Heart rate reduction by zatebradine reduces infarct size and mortality but promotes remodeling in rats with experimental myocardial infarction

Kai Hu, Anne Naumann, Daniela Fraccarollo, Peter Gaudron, Jens J. Kaden, Stefan Neubauer, and Georg Ertl. Heart rate reduction by zatebradine reduces infarct size and mortality but promotes remodeling in rats with experimental myocardial infarction. Am J Physiol Heart Circ Physiol 286: H1281–H1288, 2004; 10.1152/ajpheart.00390.2003.—The importance of heart rate for left ventricular remodeling and prognosis after myocardial infarction is not known. We examined the contribution of heart rate reduction by zatebradine, a direct sinus node inhibitor without negative inotropic effects on left ventricular function and dilatation, on mortality, energy metabolism, and neurohormonal changes in rats with experimental myocardial infarction (MI). Thirty minutes after left coronary artery ligation or sham operation, the rats were randomized to receive either placebo or zatebradine (100 mg·kg⁻¹·day⁻¹ per gavage) continued for 8 wk. Mortality during 8 wk was 33.3% in the placebo and 23.0% in the zatebradine group (P < 0.05); MI size was 36 ± 2% and 30 ± 1% (means ± SE, P < 0.05), respectively. Zatebradine improved stroke volume index in all treated rats but increased left ventricular volume in rats with small MI (2.43 ± 0.10 vs. 1.81 ± 0.10 ml/kg, P < 0.05) but not in rats with large MI (2.34 ± 0.09 vs. 2.35 ± 0.11 ml/kg, not significant). Zatebradine reduced left and right ventricular norepinephrine and increased left and right ventricular 3,4-dihydroxyphenyl ethylene glycol-to-norepinephrine ratio suggesting aggravation of cardiac sympathetic activation by zatebradine after MI. Creatine kinase and lactate dehydrogenase isoenzymes in rats with MI remained unchanged by zatebradine. Lowering heart rate per se reduces mortality and MI size in this model but induces adverse effects on left ventricular remodeling in rats with small MI.

METHODS

Animals, Experimental Myocardial Infarction, and Pharmacological Interventions

Coronary artery ligation or sham operations were performed in 12-wk-old adult female Wistar rats as described previously (10). Treatments were started by gavage 30 min after coronary artery ligation and followed daily for 8 wk. Animals were housed in polyethylene (PE) cages in climatized rooms with a 12:12-h light-dark cycle and fed standard laboratory food and tap water. All procedures conformed to the guiding principles of the American Physiological Society.

Dose-Finding Study

Dose-response curves of zatebradine on heart rate were obtained in pilot experiments. Rats were divided into four groups (n = 5 in each group): group 1 consisted of placebo-treated controls and groups 2–4 were treated with zatebradine (38, 75, and 100 mg·kg⁻¹·day⁻¹ per gavage, respectively). On the day of the study, heart rate was recorded.
under ether anesthesia at 3, 12, and 24 h with ECG in rats with various treatment protocols. A dose of 100 mg·kg⁻¹·day⁻¹ reduced heart rate by 20% over 24 h and was chosen for the main study.

**Hemodynamic Measurements and LV Volume**

Hemodynamic measurements were performed 8 wk after coronary artery ligation as described previously in detail (19, 20). LV systolic pressure (LVSP) and end-diastolic pressure (LVEDP), the maximum rate of rise of LV systolic pressure (dP/dt max), mean arterial pressure, heart rate, and mean right atrial pressure were measured under light ether anesthesia and spontaneous respiration via a short segment of fluid-filled PE-50 tubings connected to a microtip manometer (Millar). A midventral thoracotomy was performed to expose the aorta, and a precalibrated electromagnetic flow probe (2.0 mm; Statham Gould Instruments; Hato Rey, Puerto Rico) was placed on the ascending aorta for measurement of aortic flow (cardiac output excluding coronary flow), as previously described (20). Total peripheral resistance index was calculated as (mean arterial pressure – right atrial pressure)/cardiac index (CI) and was expressed as millimeters of mercury per milliliter per minute kilogram body weight. After baseline measurements, peak CI and peak stroke volume index were obtained by an acute infusion of warmed (39–40°C) Tyrode solution into a femoral vein at a rate of 40 ml·kg⁻¹·min⁻¹ for 45 s or until maximal flow was achieved. The passive pressure-volume curves of the LV were then obtained by a double-lumen catheter, as previously described (5). Briefly, the heart was arrested by potassium chloride and a double-lumen catheter (PE-50 inside PE-200) was inserted into the LV via the ascending aorta. The right ventricular free wall was incised to avoid fluid accumulation. The atroventricular groove was ligated, and isotonic saline was infused at a rate of 0.76 ml/min via one lumen while intraventricular pressure was continuously recorded through the other lumen from negative pressure to 30 mmHg. At least three reproducible pressure-volume curves were obtained within 10 min of cardiac arrest, well before the onset of rigor mortis. Operating LV end-diastolic volume was derived from the LV pressure-volume curve (19). It was defined as the volume on the pressure-volume curve corresponding to a filling pressure equal to in vivo end-diastolic pressure.

For rats assigned to biochemical measurements, on the following day after in vivo hemodynamic (heart rate, LVSP, LVEDP, and dP/dt max) measurements, the rat hearts were isolated and perfused in the Langendorff mode at a constant pressure of 100 mmHg at 37°C. It was defined as the volume on the pressure-volume curve while intraventricular pressure was continuously recorded through the corresponding to a filling pressure equal to in vivo end-diastolic pressure.

**Sample collection.** Eight weeks after coronary ligation, rats assigned to neurohormonal studies were anesthetized with an injection of pentobarbital sodium (80 mg/kg ip). A PE cannula was inserted into the trachea for artificial ventilation and a PE-50 catheter was inserted into the right carotid artery to withdraw blood. Blood samples were collected into a prechilled tube containing potassium EDTA (2 ml/g blood). Plasma was separated by centrifugation at 1,700 rpm for later measurements of endothelin-1 and H N-terminal pro-atrial natriuretic peptide (ANP) levels and plasma renin activity, as previously described (6). Plasma samples (500 μl) for catecholamine determination were extracted on aluminum oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide. Aliquots for protein were fixed in distended form in 10% buffered formalin for 24 h and were homogenized in 0.2 mol/l perchloric acid for catecholamine determination and the homogenate was centrifuged for later measurements of endothelin-1 and H N-terminal pro-atrial natriuretic peptide (ANP) levels and plasma renin activity, as previously described (6). Plasma samples (500 μl) for catecholamine determination were extracted on aluminum oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide. Plasma samples (500 μl) for catecholamine determination were extracted on aluminum oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide. Plasma samples (500 μl) for catecholamine determination were extracted on aluminum oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide. Plasma samples (500 μl) for catecholamine determination were extracted on aluminum oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide (pH 8.6) and the catecholamines were eluted with 0.1 mol/l sodium oxide.
then dissected into the LV plus interventricular septum and right ventricular free wall, which were weighed separately. The whole LV was dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Transverse serial sections of 20 µm thickness were obtained in 1-mm intervals from apex to base, mounted, and stained with sirius red (0.1% solution in saturated aqueous picric acid) to provide a clear discrimination between fibrous scar and noninfarcted tissue. Infarct size was determined by planimetric measurement with a digital imaging system (Mocha computer digitizing program) and calculated by dividing the sum of the planimetered endocardial and epicardial circumferences occupied by the infarct by the sum of the total epicardial and endocardial circumferences of the LV. Rats in hemodynamic studies were grouped as sham-operated, small myocardial infarction (MI) (<35% of LV), and large MI (≥35% of LV). Rats in energy metabolism studies were “matched” by in vivo LVEDP levels with rats in hemodynamic studies for group classification. An alternative approach was used to evaluate infarct size of rats for hormone measurements, as previously described (6). Briefly, incisions were made in the LV so that LV tissue could be pressed flat. A clear macroscopic boundary of scar could be seen, which allowed the identification of infarcted area. The endocardial and epicardial infarcted and total area were drawn onto a superimposed glass. Infarct size was determined by planimetry with a digital imaging system (Mocha computer digitizing program) and calculated as [(epicardial MI area/epicardial area + endocardial MI area/endocardial area)/2] × 100. Because this method systematically underestimates MI sizes (14), rats were grouped as sham operated, small MI (<30% of LV), and large MI (≥30% of LV).

LV shape. After separation from the right ventricle, external apex to basis distance and the maximal diameter of the LV were measured with a vernier caliper and maximal LV circumference with both a 2-0 suture and a vernier caliper. Internal LV diameter was measured as the maximal distance from the endocardial surface of the septum to the endocardial surface of the free LV wall along a line perpendicular to the septum and, accordingly, was used as a measure of aneurysmal shape distortion. LV free wall thickness, which represents scar thickness in rats with infarction, was measured at the point where the LV diameter reached the free LV wall. Average septal thickness was determined as the septal area enclosed by two lines originating from the center of gravity of endocardial circumference, which connected the two origins of right ventricular surface length. These measurements were performed with a digital imaging system (Mocha digitizing computer program).

**RESULTS**

**Mortality**

A total of 537 rats underwent coronary artery ligation, and 116 died within 30 min postoperation. The remaining 421 rats were randomized to receive either placebo (n = 186) or zatebradine (n = 235). Mortality during 8 wk was 33.3% in the placebo group and 23.0% in the zatebradine group (P < 0.05). As shown in Fig. 1, the survival benefit of zatebradine occurred on the first day after coronary ligation.

**Baseline and Peak Cardiac Performance**

Baseline and peak CI were decreased by MI (not shown). Zatebradine did not affect CI but increased baseline and peak CI in rats with small MI by zatebradine.

**Data Analysis**

The results are expressed as means ± SE. Multiple comparisons among various groups were evaluated by two-factor (MI size and treatment) factorial ANOVA (SuperANOVA, Abacus Concepts; Berkeley, CA). The mortality difference in placebo and zatebradine-treated rats was determined by χ²- and Fisher’s exact test. P < 0.05 was considered to indicate statistical significance.

**General Characteristics**

Body weights (286 ± 2) were not different among various protocols or groups of MI sizes or treatments, respectively. MI sizes and LV and RV weights were similar in the hemodynamic and neurohormonal study and pooled data are given in Table 1. MI size was substantially reduced by zatebradine in the total group. LV and right ventricular weights increased in placebo-treated rats in proportion to infarct size. Zatebradine increased LV and right ventricular weights.

**Table 1. General characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Infarction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
<td>Large</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>17</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>16</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>MI size, % Placebo</td>
<td>0</td>
<td>22±2</td>
<td>44±1</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>0</td>
<td>20±1</td>
<td>40±1</td>
</tr>
<tr>
<td>LV/BW, mg/g Placebo</td>
<td>2.26±0.06</td>
<td>2.50±0.05*</td>
<td>2.60±0.06*</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>2.74±0.07</td>
<td>2.70±0.05</td>
<td>2.70±0.05</td>
</tr>
<tr>
<td>RV/BW, mg/g Placebo</td>
<td>0.56±0.03</td>
<td>0.69±0.04</td>
<td>0.96±0.05‡</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>0.68±0.02‡</td>
<td>0.89±0.02‡</td>
<td>1.16±0.04‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. MI, myocardial infarction; LV, left ventricular weight; RV, right ventricular weight; DW, body weight. *P < 0.05 vs. sham in the same treatment group; †P < 0.05 vs. rats with small MI in the same treatment group; ‡P < 0.05 vs. placebo.
SVI in rats with MI (Fig. 2). Total peripheral resistance index tended to be higher post MI and was not affected by zatebradine (data not shown).

LV Shape and Volume

Table 3 and Fig. 3 show that LV length (LV apex-basis), width (LV maximal diameter and circumference), and volume increased in rats with MI. Zatebradine further increased diameters, circumference, and volume in sham and rats with small MI but not in rats with large MI. Free wall and septal thickness were not changed by zatebradine.

Study on Energy Metabolism

In vitro hemodynamics. As shown in Table 4, heart rate remained decreased in the isolated heart. Coronary flow (CF) increased in zatebradine-treated rats with large MI.

Pressure-volume relations. LV developed pressure or dP/dt max (performance) were related to volume (preload). These relations were shifted to the lower right in proportion to MI size (less performance at more preload). Zatebradine shifted the curves to the right in sham rats and rats with small MI but shifted the curves to the left in hearts with large MI (Fig. 4, A and B). MV˙O2-PVA relations shifted to the right after MI.
and were shifted to the left by zatebradine in all treated hearts (Fig. 5).

Parameters of Energy Metabolism

After MI, CK, CK-BB, and CK-MB isoenzymes increased, CK-mito and total creatine decreased in proportion to MI size (data not shown). CK-MB increased from 15 ± 1% of total CK (placebo) to 20 ± 1% (zatebradine, \( P < 0.05 \) vs. placebo) in sham-operated hearts; it remained unchanged in animals with large MI (placebo 21 ± 1%, \( P < 0.05 \) vs. sham; zatebradine 19 ± 1%, not significant vs. placebo). The LDH5-to-LDH1 ratio increased after large MI and was not changed by zatebradine. Myocardial ATP values decreased after large MI both in placebo and in zatebradine-treated rats (Table 5).

Hormone Study

Plasma hormone and catecholamine measurements. Plasma norepinephrine, epinephrine, and endothelin-1 tended to be higher and plasma renin activity increased in proportion to MI sizes but were not affected by zatebradine (data not shown). Circulating NH2 terminal pro-ANP was increased in proportion to MI size and by zatebradine (Table 5). LV norepinephrine remained unchanged post-MI but was decreased by zatebradine in MI rats. Right ventricular norepinephrine decreased in proportion to MI size and was further decreased by zatebradine in sham rats and rats with small MI. LV and right ventricular 3,4-dihydroxyphenyl ethylene glycol (DHPG)-to-norepinephrine ratio increased post MI and were further increased by zatebradine (Table 5).

DISCUSSION

The major results of this study were that zatebradine reduced "acute" mortality and MI size and improved LV performance post MI, but induced LV dilatation in sham rats and rats with small MI. Accordingly, a shift of myocardial CK isoenzymes toward an "embryonic" pattern and shift of LDH isoenzymes toward the "hypoxic" enzymes were induced by zatebradine in sham rats as observed in models of hypertrophy or failure (13,
The ambivalent effect of zatebradine is underlined by an increase in NH$_2$ terminal pro-ANP, which is a marker for late prognosis in patients with LV dysfunction post MI (8). In addition, LV and right ventricular norepinephrine was decreased and the DHPG-to-norepinephrine ratio was increased by zatebradine.

**Mortality**

In a recent study, Opitz and collaborators (18) demonstrated that acute and subacute death in this model was an arrhythmic event (ventricular fibrillation). Bril and coworkers (1) studied the effects of zatebradine (750 mg/kg iv) 20 min after left circumflex coronary artery ligation in rabbit and found that the incidence of ventricular fibrillation was reduced by zatebradine by 50%. Because this effect was completely reversed by atrial pacing to the preload heart rate (1), direct antiarrhythmic effects of zatebradine were unlikely. In animals with large MI, zatebradine improved global CF. Thus the reduction of acute mortality by lowering heart rate might be related to an improvement of energy balance by reducing O$_2$ consumption and improving coronary flow at longer diastoles. In addition, MI size for the total group was reduced by zatebradine, thus most likely contributing to the lower mortality rate. Reduction of acute mortality by zatebradine was in part outweighed by later excess mortality. Early survival of animals with infarcts, which would not have survived without treatment, might be an explanation or the adverse effect of zatebradine on remodeling in animals with small MI. Moreover, in agreement with a previous study in this model (9), norepinephrine levels were reduced in tissue from the right ventricle in MI rats. The DHPG-to-norepinephrine ratio was also increased in these rats, reflecting an increased norepinephrine turnover (9). Because this ratio was further increased by zatebradine in MI hearts, a further increased norepinephrine turnover is suggested. Thus sympathetic activation could contribute to the late deaths by zatebradine.

**Remodeling and Performance**

Zatebradine increased LV weight and volume in sham-operated rats and rats with small MI at a constant ratio of LV weight to volume and an unchanged wall thickness (“eccentric hypertrophy”). In fact, animals with small MI treated with zatebradine ended up with an increased filling pressure and the same LV volumes as animals with large MI. The exact mechanism of LV dilation by heart rate reduction remains unknown.

### Table 4. *In vitro hemodynamics and oxygen consumption*

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Small</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Numbers of animals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>11</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>11</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td><strong>HR, min$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>264±8</td>
<td>256±9</td>
<td>246±12</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>216±8</td>
<td>199±9</td>
<td>192±9</td>
</tr>
<tr>
<td><strong>dP/dt$_{max}$, mmHg/s</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>2.255±88</td>
<td>1.497±137*</td>
<td>1.193±243*</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>2.468±136</td>
<td>1.723±117*</td>
<td>1.600±163*</td>
</tr>
<tr>
<td><strong>CF, ml/min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>17.5±1.2</td>
<td>16.1±1.1</td>
<td>17.7±1.8</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>19.3±0.9</td>
<td>17.1±0.6</td>
<td>21.3±0.8‡</td>
</tr>
<tr>
<td><strong>MVO$_2$, µmol·g dry wt$^{-1}$·min$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>28.3±1.9</td>
<td>22.8±1.5*</td>
<td>23.0±2.4</td>
</tr>
<tr>
<td>Zatebradine</td>
<td>31.8±1.8</td>
<td>20.2±1.0*</td>
<td>21.4±1.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE. CF, coronary flow; MVO$_2$, myocardial O$_2$ consumption. *P < 0.05 vs. sham in the same treatment group; †P < 0.05 vs. rats with small MI in the same treatment group; ‡P < 0.05 vs. placebo rats with comparable MI size.
But it was also recently reported as an acute effect of zatebradine. The fundamental variables like blood pressure and cardiac output were not altered despite lower heart rate. In acute experiments, zatebradine reduces, along with heart rate, cardiac output and increases LV filling pressure (22). Increased filling pressure may have been the stimulus for structural LV dilatation, which in turn normalized filling pressure (29). The increase in chamber volume probably contributed to normalize cardiac output and thus blood pressure by the geometric advantage of a larger ventricle (11). In addition, however, isolated MI hearts even at the same preload and working isovolumetrically, developed more pressure when pretreated with zatebradine. Zatebradine shifted developed pressure-diastolic volume relations to the left. Thus, in sham and small MI rats, LV enlargement appeared to be an adaptation to lower heart rate. However, MVO$_2$-PVA relations were shifted to the left by chronic zatebradine treatment, suggesting reduced efficiency of electromechanical coupling or increased basal MVO$_2$ in these hearts (Fig. 5) (28), which was another unexpected fundamental difference to the acute effects of lowering heart rate (25). Zatebradine did not change the slope of the MVO$_2$/PVA relations at least in infarcted hearts. Thus an effect on contractile efficiency appears not to be a mechanism of action of zatebradine.

It remained unclear why LV dilatation was not aggravated by zatebradine in the animals with large MI. An extensive MI might provide maximal stimulus for dilatation and thus might prevent further dilatation by other stimuli like lowering heart rate. One also may speculate that improved coronary flow and metabolic balance was protective. The LDH5-to-LDH1 shift toward the anaerobic enzymes observed in large MI was however, not prevented by zatebradine. How did hearts with large infarcts maintain their stroke volume at lower heart rate? In rats with large MI, zatebradine did not change mean arterial pressure or total peripheral resistance index. Thus afterload appeared to be unchanged. Ejection fraction was increased and dP/dt$_{max}$ unchanged despite lower heart rate. One explanation might be a beneficial effect of longer ejection time in these enlarged hearts.

**Zatebradine Versus β-Blocker**

Previous studies (4, 10) have shown that β-blockers may promote LV dilatation in rats with MI. The effect is dependent on MI size similar to that of zatebradine observed in the present study. Cardiac output was also maintained despite reduction of heart rate by β-blockers. Finally, promotion of LV dilatation by β-blockers was also not seen in rats with large MI (10). There were, however, distinct effects of β-blockers not observed with zatebradine. The β-blocker bisoprolol prevented the shift of CK isoenzymes to an embryonic pattern, the shift of LDH isoenzymes to a hypoxic pattern, and increased total myocardial creatine (13). All of these effects were not observed with zatebradine. Thus lowering heart rate per se reduces early mortality and MI size but induces adverse effects on LV remodeling in rats with small MI. Additional effects of β-blockers independent of lowering heart rate, such as an increase in myocardial creatine content (13), may be more important for long-term processes. Future experimental studies are needed comparing directly drugs only lowering heart rate with β-blockers. The biochemical effects of β-blockers on the myocardium not shared by zatebradine suggest superiority of β-blockers for the treatment of heart failure. Especially,
changes of cardiac neurotransmitters and their metabolites after zatebradine suggest caution with an uncritical application of this therapy to patients with heart failure.

**Clinical Relevance**

Zatebradine has not been further developed to clinical use for various reasons. The successor drug ivabradine is, however, now evaluated in phase III studies (26). The present study may contribute to a better understanding of this therapeutic principle. So far, no clinical experience exists on the chronic use of pure heart rate-lowering drugs in patients with heart failure. Short-term use appears promising (25).

**ACKNOWLEDGMENTS**

Zatebradine was a gift of Dr. Karl Thomae, Biberach/Riss, Germany.

**GRANTS**

This study was supported by Deutsche Forschungsgemeinschaft, Sonderforschungsbereich “Pathophysiologie der Herzinsuffizienz” SFB 355 Würzburg.

**REFERENCES**