Comparison of cardioprotective effects of mibefradil and ramipril in stroke-prone spontaneously hypertensive rats

Qin-gui XIA[1,2], Alexander REINECKE[3], Marc DORENKAMP[2], Christain STORZ[2], Havvo BITTERLING[2], Susanne PENZ[2], Jack CLEUTJENS[2], Mat JAP DAEMEN[4], Reichiger SIMON[3], Thomas UNGER[2]

[1]Department of Physiology, Wenzhou Medical College, Wenzhou 325027, China; [2]Institute of Pharmacology, University of Kiel, 24105 Kiel, Germany; [3]Clinic of Cardiology, University of Kiel, 24105 Kiel,Germany; [4]Department of Pathology, University of Maastricht, Maastricht, The Netherlands

KEY WORDS mibefradil; ramipril; angiotensin-converting enzyme inhibitors; calcium channel blockers; myocardial infarction; heart failure; cardiac remodeling; ventricular function; SHR-SP rats

ABSTRACT

AIM: To elucidate the cardioprotective effects of T-type calcium channel blocker mibefradil and compare with that of the angiotensin-converting enzyme inhibitor ramipril in a stroke-prone spontaneously hypertensive rats (SHR-SP) model of congestive heart failure (CHF) after myocardial infarction (MI). **METHODS:** SHR-SP rats were subjected to permanent ligation of the left anterior decending coronary artery. Treatment with mibefradil (10 $mg \cdot kg^{-1} \cdot d^{-1}$), ramipril (10 $mg \cdot kg^{-1} \cdot d^{-1}$), or placebo was initiated 4 weeks before surgery and continued up to 6 weeks after induction of MI. Sham-operated rats served as controls. **RESULTS:** In placebo-treated MI rats, six weeks after MI, left ventricular circumference, inner diameter, and left ventricular end-diastolic pressure (LVEDP) were increased, whereas mean arterial blood pressure (MAP) and maximum rate of rise of left ventricular pressure (dp/dt_{max}) were decreased compared with sham-operated controls ($P<0.01$). In ramipril-treated MI rats, heart weight, heart weight to body weight ratio and interstitial collagen content were reduced ($P<0.05$, $P<0.01$), LVEDP was slightly decreased ($P>0.05$), and dp/dt_{max} was improved ($P<0.01$) compared with placebo-treated MI rats. In contrast, in mibefradil-treated MI rats, heart weight, heart weight to body weight ratio were slightly but not significantly reduced, LVEDP was slightly elevated compared with placebo-treated MI rats, and was elevated ($P<0.05$) compared with ramipril-treated MI rats, although interstitial collagen content were reduced ($P<0.01$) compared with placebo-treated MI rats.**CONCLUSION:** Chronic treatment with ramipril diminishes cardiac remodeling of heart failure after MI to a greater extent than mibefradil. Moreover, 6 weeks after MI, mibefradil treatment results in a slight rise in LVEDP compared with placebo-treated rats. Therefore, treatment with mibefradil might be deleterious rather than beneficial compared with ramipril or even placebo treatment in experimental MI.

INTRODUCTION

Acute myocardial infarction (MI), particularly large and transmural infarctions, can result in complex alterations of cardiac architecture involving both the infarcted and the noninfarcted myocardium. These alterations, referred to as cardiac remodeling, profoundly affected the cardiac function and the prognosis for survival. A large amount of data demonstrated that ACE inhibitors prevented MI-induced cardiac remodeling and thus improved left ventricular function and survival. They have become a cornerstone of therapy for patients with left ventricular dysfunction for nearly 2 decades. Calcium channel blockers are frequently

[1] Correspondence to Prof Qin-gui XIA, MD.
Phn 86-577-8885-6805. E-mail xiaqingui@yahoo.com
Received 2003-04-14 Accepted 2003-10-9

used in the treatment of clinical myocardial ischemia. However, their therapeutic properties in myocardial infarction-induced heart failure is not clearly defined. L-type calcium channel blockers have, to date, shown disappointing effect in chronic heart failure, one explanation for their lack of benefit is that this class of agents exert substantial negative inotropic effects leading to left ventricular dysfunction and/or reflex increases in sympathetic tone. Mibefradil is a selective T-type calcium channel blocker, which does not exert substantial negative inotropic effect, nor does it stimulate the neurohormoral system[1,2]. This suggested that mibefradil might offer unique advantage over previous L-type calcium channel blockers. Mibefradil was found to attenuate cardiac remodeling and improve cardiac function in infarcted normotensive rats[3] and limit infarct size in short-term ischemic/reperfused myocardium in rats[4], dogs[5], and pigs[6]. A clinical trial, the MACH-1 study[7], however, found a trend towards to higher mortality on mibefradil than on placebo in patients with heart failure (NYHA class II to IV; left ventricular ejection fraction <35 %).

Why did good news in experimental study become bad news after clinically effective therapy? Normotensive animal models, which were used in most experimental protocols investigating the effects of mibefradil on chronic heart failure (CHF) after MI, might not fully represent the gradually deteriorated and complex clinical situation of heart failure. This could be one explanation for the contrary data from experimental and clinical use. Ischemic and hypertensive heart diseases are commonly associated pathophysiological conditions of heart failure in humans. Stroke-prone spontaneously hypertensive rats (SHR-SP), a genetic model of hypertension, combined these two parameters. This study therefore was performed to determine whether the T-type calcium channel blocker mibefradil prevents cardiac remodeling and improves left ventricular dysfunction in SHR-SP model of MI-induced heart failure in comparison with the ACE inhibitor ramipril.

MATERIALS AND METHODS

Animals and model of myocardial infarction Male SHR-SP rats weighing 280-300 g were used for the experiments. The rats were housed under controlled conditions of constant temperature and humidity and exposed to a 12-h light/dark cycle. The rats had free access to a standard rat chow (Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. The investigation was under the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was performed in accordance with the German law on animal protection as released in the new version in 1993.

MI was induced by ligation of the left anterior descending coronary artery (LAD) and sham operations were performed as previously described[8].

Experimental protocol Rats were divided randomly into four groups and treated according to the following protocol (n=8-10):

1) Sham operation+placebo (saline 1 mL·kg^{-1}·d^{-1} ig).

2) MI+placebo (saline 1 mL·kg^{-1}·d^{-1}, ig).

3) MI+ramipril (10 mg·kg^{-1}·d^{-1}, ig).

4) MI+mibefradil (10 mg·kg^{-1}·d^{-1}, ig).

The ACE inhibitor ramipril was supplied by Aventis Pharma Deutschland GmbH (Frankfurt/Main, Germany). Mibefradil was provided by Hoffmann-La Roche (Grenzach-Wyhlen, Germany). Ramipril and mibefradil were dissolved in normal saline at a concentration of 10 g/L and administered intragastrically using a gavage needle once daily during the treatment period, and normal saline served as placebo. Treatment was started 4 weeks pre-MI and continued up to 6 weeks post-MI.

The doses of ramipril and mibefradil in the present study were chosen, because after 4 weeks of treatment with ramipril or mibefradil 10 mg·kg^{-1}·d^{-1}, MAP was lowered to a similar extent (135 mmHg) in SHR-SP before MI (unpublished data).

At the end of the treatment period, arterial, venous and left ventricular catheters were chronically implanted. Twenty-four hours later, hemodynamic signals were recorded in conscious rats. After recording, rats were sacrificed and hearts were taken out for morphological examinations.

Hemodynamic studies Under chloral hydrate (200 mg·kg^{-1}, ip) anaesthesia, polypropylene tubes (Portex, London, UK) were inserted into the right femoral artery and vein. A pig-tail catheter consisting of a PP 10 and PP 50 polypropylene tubes was introduced in the left ventricle via the right carotid artery. The catheters were exteriorized and anchored at the posterior neck region.

Blood pressure, HR, and left ventricular pressure were recorded 24 h after catheterization in conscious rats by a computer-based recording and analysing

system. Left ventricular end-diastolic pressure (LVEPP) and maximum rate of rise of left ventricular pressure (dp/dt_{max}) were calculated by offline analysis of digitized pressure signal. MAP, HR, LVEDP, and dp/dt_{max} were averaged over 5-min periods in the statistical analysis.

Cardiac morphological examinations After hemodynamic measurements, rats were anaesthetized with ether and the hearts were arrested in diastole by an intravenous injection of KCl solution. The hearts were excised and the atria and large vessels were removed. The hearts were weighed and then placed in 4 % phosphate-buffered formalin for at least 24 h, and cut transversely into five sections of approximately identical thickness 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 7-μm-thick slices. The slices were mounted onto glass slides and were stained with picrosirius red. Those transections of the midsagittal level were used for morphological analysis with a computerised morphometric system (Quantimet 570, Leica, Cambridge, UK)[8].

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

RESULTS

Effects of mibefradil and ramipril on cardiac morphology After MI, food and water intake was markedly reduced in all animals followed by a decrease of body weight (data not shown). One week post infarct, the food and water intake was normalised and was not different from sham-operated animals any more. At the end of the experiment, no significant difference in body weight was found in the groups (Tab 1).

Cardiac hypertrophy was evaluated by comparing heart weight and heart weight to body weight ratio. Six weeks after MI, heart weight and heart weight to body weight ratio were slightly increased in placebo-treated infarct animals compared with the sham-operated group. Ramipril but not mibefradil decreased both parameters compared with placebo-treated MI group ($P<0.05$, $P<0.01$, Tab 1).

Circumference and inner diameter of left ventricle were used to determine left ventricular dilatation. Myocardial infarction resulted in a marked increase of left ventricular circumference and inner diameter. Both parameters were not modified by ACE inhibitor ramipril or calcium channel blocker mibefradil compared with the placebo treated infarct rats. Septal thickness was reduced in placebo-treated infarct group. This parameter was slightly but not significantly increased in both treatment groups *vs* placebo-treated MI group (Tab 1).

Interstitial collagen content (ICC) was increased after MI in plocebo-treated SHR-SP. The ACE inhibitor ramipril as well as calcium channel blocker mibefradil lowered the deposition of interstitial collagen compared with placebo-treated MI control group ($P<0.01$, Tab 1).

Infarct size was measured at the end of the experiment in all animals. None of the sham-operated rats had evidence of MI, while the coronary ligation animals

Tab 1. Effects of ramipril or mibefradil treatment on morphological parameters. Mean±SEM. $^cP < 0.01$ *vs* sham-operated group. $^eP<0.05$, $^fP<0.01$ *vs* placebo-treated infarct group.

	Sham (n=10)	Placebo (n=9)	Ramipril (n=8)	Mibefradil (n=8)
Body Weight (g)	327±3	324±3	314±2	318±2
Heart weight (g)	1.36±0.04	1.39±0.04	1.15±0.05^e	1.24±0.06
HW/BW (mg/g)	3.98±0.10	4.08±0.14	3.54±0.08^e	3.91±0.15
LV inner diameter (mm)	4.42±0.18	6.66±0.14^c	6.46±0.10	6.70±0.11
LV circumference (mm)	20.8±0.5	24.8±0.8^c	25.2±0.8	26.1±0.7
Septal thickness (mm)	2.81±0.09	2.45±0.10	2.67±0.11	2.53±0.09
ICC (%)	5.19±0.06	6.85±0.13^c	3.68±0.23^f	4.00±0.21^f
Infarct size (%)		26.7±3.0	40.6±2.8^f	42.3±2.0^f

LV, left ventricular; ICC, interstitial collagen content.

had microscopic evidence of transmural infarction. Infarct size was expressed as a percentage of the total circumference of the left ventricle. Infarct size was 26.7 %±3.0 % in placebo treated-MI group, 40.6 %± 2.8 % in ramipril-treated group, and 42.3 %±2.0 % in mibefradil-treated group, respectively. Thus, the infarct size of both treatment groups was larger than that of placebo-treated MI group (P<0.01). Previous studies from Pfeffer's laboratory had demonstrated that the impairment of LV function was directly related to infarct size. In the present study, the infarct size in ramipril or mibefradil treatment groups was larger than that in placebo-treated group, indicating that mibefradil, similar to ramipril, markedly increased the tolerance to infarct size in this experimental model of heart failure (Tab 1).

Effects of mibefradil and ramipril on hemodynamics and left ventricular function MAP was decreased in placebo-treated infarct animals compared with sham-operated rats. Six weeks after MI, ramipril and mibefradil decreased MAP to a similar extent compared with placebo treated infarct rats (P<0.01, Tab 2).

HR did not differ between all groups (Tab 2).

LVEDP was markedly elevated in placebo-treated MI rats; LVEDP was slightly reduced under treatment with ACE inhibitor ramipril, and was slightly increased under treatment with calcium channel blocker mibefradil compared with the placebo-treated infarct group. LVEDP was increased under treatment with mibefradil compared with ramipril-treated group (P<0.05, Tab 2).

The dp/dt_{max} was severely impaired in placebo-treated MI rats; mibefradil slightly reduced dp/dt_{max} and ramipril significantly improved myocardial contractility (Tab 2).

DISCUSSION

The primary purpose of this study was to elucidate why mibefradil failed to improve the outcome of CHF in the MACH-1 study[7], but gave promising data in experimental studies[3-6]. To address this question, we used SHR-SP as a genetic model of arterial hypertensive and cardiac hypertrophy for induction of MI.

Six weeks after MI, in placebo treated MI rats, although almost one third of the left ventricular myocardium was replaced by paper-thin scar tissue, the heart weight and heart weight to body weight ratio was still slightly increased compared with age-matched sham-operated SHR-SP, indicating reactive hypertrophy in the remaining non-infarcted myocardium. In addition, in all MI rats, the interstitial collagen content as well as the left ventricular inner diameter were markedly increased. Furthermore, in placebo-treated MI rats, due to CHF, the MAP was reduced by 32 mmHg, whereas LVEDP was elevated 10-fold, left ventricular dp/dt_{max} was decreased by 80% compared with sham-operated rats. Pfeffer model of heart failure has shown that the moderate infarct size (ie, infarct size was 21 %-39 %), LVEDP was increased by 10-15 mmHg in Wistar rat model of MI-induced CHF. In SHR-SP with similar infarct size, LVEDP was elevated by 25 mmHg in present study. These data indicated that six weeks after MI, SHR-SP had developed more severe LV dysfunction and CHF than the normotensive strains.

Calcium channel blocker therapy in chronic congestive heart failure have yielded disappointing results, because their negative inotropy, sympathetic activation, and cytokine mechanisms worsen cardiac function[9]. Traditional calcium antagonists act on the L-type calcium channel. However, both L- and T-type channels

Tab 2. Effects of ramipril or mibefradil treatment on hemodynamic parameters. Mean±SEM. ^{c}P<0.01 *vs* sham-operated group. ^{f}P<0.01 *vs* placebo-treated infarct group. ^{h}P<0.05, ^{i}P<0.01 *vs* ramipril-treated group.

	Sham (n=10)	Placebo (n= 9)	Ramipril (n=8)	Mibefradil (n=8)
MAP (mmHg)	172±3	140±4^{c}	116±6^{cf}	113±5^{cf}
Heart rate (min^{-1})	413±7	407±15	397±11	386±16
LVEDP (mmHg)	2.6±0.7	28±4^{c}	22±4^{c}	33.5±2.3^{ch}
dp/dt_{max} (mmHg/s)	10360±470	1850±400^{c}	5800±490^{cf}	1640±320^{ci}

MAP, mean arterial blood pressure; LVEDP, left ventricular end-diastolic pressure; dp/dt_{max}, the maximum rate of rise of the left ventricular pressure.

can be found in cardiovascular tissues. L-type channels are found in both adult myocardium and vascular smooth muscle[10]. T-type channels occur in vascular smooth muscle and the pacemaker cells of the sinoatrial node, mediating vascular tone and nodal autoarrhythmicity[11]. In contrast, in atrial and ventricular myocytes, T-type channels are absent, and occur in only under pathophysiological conditions and may affect cellular responses to hypertrophic stimuli[12]. Mibefradil is a selective T-type calcium channel blocker. It does not exert negative inotropic effects nor does it stimulate the neurohormonal system[2,13]. Thus, mibefradil might offer advantage over L-type calcium channel blockers. However, the MACH-1 study showed a slightly higher mortality in CHF patients (NYHA class II to IV) treated with mibefradil within the first 3 months. The interaction of mibefradil with antiarrhythmic drugs (class I or III), including amiodarone, was thought to be the main reason of the poor outcome[7], because the metabolic elimination of many antiarrhythmics was via hepatic cytochrome P-450 enzymes that were competitively inhibited by mibefradil[14]. Thus, coadministration of these drugs with mibefradil may lead to toxic plasma concentrations. Moreover, mibefradil resulted in a higher incidence of first-degree AV block and sinus bradycardia[7]. A study[6] has demonstrated that the infarct size-limiting effect of mibefradil in the ischemic/reperfused pig hearts is via the activation of ATP-sensitive potassium channels (K_{ATP}). However, K_{ATP} openers, such as cromakalim, have been shown to increase the incidence of ventricular arrhythmias and fibrillation in the ischemic/reperfused myocardium[15]. Therefore, a proarrhythmic effect of mibefradil might be one explanation for poor survival after MI. However, we did not investigate survival in the study because of the limited group size. Further studies will address the question.

The results of the present study showed that ramipril treatment significantly decreased heart weight, heart weight to body weight ratio, and interstitial collagen content compared with placebo-treated MI rats. In contrast, mibefradil treatment only slightly but not significantly decreased heart weight and heart weight to body weight ratio, although the interstitial collagen content was reduced. Thus, cardiac remodeling was better diminished under treatment with the ACE inhibitor ramipril than under treatment with mibefradil. Ramipril treatment slightly decreased LVEDP and improved myocardial contractility (LV dp/dt_{max}) compared with placebo-treated infarct rats; whereas LVEDP was even elevated by 19.6 % under mibefradil after MI compared with placebo-treated rats and by 48.9 % compared with ramipril treatment. Therefore, mibefradil does not significantly prevent cardiac remodeling and worsens left ventricular function after MI. These mechanisms may also be relevant for the poor outcome of mibefradil treatment in the MACH-1 study.

In conclusion, the present results showed that the chronic treatment with the ACE inhibitor ramipril prevented cardiac remodeling and improved left ventricular dysfunction, whereas calcium channel blocker mibefradil failed to exert beneficial effects. Moreover, six weeks after MI, mibefradil showed even adverse effects on cardiac hemodynamics compared with placebo treatment in MI-induced heart failure in SHR-SP.

REFERENCES

1 Mulder P, Richard V, Thuillez C. Different effects of calcium antagonists in rat model of heart failure. Cardiology 1998; 89 (Suppl 1): 33-7.

2 Clozel JP, Ertel EA, Ertel SI. Voltage-gated T-type Ca^{2+} channel and heart failure. Proc Assoc Am Physicians 1999; 111: 429-37.

3 Sandmann S, Class R, Cleutjens JP, Daemen MJ, Unger T. Calcium channel blockade limits cardiac remodeling and improves cardiac function in myocardial infarction-induced heart failure in rats. J Cardiovasc Pharmacol 2001; 37: 64-77.

4 Mocanu MM, Gadgil S, Yellon DM, Baxter GF. Mibefradil, a T-type and L-type calcium blocker, limits infarct size through a glibenclamide-sensitive mechanism. Cardiovasc Drugs Ther 1999; 13: 115-22.

5 Vander Heide RS, Schwartz LM, Reimer KA. The novel calcium channel antagonist Ro 40-5967 limits myocardial infarct size in the dog. Cardiovasc Res 1994; 28: 1526-32.

6 Schulz R, Post H, Jalowy A, Backenkohler U, Dorge H, Vahlhaus C, *et al*. Unique cardioprotective action of the new calcium channel antagonist mibefradil. Circulation 1999; 99: 305-11.

7 Levine TB, Bernink PJ, Caspi A, Elkayam U, Geltman EM, Greenberg B, *et al*. Effect of mibefradil, a T-type calcium channel blocker, on morbidity and mortality in moderate to severe congestive heart failure: the MACH-1 study. Mortality assessment in congestive heart failure trial. Circulation 2000; 101: 758-64.

8 Xia QG, Chung O, Spitznagel H, Sandmann S, Illner S, Rossius B, *et al*. Effects of a novel angiotensin AT_1 receptor antagonist, HR720, on rats with myocardial infarction. Eur J Pharmacol 1999; 385 (2-3): 171-9.

9 Cohn J. The management of chronic heart failure. N Engl J Med 1996; 335: 490-8.

10 Katz A. Calcium channel diversity in the cardiovascular system. J Am Coll Cardiol 1996; 28: 522-9.

11 Ertel SI, Ertel EA, Clozel JP. T-type Ca^{2+} channels and pharmacological blockade: potential pathophysiological

relevance. Cardiovasc Drugs Ther 1997; 11: 723-39.
12 Sen L, Smith TW. T-type Ca^{2+} channels are abnormal in genetically determined cardiomyopathic hamster hearts. Circ Res 1994; 75: 149-55.
13 Billups SJ, Carter BL. Mibefradil: a new class of calcium-channel antagonists. Ann Pharmacother 1998; 32: 659-71.
14 Brogden R, Markham A. Mibefradil: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of hypertension and angina pectoris. Drugs 1997; 54: 774-93.
15 Wilde AAM. Role of ATP-sensitive K^+ channel current in ischemic arrhythmias. Cardiovasc Drugs Ther 1993; 7 (Suppl 3): 521-6.