Effects of the calcium channel antagonist mibefradil on haemodynamic and morphological parameters in myocardial infarction-induced cardiac failure in rats

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Abstract

Objective: Calcium channel antagonists (CCA) have been proposed for the prevention of cardiac events after myocardial infarction (MI). Mibefradil is a CCA featuring a selective blockade of T-type Ca\textsuperscript{2+}-channels. The aim of the study was to characterize the effects of mibefradil on haemodynamic and morphological parameters in a model of postMI chronic heart failure and to establish the "therapeutic window" for the start of therapy.

Methods: MI was induced by permanent ligation of the left coronary artery in male normotensive Wistar rats. Animals were assigned to placebo- or mibefradil-treated (10 mg/kg/day p.o.) groups as follows: (1) sham operation; (2) MI placebo treatment; (3) 7 days preMI start of treatment; (4) 3 h postMI start of treatment; (5) 24 h postMI start of treatment; (6) 3 days postMI start of treatment; (7) 7 days postMI start of treatment. Treatment was continued for 6 weeks postMI. At this time point, mean arterial blood pressure (MAP), heart rate, left ventricular enddiastolic pressure (LVEDP) and contraction force (dP/dt) were measured in conscious rats at baseline and after methoxamine (MEX; 0.5–1.0 mg/h i.v.) stimulation to increase afterload. The hearts were subjected to histological determination of infarct size (IS), infarct length (IL), noninfarcted length (NL), left ventricular circumference (LVC), inner LV-diameter (LVD) and septal thickness (ST).

Results: Six weeks after MI, MAP was lowered, LVEDP increased and dP/dt reduced. Mibefradil treatment increased basal MAP in groups 3–5 compared to the placebo-treated MI group. Under mibefradil, LVEDP was reduced at baseline in groups 3–6 and, after MEX, in all groups. dP/dt was reduced in groups 3–4 at baseline and after MEX. In the placebo-treated MI group, the infarcted area was 39% of the LV and heart weight, LVD and LVC were increased. Heart weights of mibefradil-treated rats (groups 3–6) did not differ from those of the placebo-treated group. Early onset of treatment with mibefradil reduced IS and IL and increased NL in groups 3–4. LVD and LVC were decreased in group 3 only. ST was increased in groups 3–5.

Conclusion: Chronic treatment with mibefradil exerts beneficial actions on cardiac structure and performance in postMI cardiac failure in rats, especially when the onset of treatment is either prior to or within hours after the acute ischemic event.

Keywords: Calcium channel blockade; T-type calcium channel; Mibefradil; Myocardial infarction; Rat

1. Introduction

Calcium channel antagonists (CCA), potent vasodilators widely used in the treatment of hypertension and angina pectoris [1], have also been proposed for the prevention of cardiac events after myocardial infarction (MI) [2]. Despite different chemical structures, most of the CCA used today act via inhibition of the slow Ca\textsuperscript{2+}-inward current through L-type Ca\textsuperscript{2+}-channels [3,4]. Three main classes of CCA have been described: dihydropyridines (e.g. nifedipine), phenylalkylamines (e.g. verapamil) and benzoiazepines (e.g. diltiazem). Whereas each of these drug classes has its own advantages, unwarranted effects, e.g.,
neurohormonal activation, AV-blockade, arrhythmias or negative inotropic actions, often limit their use in cardiac therapy. Dihydropyridine-type CCA are very potent peripheral vasodilators and can, therefore, produce reflex tachycardia [5]. In addition, negative inotropic effects have been demonstrated in vitro and in vivo with CCA such as nifedipine [6] and verapamil [7,8], which render these compounds potentially dangerous in patients with a compromised myocardial function [9,10]. Diltiazem appears to exert less negative inotropic effect than other CCA in normal animals [11,12], but has been shown to impair left ventricular function in dogs with volume overload-induced cardiac hypertrophy and to produce severe bradycardia [13]. Furthermore, diltiazem has been shown to increase the mortality in patients with clinical signs of heart failure following MI [14]. Possible explanations of these adverse effects of CCA are their depressant effects on cardiac function through direct negative inotropic and dromotropic actions on the myocardium and neurohormonal activation which can produce cellular damage [15,16].

Mibefradil is a novel tetrazolium CCA featuring an inhibition of both, the L- and T-type Ca$^{2+}$-channels, with a higher selectivity for T-type Ca$^{2+}$-channels with respect to L-type Ca$^{2+}$-channels [17]. Mibefradil has preferential coronary vasodilative effects and, in animal experiments, this compound was shown to increase coronary blood flow during ischemia induced by lowering coronary perfusion pressure in dogs [18]. Pharmacological and clinical evidence has been presented that mibefradil does not exert negative inotropic actions in isolated rat hearts [19] and in patients with stable angina pectoris [20]. Thus, mibefradil might be a safer drug than other CCA in patients with MI, especially when left ventricular dysfunction occurs.

The aim of the present study was to characterize the effects of a long-term treatment with mibefradil on cardiac function and morphology in a rat model of chronic heart failure after MI. Mibefradil treatment was started at different time points prior to and after permanent coronary ligation to establish the optimal time point for commencing therapy.

2. Methods

Male normotensive Wistar rats (Charles River Viga, Sulzfeld, Germany) initially weighing 230–270 g were used in the study. All experiments were performed in accordance with the German law on animal protection as released in its new version in 1993. The animals were housed individually at controlled temperature and humidity under a 12-h-light/dark-cycle. Rats had free access to a standard diet (Altromin®, Altromin International GmbH u.Co.Kg., PF 1120, 32770 Lage-Lippe, Germany) and to drinking water. They were divided randomly into seven groups: sham operation, control MI and placebo treatment and groups subjected to mibefradil treatment (10 mg/kg/day p.o.) with the start of treatment at different time points before and after MI as indicated below (see Fig. 1):

1. sham (sham surgery, without treatment, $n=15$)
2. placebo-treated MI (control MI, $n=15$)
3. 7d preMI (MI, start of treatment 1 week prior to infarction, $n=13$)
4. 3h postMI (MI, start of treatment 3 h after infarction, $n=13$)
5. 24h postMI (MI, start of treatment 24 h after infarction, $n=14$)
6. 3d postMI (MI, start of treatment 3 days after infarction, $n=13$)
7. 7d postMI (MI, start of treatment 7 days after infarction, $n=13$).

After one week in single cages, rats in group 1 underwent a sham operation. In the remainder of rats, MI was induced by permanent ligation of the left coronary artery. Food and water intake as well as body weight (BW) were function through direct negative inotropic and dromotropic went a sham operation. In the remainder of rats, MI was

Mibefradil treatment was started at different time points prior to and after permanent coronary ligation to establish the optimal time point for commencing therapy.

2.1. Surgical procedures

MI was induced by permanent ligation of the left coronary artery using a modified version of the technique described by Johns and Olson [21]. Briefly, rats were anaesthetized with ether to cannulate the tail vein for intravenous injections of methohexital-sodium (10 mg/kg). The chest of the animals was shaved and disinfected. Rats were intubated and artificially ventilated with room air (50 hubs/min, 200 mmH$_2$O, 1.5 ml/hub). The electrocardiogram (ECG) was monitored continuously during surgery. A left thoracotomy was started by incising the skin 2 cm parallel to the third and fourth rib. The pectoral muscles were dislocated to expose the ribs and the incision was done at the fourth intercostal space to insert a rib-spreadng chest retractor. After anterior pericardectomy, the heart was exposed. The left coronary artery was then ligated intrathoracically using sterile 6-0 suture material (Ethibond, Ethicon, Norderstedt, Germany) under a stereomicroscope. Successful ligation of the coronary artery was verified by the occurrence of arrhythmias in the
Study Design:

-1  MI  1  2  3  4  5  6 weeks

Start of treatment:

control MI, placebo (2), n=15/47
Mibefradil 7 d pre (3), n=13/31
Mibefradil 3 h post (4), n=13/34
Mibefradil 24 h post (5), n=14/35
Mibefradil 3 d post (6), n=13/34
Mibefradil 7 d post (7), n=13/39

End of study

Fig. 1. Time-table of experimental protocol (study design).

ECG and, visually, by observing the colour changes of the ischemic area. In rats that underwent sham surgery, ligation was placed beside the coronary artery. The thoracic cavity was closed during expiration hold. At the end of the operation procedure, analgesia was induced by a subcutaneous injection of buprenorphine-HCl (0.2 mg/kg).

2.2. Arterial, venous and left ventricular catheters

At the end of the study, 6 weeks after MI, the animals were anaesthetized with ether. Anaesthesia was continued by intravenous injection of methohexitol-sodium (10 mg/kg). Femoral arterial and venous catheters were chronically implanted using a procedure described elsewhere [22]. Briefly, polypropylene tubes (Portex, London, UK) were inserted into the right femoral artery and vein and exteriorized at the nape of the neck. The left ventricle was then cannulated using a specially constructed pig-tail catheter consisting of a PP10 tube 60 mm in length welded to a 350 mm length of PP50 tube [23]. The pig-tail at the end of the PP10 portion was inserted into the right carotid artery and advanced into the left ventricle via the ascending aorta. During cannulation, the catheter was connected to a transducer and blood pressure monitor to verify the position of the tip of the catheter. The left ventricle was considered to be reached when the pulse pressure had a typical left ventricular configuration. The PP50 portion was then tunnelled under the skin and anchored at the posterior neck region.

2.3. Haemodynamic measurements

In preliminary experiments, the femoral artery was cannulated to measure arterial blood pressure and heart rate (HR) during surgery and during the 48 h time interval after induction of MI or sham operation.

Six weeks after induction of MI haemodynamic measurements were performed 24 h after the implantation of the catheters in conscious rats as described previously [23]. The femoral artery- and left ventricular-catheter were connected to the pressure transducers (DTX/Plus, Spectramed, Oxnard, CA, USA). Mean arterial blood pressure (MAP), HR and left ventricular pressure (LVP) were obtained using two pressure processors (Gould, Valley View, OH, USA). The output signals were recorded on a pen recorder (Gould Series 2000, Gould) and analysed by a computer-based system MEGA [24] at a rate of 800 Hz. The computer program calculated the left ventricular enddiastolic pressure (LVEDP) and the maximum positive change in the left ventricular pressure signal (dP/dt_{max}) (1000 mmHg/s). The last parameter was considered as a marker of the myocardial contractility. Since ECG was not recorded in this part of the study, LVEDP was measured at the point where the slope of the ventricular pressure signal changed from the slow to the rapidly increasing portion.
This point has been shown to be closely linked to the R-wave of the ECG and to represent LVEDP in rats [23]. MI was clearly detectable with scar tissue showing a green colour and the remaining myocardium a red colour. For morphometric measurement of IS and the extent of ventricular dilatation, a computerised 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. Endocardial and epicardial circumference of the whole left ventricle and of the scar tissue were outlined by the computer. The borderline between the infarcted area and the remaining myocardial muscle was exactly marked with a pointer. Thereafter, the IS was calculated by the system as the percentage of the LVC [27,28].

$$\text{IS} \, (\%) = \left[ \left( \frac{\text{epicardial infarct length}}{\text{total epicardial circumference}} + \frac{\text{endocardial infarct length}}{\text{total endocardial circumference}} \right) \times \frac{1}{2} \right] \times 100$$

In addition, the average ST was determined as the septal area enclosed by two lines originating from the center of gravity of the left ventricular endocardial circumference, which connected the two origins of right ventricular free wall, divided by the average of right and left ventricular surface length [29]. The determination of IT was measured in the same way as ST. Total IL, NL, mean LVC and LVD of the transsection were also calculated by the computer according to the formulae below:

$$\text{ST} \, (\text{mm}) = \frac{\text{area of the septum}}{\text{mean circumference length of the septum}}$$

$$\text{IT} \, (\text{mm}) = \frac{\text{infarction area}}{\text{mean circumference length of the infarction area}}$$

$$\text{IL} \, (\text{mm}) = \left[ \text{endocardial} + \text{epicardial infarct length} \right] \times \frac{1}{2}$$

$$\text{NL} \, (\text{mm}) = \left[ \text{endocardial} + \text{epicardial length of noninfarcted myocardium} \right] \times \frac{1}{2}$$

$$\text{LVC} \, (\text{mm}) = \left[ \text{endocardial} + \text{epicardial length of the left ventricle} \right] \times \frac{1}{2}$$

$$\text{LVD} \, (\text{mm}) = \frac{\text{endocardial circumference}}{\pi}$$

2.6. Survival rate

The survival rate was calculated for each group 1 day, 3 days, 7 days, 3 weeks and 6 weeks after induction of MI or sham operation and is expressed as decimal fraction of the number of animals included in the study. No statistical analysis of survival rates was performed because the
number of rats in each group was too low to yield statistically valid data.

2.7. Drugs

Mibefradil was kindly provided by Hoffmann-La Roche (Grenzach-Wyhlen, Germany). In preliminary experiments, the dosage of 10 mg/kg/day mibefradil chronically administered via gastric gavage did not significantly influence MAP in infarcted or sham-operated animals. In these experiments, a 24 h blood pressure profile was measured in conscious rats beginning immediately after p.o. application of mibefradil (5–15 mg/kg/day) to determine a dose which did not affect cardiac function under control conditions.

2.8. Statistical analysis

Statistical evaluation of obtained haemodynamic and morphometric data was performed using one-way analysis of variance with repeated measures. Means shown to be different between individual groups were compared using the posthoc Student’s t-test and were considered significant at p<0.05. Data were expressed as mean±standard error of the mean (S.E.M.).

3. Results

3.1. Morphology

After induction of MI, food and water intake was reduced in all animals followed by a decrease of BW (data not shown). Seven days after MI the food and water intake was normalized and was not different from sham-operated animals any more. During the following weeks of the experiment, no differences in food and water intake as well as BW were observed between the groups.

Cardiac weight was examined by determining the ratio of THW to BW. THW/BW was increased in the placebo-treated MI group when compared to the sham-operated days after induction of MI. IT was not different between the placebo-treated MI group and in the 7d postMI mibefradil-treated group compared to the sham-operated group. Cardiac weights of 7d preMI, 3h postMI, 24h postMI and 3d postMI mibefradil-treated groups tended to decrease when compared to the placebo-treated MI group but this difference was not statistically significant (Table 1).

The IS is shown in Fig. 2 and the IL in Table 1. The IS was determined as percentage of left ventricular circumference (%) 6 weeks after induction of myocardial infarction (MI). Indicated are animals subjected to control MI animals (placebo, black column) and mibefradil-treated animals (10 mg/kg/day p.o.) started at different time points before and after induction of MI (striped columns, 7d preMI, 3h postMI, 24h postMI, 3d postMI, 7d postMI). Data represent mean±S.E.M., n=12–15. * Significant versus control MI (p<0.05).

The LVD and LVC were used to determine left ventricular expansion. LVD (Fig. 3) and LVC (Table 1) were increased in the placebo-treated MI group when compared to the sham group. Both parameters, were significantly increased in the placebo-treated MI group when compared to the sham group. Both parameters, were significantly

![Graph showing infarct size (IS) as percentage of left ventricular circumference (%) 6 weeks after induction of myocardial infarction (MI). Indicated are animals subjected to control MI animals (placebo, black column) and mibefradil-treated animals (10 mg/kg/day p.o.) started at different time points before and after induction of MI (striped columns, 7d preMI, 3h postMI, 24h postMI, 3d postMI, 7d postMI). Data represent mean±S.E.M., n=12–15. * Significant versus control MI (p<0.05).](https://academic.oup.com/cardiovascres/article-abstract/39/2/339/286805/312x561)
reduced by mibefradil treatment when begun 7 days preMI and tended to decrease when mibefradil treatment was commenced 3 h postMI as compared to the placebo-treated MI group. No differences in LVD and LVC with respect to the placebo-treated MI group were detected when mibefradil treatment was commenced 24 h, 3 days and 7 days postMI.

NL was increased in 7d preMI and 3h postMI mibefradil-treated groups as compared to the placebo-treated MI group. No differences in NL compared to the placebo-treated MI group were detected when mibefradil treatment was commenced between 24 h postMI and 7 days postMI (Table 1).

ST was reduced in the placebo-treated MI group as compared to the sham group (Fig. 4). ST was increased in all mibefradil-treated groups as compared to the placebo-treated MI group and was, in addition, increased in 7d preMI and 3h postMI mibefradil-treated groups as compared to the sham group. Left ventricle noninfarcted area (NL×ST) was increased in 7d preMI, 3h postMI and 24h postMI mibefradil-treated groups compared to the placebo-treated MI group (Table 1).

### 3.2. Haemodynamics

MAP, measured in separate groups (n=6–8) during surgery and during the 48 h time interval after operation, was reduced in the placebo-treated MI group and in mibefradil-treated groups (7d preMI and 3h postMI) immediately after MI and during the following 48 h period as compared to sham-operated animals. No significant differences were seen between the placebo-treated MI group and the mibefradil-treated groups (Fig. 5A). HR of the placebo-treated MI group and 7d preMI and 3h postMI mibefradil-treated groups was increased compared to the sham group. Animals of the 7d preMI mibefradil-treated group showed a tendency for reduction in HR compared to the placebo-treated MI group (Fig. 5B).

Six weeks after surgery, a decrease of MAP (Table 2), increase of LVEDP and decrease of cardiac contractility at baseline (Fig. 6A, Fig. 7A) and after methoxamine stimulation (Fig. 6B, Fig. 7B) were observed in the placebo-treated, infarcted animals when compared to sham-operated animals. To test cardiac function after increased afterload, a methoxamine infusion was initiated and steadily increased until MAP was elevated in all groups by 20 mmHg as compared to baseline. Animals of the placebo-treated MI group had to be infused with higher doses of methoxamine to increase blood pressure by 20 mmHg as compared to sham-operated animals (Table 2). In contrast, the consumption of methoxamine to increase blood pressure by 20 mmHg was reduced in the mibefradil-treated groups when treatment was begun 7 days preMI or 3 h postMI (Table 2).

Six weeks after induction of MI, MAP was decreased in all infarcted groups as compared to the sham-operated group. The mibefradil-treated animals in the 7d preMI, 3h postMI and 24h postMI treated groups showed higher MAP values at baseline compared to the placebo-treated MI group (Table 2).

HR at week 6 after MI/sham surgery did not differ significantly among the seven groups at baseline or after methoxamine infusion (Table 2).

Placebo-treated MI rats had higher LVEDPs at baseline and during increased afterload than those that underwent sham surgery. In the mibefradil-treated groups 7d preMI,
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=15)</th>
<th>Placebo-treated MI (n=15)</th>
<th>7d preMI (n=13)</th>
<th>3h post MI (n=12)</th>
<th>24h postMI (n=14)</th>
<th>3d postMI (n=13)</th>
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<tr>
<td>MAP (mmHg) baseline</td>
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<td>102±3</td>
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<td>MAP (mmHg) stimulation</td>
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<td>115±4</td>
<td>125±2</td>
<td>123±3</td>
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<td>HR (beats/min) stimulation</td>
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<td>329±6</td>
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<tr>
<td>MEX infusion (mg/h)</td>
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<td>0.91±0.132</td>
<td>0.67±0.059</td>
<td>0.71±0.062</td>
<td>0.74±0.124</td>
<td>0.79±0.117</td>
<td>0.81±0.127</td>
</tr>
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</table>

* Significant versus placebo-treated MI (p<0.05); ** significant versus sham (p<0.05).

Indicated are animals that underwent sham surgery (sham), placebo-treated MI rats and rats treated with mibefradil (10 mg/kg/day) beginning 7 days before (7d preMI), 3 h, 24 h, 3 days and 7 days postinduction of MI. Data are presented as mean±S.E.M.: mean arterial blood pressure (MAP) and heart rate (HR) under basal conditions (baseline) and after methoxamine infusion (stimulation) and methoxamine infusion (MEX).
Fig. 6. (A) Basal left ventricular enddiastolic pressure (LVEDP) 6 weeks after induction of myocardial infarction (MI). Indicated are animals subjected to sham surgery (sham, white column), control MI animals (placebo, black column) and mibefradil-treated animals (10 mg/kg/day p.o.) started at different time points before and after induction of MI (striped columns, 7d preMI, 3h postMI, 24h postMI, 3d postMI, 7d postMI). Data represent mean±SEM, n=12±15. * Significant versus control MI (p<0.05), † significant versus sham (p<0.05). (B) Left ventricular enddiastolic pressure after increased afterload (methoxamine stimulation) (LVEDP) 6 weeks after induction of myocardial infarction (MI). Indicated are animals subjected to sham surgery (sham, white column), control MI animals (placebo, black column) and mibefradil-treated animals (10 mg/kg/day p.o.) started at different time points before and after induction of MI (striped columns, 7d preMI, 3h postMI, 24h postMI, 3d postMI, 7d postMI). Data represent mean±SEM, n=12±15. * Significant versus control MI (p<0.05), † significant versus sham (p<0.05).

Fig. 7. (A) Basal cardiac contractility (dP/dt<sub>max</sub>) 6 weeks after induction of myocardial infarction (MI). Indicated are animals subjected to sham surgery (sham, white column), control MI animals (placebo, black column) and mibefradil-treated animals (10 mg/kg/day p.o.) started at different time points before and after induction of MI (striped columns, 7d preMI, 3h postMI, 24h postMI, 3d postMI, 7d postMI). Data represent mean±SEM, n=12±15. * Significant versus control MI (p<0.05), † significant versus sham (p<0.05). (B) Cardiac contractility after increased afterload (methoxamine stimulation) (dP/dt<sub>max</sub>) 6 weeks after induction of myocardial infarction (MI). Indicated are animals subjected to sham surgery (sham, white column), control MI animals (placebo, black column) and mibefradil-treated animals (10 mg/kg/day p.o.) started at different time points before and after induction of MI (striped columns, 7d preMI, 3h postMI, 24h postMI, 3d postMI, 7d postMI). Data represent mean±SEM, n=12±15. * Significant versus control MI (p<0.05), † significant versus sham (p<0.05).

4. Discussion

The purpose of this investigation was to examine the effects of chronic treatment with the CCA, mibefradil, on haemodynamic and morphological parameters following MI. Six weeks after permanent ligation of the left anterior coronary artery, ventricular performance was assessed at baseline and after increased afterload induced by methoxamine infusion. Morphometric examinations were performed to determine the degree of alterations of the morphological parameters (remodeling) of the left ventricle after MI.

The experimental model used in the present experiments features pronounced heart failure 6 weeks after the induction of MI. At this time point, remodeling usually has taken place and stable haemodynamic conditions can be expected [30]. Our results show an increased LVEDP and an impaired myocardial contractility (dP/dt<sub>max</sub>) in animals of the placebo-treated MI group. The changes of both
parameters were accompanied by a reduction of MAP. Eccentric cardiac hypertrophy was present, which was evidenced by a left ventricle dilatation and increase in cardiac weight without an increase in ST thickness. Results of several studies demonstrated that this dilative hypertrophy is accompanied by cardiac fibrosis as evidenced by the accumulation of collagen and an elevated stiffness of the infarcted and noninfarcted hypertrophied areas [31,32]. Compared to placebo-treated MI rats, LVEDP at baseline was significantly lowered in mibefradil-pretreated animals and when treatment was started within 3 days after MI at baseline, and also in all mibefradil-treated groups during increased afterload. The observed effects of mibefradil on LVEDP are partly attributed to a reduction in afterload. However, preload plays also an important role in influencing LVEDP and might contribute to the reduction of cardiac hypertrophy [60]. The effect on LVEDP was accompanied by an increased \( \frac{dP}{dt_{\text{max}}} \) under basal conditions and during increased afterload in animals in which treatment was started 7 days prior to and 3 h after coronary artery ligation. This finding indicates an improved myocardial contractility in animals with early-onset treatment. Indeed, LVEDP and \( \frac{dP}{dt_{\text{max}}} \) of 7 day pretreated rats were not significantly different from sham-operated animals.

The lack of a negative inotropic effect of mibefradil in our experiments is in agreement with results of other studies, which have shown that mibefradil had no adverse effect on contractile function in a rat model of heart failure, whereas verapamil depressed cardiac function in this model [33,34], and that mibefradil exerted less negative inotropic actions than diltiazem [35]. Another study has shown that mibefradil had a tenfold lower potency than verapamil for reducing myocardial contractility [36]. Negative inotropy has also been demonstrated in vitro and in vivo in normal hearts with CCA such as nifedipine and verapamil [6,7]. Diltiazem appears to afford less cardio-suppression than other CCA in normal animals [6,11,12] but impaired left ventricular function in conscious dogs with volume overload-induced cardiac hypertrophy [13] and in the isolated hypertrophied failing heart [37] at a dose which did not affect cardiac function in the control state.

Some CCA of the dihydropyridine type stimulate the sympathetic nervous system which is reflected by an elevation of HR as well as of circulating adrenaline- and noradrenaline levels [38–40]. A tachycardic response to mibefradil was not observed in the present study, rather a tendency to a decreased HR which was, however, statistically not significant. In addition, mibefradil pretreated MI animals showed a slight decrease in HR during the acute phase after operation and during the 48 h time interval after induction of MI. These findings could be partly explained by the fact that cardiac T-channels, preferentially blocked by mibefradil, are located primarily in the sinus node [41]. Our data are in agreement with results of other studies showing that mibefradil prevented ischemia-induced sympathetic activation and reflex tachycardia [18,34,42].

Coronary occlusion may induce hypotension, which results in baroreceptor activation and subsequent systemic reflex vasoconstriction thus worsening the imbalance between myocardial oxygen demand and supply [43–45]. Moreover, after coronary occlusion, lactate, the end product of glycolysis, accumulates in the tissue as a consequence of the anaerobic metabolism in the myocytes. In addition, myocardial injury during ischemia or after infarction is thought to be associated with a cellular accumulation of calcium [46]. It is possible that, in our experiments, early-onset of mibefradil treatment protected the heart by increasing oxygen supply due to an improved perfusion of the myocardium in the noninfarcted area and, especially, in the marginal zone of the infarcted area (“area at risk”). The dilatative action of mibefradil on coronary arteries is thought to be mediated by a direct inhibition of vascular smooth muscle contraction via L- and/or T-type Ca\(^{2+}\)-channels as well as an endothelium-dependent relaxation by increasing the release of endothelium-derived relaxing factor [66]. A marked vasodilatory action of mibefradil on the coronary arteries has previously been demonstrated in isolated, perfused guinea pig hearts showing that mibefradil produced a larger increase in coronary blood flow and, at the same time, a smaller decrease in left ventricular pressure than other CCA [36]. Furthermore, in contrast to mibefradil, the doses needed to double coronary blood flow of other, nondihydropyridine CCA such as verapamil and diltiazem, were very close to those producing first-degree AV-block which limited the therapeutic use of these drugs [47,48]. Mibefradil treatment might thus have resulted in a reduction in the rate of the ischemic anaerobic metabolism of the myocardium after MI reducing the calcium overload of the myocytes. The latter effect engenders a lowering of the energy demand for the maintenance of the calcium homeostasis thereby improving the energy state of the myocytes. In sustained ischemia, a reduction of energy metabolism has been shown to reduce cellular damage [49].

An IS limiting property of mibefradil has already been shown in the reperfused infarction model of dogs [50]. In our experiments, when mibefradil treatment was commenced 7 days prior to and 3 h after induction of MI, IS and IL were significantly reduced accompanied by a significant increase of the NL in these two groups. In addition, LVD and LVC were reduced when mibefradil treatment was started 7 days prior to and 3 h after coronary artery ligation. These data indicate that myocardial structure postMI is best preserved when mibefradil treatment has already been commenced before or is installed within 3 h after the acute event.

An interesting finding of this study is that in all mibefradil-treated groups except the 7d postMI group, heart weight was not increased over sham-operated animals. If an increase of total cardiac weight can be taken as
a reflection of cardiac remodeling, these findings suggest that remodeling was not entirely dependent on the effects of treatment on IS but seemed to have an independent drug-related component. Evidence has been presented that T-type Ca\(^{2+}\)-channels can promote cell growth and proliferation [51,52]. In addition, T-type Ca\(^{2+}\)-channels are overexpressed in failing hearts [53] but not in healthy adult rat hearts [64,65]. In support of these findings, in myocytes of cardiomyopathic hamsters the density of T-type Ca\(^{2+}\)-channel currents was increased, and abnormal channel activity and inactivation kinetics were observed, whereas the density of L-type Ca\(^{2+}\)-channel currents and activity were constant [61]. Thus, the cardioprotective effects of mibefradil might be more pronounced in the failing heart which is related to an increased expression of T-type Ca\(^{2+}\)-channels. An antiproliferative effect of mibefradil in rat arteries has already been reported [54]. Based on these findings, it is tempting to speculate that mibefradil, through its effect on T-type Ca\(^{2+}\)-channels, reduced postMI cardiac hypertrophy to a greater extent than what could have been expected by the reduction of IS alone. The higher number and activity of T-type Ca\(^{2+}\)-channels under hypertrophic conditions suggests an influence of this channel type on intracellular Ca\(^{2+}\)-levels. A contribution of this channel type in remodeling processes in heart failure could explain the antihypertrophic and antiproliferative effects of mibefradil due to its T-channel blocking action. The effects of mibefradil are not only related to T-type Ca\(^{2+}\)-channels because mibefradil has recently been shown to inhibit calcium-activated Cl\(^{-}\}-channels in endothelial cells followed by a concomitant hyperpolarisation indicating a modulation of the Ca\(^{2+}\)-signalling mechanism [62]. This fact might contribute to the complex cardiovascular actions of the compound.

The specificity of the beneficial effects of CCA in the ischemic heart is still a matter of debate. According to a widely discussed hypothesis, increased intracellular calcium after ischemia is a major mediator of structural and functional deterioration of the myocardium. Previous studies have demonstrated that cardiac necrosis can be prevented by verapamil [55,56]. Studies using a model of permanent coronary occlusion in the rat demonstrated in animals receiving an intravenous infusion of diltiazem 20 min before and during 60 min after ligation, that myocardial necrosis was significantly reduced and the cardiac metabolic status was improved [57]. In another study, a single injection of diltiazem significantly preserved total myocardial creatine kinase activity and reduced IS [58]. These results suggest but do not prove directly that cardiac protection by CCA is afforded by their inhibitory action on ischemia-induced calcium overload. More direct support for the above hypothesis comes from studies with mibefradil. Pretreatment of dog coronary arterial vascular muscle cells with this drug significantly reduced intracellular calcium activity during stimulation with noradrenaline [59]. In addition, our own unpublished findings show that mibefradil pretreatment prevented an increase of intracellular calcium concentration after MI in the rat papillary muscle (Sandmann et al., in preparation). The inhibition of calcium entry into ischemic myocytes could stem from the fact that mibefradil blocks calcium entry more potently in depolarised cells, because ischemic myocardial tissue is depolarised [67].

The present study demonstrated that mibefradil treatment starting either 7 days prior to or within 3 days postMI improved morphological and haemodynamic parameters after 6 weeks. In these four groups, fewer animals had to be operated in order to obtain adequate numbers of animals surviving the protocol as compared to placebo-treated or 7d postMI mibefradil-treated groups. This suggests that mibefradil treatment may reduce mortality and prolong life in rats when initiated within the first days after MI. This hypothesis is supported by experiments performed over a 9 month period in rats with chronic heart failure demonstrating that mibefradil improved survival to the same extent as the angiotensin-conversion-enzyme (ACE) inhibitor cilazapril [63].

In conclusion, our data demonstrate that early-onset treatment with the preferentially T-type Ca\(^{2+}\)-channel blocking agent, mibefradil, improves cardiac performance and reduces IS as well as cardiac remodeling in chronic heart failure postMI. Cardioprotection was greatest when treatment was begun before or within 3 h after the acute ischemic event. Several features of selective T-type Ca\(^{2+}\)-channel blockade may have contributed to mibefradil’s beneficial action on the heart including the lack of sympatho-activation and of negative inotropy as well as an inhibition of growth effects mediated through this particular calcium channel. Whether or not an improved coronary perfusion in the marginal zone of the infarct and/or a prevention of ischemia-induced cellular calcium overload contribute to the effects of mibefradil needs to be further investigated.

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