnston, C.I., 1993. Blockade of the renin–angiotensin system at different sites: effect on renin, angiotensin and aldosterone. J. Hypertens. 11 (Suppl. 3), S23–S26.

Ambrose, J., Pribnow, D.G., Giraud, G.D., Perkins, K.D., Muldoon, L., Greenberg, B.H., 1999. Angiotensin type 1 receptor antagonism with irbesartan inhibits ventricular hypertrophy and improves diastolic function in the remodeling post-myocardial infarction ventricle. J. Cardiovasc. Pharmacol. 33, 433–439.

Baker, K.M., Singer, H.A., 1988. Identification and characterization of guinea pig angiotensin II ventricular and atrial receptors: coupling to inositol phosphate production. Circ. Res. 62, 896–904.

Baker, K.M., Booz, G.W., Dostal, D.E., 1992. Cardiac actions of angiotensin II: role of an intracardiac renin–angiotensin system. Annu. Rev. Physiol. 54, 227–241.

Booz, G.W., Baker, K.M., 1996. The role of the renin–angiotensin system in the pathophysiology of cardiac remodeling. Blood Pressure 5 (Suppl. 2), 10–18.

Campbell, D.J., Kladis, A., Valentijn, A.J., 1995. Effects of losartan on angiotensin and bradykinin peptide and angiotensin-converting enzyme. J. Cardiovasc. Pharmacol. 26, 233–240.

Capasso, J.M., Anversa, P., 1992. Mechanical performance of spared myocytes after myocardial infarction in rats: effects of captopril treatment. Am. J. Physiol. 263, H841–H849.

Capasso, J.M., Li, P., Meggs, L.G., Herman, M.V., Anversa, P., 1994. Efficacy of angiotensin-converting enzyme inhibition and AT_1 receptor blockade on cardiac pump performance after myocardial infarction in rats. J. Cardiovasc. Pharmacol. 23, 584–593.

Cheng, C.P., Li, P., Zhang, X., Ohno, M., Wang, Z.M., Little, W.C., 1996. Altered ventricular and myocyte response to angiotensin II in pacing-induced heart failure. Circ. Res. 78, 880–892.

Chung, O., Stoll, M., Unger, T., 1996. Physiologic and pharmacologic implications of AT_1 versus AT_2 receptors. Blood Pressure 5 (Suppl. 2), 47–52.

Dubey, R.K., Jackson, E.K., Lüscher, T.F., 1995. Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell. J. Clin. Invest. 96, 141–149.

Emori, Y., Hirata, Y., Ohta, K., Shichiri, M., Marumo, F., 1989. Secretary mechanism of immunoreactive endothelin in cultured bovine endothelial cells. Biochem. Biophys. Res. Commun. 160, 93–100.

Fraccarollo, D., Hu, K., Galuppo, P., Gaudron, P., Ertl, G., 1997. Chronic endothelin receptor blockade attenuates progressive ventricular dilation and improves cardiac function in rats with myocardial infarction, possible involvement of myocardial endothelin system in ventricular remodeling. Circulation 96, 3963–3973.

Frimm, C.C., Sun, Y., Weber, K.T., 1997. Angiotensin II receptor blockade and myocardial fibrosis of the infarcted rat heart. J. Lab. Clin. Med. 129, 439–446.

Gohlke, P., Pees, C., Unger, T., 1998. AT_2 receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism. Hypertension 31, 349–355.

Gray, M.O., Long, C.S., Kalinyak, J.E., Li, H.T., Karliner, J.S., 1998. Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-β1 and endothelin-1 from fibroblasts. Cardiovasc. Res. 40, 352–363.

Hirsch, A.T., Talsness, C.E., Schunkert, H., Paul, M., Dzau, V.J., 1991. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. Circ. Res. 69, 475–482.

Hou, J., Kato, H., Cohen, R.A., Chobanian, A.V., Brecher, P., 1995. Angiotensin II-induced cardiac fibrosis in the rat is increased by chronic inhibition of nitric oxide synthase. J. Clin. Invest. 96, 2469–2477.

Ito, H., Hirata, Y., Adachi, S., Tanaka, M., Tsujino, M., Koike, A., Nogami, A., Murumo, F., Hiroe, M., 1993. Endothelin-1 is an autocrine/paracrine factor in mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. J. Clin. Invest. 92, 398–403.

Jin, D., Song, K., Oka, Y., Takai, S., Shiota, N., Miyazaki, M., 1997. Pharmacological profiles of a novel non-peptide angiotensin II type 1 receptor antagonist HR720 in vitro and in vivo. Jpn. J. Pharmacol. 75 (3), 259–266.

Ju, H., Zhao, S., Jassal, D.S., Dixon, I.M.C., 1997. Effect of AT_1 receptor blockade on cardiac collagen remodeling after myocardial infarction. Cardiovasc. Res. 35, 223–232.

Junqueira, L.C., Bignolas, G., Brentani, R.R., 1979. Red sirius staining plus polarizing microscopy: a specific method for collagen detection in tissue sections. Histochem. J. 79, 445–447.

Kato, H., Hou, J., Chobanian, A.V., Brecher, P., 1996. Effects of angiotensin II infusion and inhibition of nitric oxide synthase on the rat aorta. Hypertension 28, 153–158.

Kojima, M., Shiojima, I., Yamazaki, T., Komuro, I., Zou, Z., Wang, Y., Mizuno, T., Ueki, K., Tobe, K., Kadowaki, T., Nagai, R., Yazaki, Y., 1994. Angiotensin II receptor antagonist TCV-116 induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signaling pathway of stretch-mediated cardiomyocyte hypertrophy in vitro. Circulation 89, 2204–2211.

Li, P., Sonnenblick, E.H., Anversa, P., Capasso, J., 1994. Length-dependent modulation of angiotensin II inotropism in rat myocardium: effects of myocardial infarction. Am. J. Physiol. 266, H779–H786.

Liu, Y.H., Yang, X.P., Sharov, V.G., Nass, O., Sabbah, H.N., Peterson, E., Carretero, O.A., 1997. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure. Role of kinin and angiotensin II type 2 receptors. J. Clin. Invest. 99, 1926–1935.

Lopez, J.J., Lorell, B.H., Ingelfinger, J.R., Weinberg, E.O., Schunkert, H., Diamant, D., Tang, S.S., 1994. Distribution and function of cardiac angiotensin AT_1- and AT_2-receptor subtypes in hypertrophied rat hearts. Am. J. Physiol. 267, H844–H852.

Makino, N., Hata, T., Sugano, M., Dixon, I.M., Yanaga, T., 1996. Regression of hypertrophy after myocardial infarction is produced by the chronic blockade of angiotensin type 1 receptor in rats. J. Mol. Cell Cardiol. 28, 507–517.

Meggs, L.G., Coupet, J., Huang, H., Cheng, W., Li, P., Capasso, J.M., Homcy, C.J., Anversa, P., 1993. Regulation of angiotensin II receptors on ventricular myocytes after myocardial infarction in rats. Circ. Res. 72, 1149–1162.

Moraves, C.S., Schluchter, M.D., Paranandi, L., Czerska, B., Stewart, R.W., Rosenkranz, E., Bond, M., 1990. Inotropic effects of angiotensin II on human cardiac muscle in vitro. Circulation 82, 1973–1984.

Nakajima, M., Hutchinson, H.G., Fujinaga, M., Hayashida, W., Morishita, R., Zhang, L., Horiuchi, M., Pratt, R.E., Dzau, V.J., 1995. The angiotensin II type 2 (AT_2) receptor antagonizes the growth effects of AT_1 receptor: gain-of-function study using gene transfer. Proc. Natl. Acad. Sci. U.S.A. 92, 10663–10667.

Pfeffer, M.A., Braunwald, E., 1990. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. Circulation 81, 1161–1172.

Pfeffer, J.M., Pfeffer, M.A., Braunwald, E., 1985. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ. Res. 57, 84–95.

Pfeffer, J.M., Pfeffer, M.A., Fletcher, P.J., Braunwald, E., 1991. Progressive ventricular remodeling in rat with myocardial infarction. Am. J. Physiol. 260, H1406–H1414.

Pitt, B., Segal, R., Martinez, F.A., Meurers, G., Cowley, A.J., Thomas, I., Deedwania, P.C., Ney, D.E., Snavely, D.B., Chang, P.I., 1997. Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan in the Elderly Study ELITE). Lancet 349, 747–752.

Raya, T.E., Gay, R.G., Aguire, M., Goldman, S., 1989. Importance of venodilatation in prevention of left ventricular dilatation after chronic large myocardial infarction in rats: a comparison of captopril and hydralazine. Circ. Res. 64, 330–337.

Raya, T.E., Fonken, S.J., Lee, R.W., Daugherty, S., Goldman, S., Wong, P.C., Timmermans, P.B., Morkin, E., 1991. Hemodynamic effects of direct angiotensin II blockade compared to converting enzyme inhibition in rat model of heart failure. Am. J. Hypertens. 4, 334S–340S.

Richer, C., Fornes, P., Cazaubon, C., Domergue, V., Nisato, D., Giudicelli, J.F., 1999. Effects of long-term angiotensin II AT_1 receptor blockade on survival, hemodynamics and cardiac remodeling in chronic heart failure in rats. Cardiovasc. Res. 41, 100–108.

Richer-Giudicelli, C., Fornes, P., 1997. Effects of angiotensin II AT_1 receptor blockade on survival, systemic and coronary hemodynamics and cardiac remodeling in post-ischemic heart failure in rats (Abstract). Circulation 96, I-644, (Suppl).

Sadoshima, J., Izumo, S., 1993. Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT_1 receptor subtype. Circ. Res. 73, 413–423.

Sakai, S., Miyauchi, T., Sakurai, T., Kasuya, Y., Ihara, M., Yamaguchi, I., Goto, K., Sugishita, Y., 1996. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats with congestive heart failure. Marked increase in endothelin-1 production in the failing heart. Circulation 93, 1214–1222.

Sandmann, S., Spitznagel, H., Chung, O., Xia, Q.G., Illner, S., Jänichen, G., Rossius, B., Daemen, M.J.A.P., Unger, T., 1998. Effects of the

calcium channel antagonist mibefradil on haemodynamic and morphological parameters in myocardial infarction-induced cardiac failure in rats. Cardiovasc. Res. 39, 339–350.

Schieffer, B., Wirger, A., Meybrunn, M., Seitz, S., Holtz, J., Riede, U.N., Drexler, H., 1994. Comparative effects of chronic angiotensin converting enzyme inhibition and angiotensin II type 1 receptor blockade on cardiac remodeling after myocardial infarction in the rat. Circulation 89, 2273–2282.

Seyedi, N., Xu, X.B., Nasiletti, A., Hintze, T.H., 1995. Coronary kinin generation mediates nitric oxide release after angiotensin receptor stimulation. Hypertension 26, 164–170.

Shimizu, M., Wang, Q.D., Sjöquist, P.O., Ryden, L., 1998. Angiotensin II type 1 receptor blockade with candesartan protects the porcine myocardium from reperfusion-induced injury. J. Cardiovasc. Pharmacol. 32, 231–238.

Shubeita, H.E., McDonough, P.M., Harris, A.N., Knowlton, K.U., Glembotski, C.C., Brown, J.H., Chien, K.R., 1990. Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes. A paracrine mechanism for myocardial cell hypertrophy. J. Biol. Chem. 265, 20555–20562.

Sladek, T., Sladkova, J., Kolar, F., Papousek, F., Cicutti, N., Korecky, B., Rakusan, K., 1996. The effect of AT_1 receptor antagonist on chronic cardiac response to coronary artery ligation in rats. Cardiovasc. Res. 31, 568–576.

Stauss, B., Itoi, K., Stauss, H., Unger, T., 1990. A novel inexpensive computer system to record and analyze hemodynamic data in conscious animals. Eur. J. Pharmacol. 183, 863–864.

Stauss, H.M., Zhu, Y.C., Redich, T., Unger, T., 1994. Angiotensin-converting enzyme inhibition in infarct-induced heart failure in rats: bradykinin versus angiotensin II. J. Cardiovasc. Risk 1, 255–262.

Stoll, M., Steckelings, U.M., Paul, M., Bottari, S.P., Metzger, R., Unger, T., 1995. The angiotensin AT_2 receptor mediates inhibition of cell proliferation in coronary endothelial cells. J. Clin. Invest. 95, 651–657.

Sudhir, K., MacGregor, J.S., Gupta, M., Barbant, S.D., Redberg, R., Yock, P.G., Chatterjee, K., 1993. Effect of selective angiotensin II receptor antagonism and angiotensin converting enzyme inhibition on the coronary vasculature in vivo. Intravascular two-dimensional and Doppler ultrasound studies. Circulation 87, 931–938.

Unger, T., Chung, O., Csikos, T., Culman, J., Gallinat, S., Gohlke, P., Meffert, S., Stoll, M., Stroth, U., Zhu, Y.Z., 1996. Angiotensin receptors. J. Hypertens. 14 (Suppl. 5), S95–S103.

Unger, T., Culman, J., Gohlke, P., 1998. Angiotensin II receptor blockade and end-organ protection: pharmacological rationale and evidence. J. Hypertens. 16 (Suppl. 7), S3–S9.

Wiemer, G., Schölkens, B.A., Wagner, A., Heitsch, H., Linz, W., 1993. The possible role of angiotensin II subtype AT_2 receptors in endothelial cells and isolated ischemic rat hearts. J. Hypertens. 11 (Suppl. 5), S234–S235.

Yamagishi, H., Kim, S., Nishikimi, T., Takeuchi, K., Takeda, T., 1993. Contribution of cardiac renin–angiotensin system to ventricular remodeling in myocardial-infarcted rats. J. Mol. Cell Cardiol. 25, 1369–1380.

Zou, A.P., Wu, F., Cowley, A.W. Jr., 1998. Protective effect of angiotensin II-induced increase in nitric oxide in the renal medullary circulation. Hypertension 31, 271–276.