Unique Cardioprotective Action of the New Calcium Antagonist Mibefradil

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Background—Mibefradil is a calcium antagonist with few negative inotropic effects at therapeutic concentrations.

Methods and Results—The effect of mibefradil on infarct size (IS) was compared with those of placebo, amlodipine, and verapamil in 64 anesthetized pigs. In placebo pigs, after 90 minutes of ischemia and 120 minutes of reperfusion, IS (by triphenyl tetrazolium chloride staining) was 15.3\(\pm\)10.8\% (SD) of the area at risk. Mibefradil (0.60 mg/kg IV) reduced heart rate and left ventricular (LV) pressure, and IS was 1.9\(\pm\)3.9\% (\(P<0.05\) versus placebo). Verapamil (0.15 mg/kg IV) also decreased heart rate, LV pressure, and IS (6.1\(\pm\)4.2\%, \(P<0.05\) versus placebo). Amlodipine (0.20 mg/kg IV) did not alter heart rate, LV pressure, or IS (9.9\(\pm\)5.4\%, \(P=NS\) versus placebo). When heart rate was maintained constant by left atrial pacing and LV pressure was adjusted to that of the placebo group by an intra-aortic balloon, mibefradil still decreased IS (3.8\(\pm\)3.0\%, \(P<0.05\) versus placebo), but verapamil did not (11.6\(\pm\)8.3\%, \(P=NS\) versus placebo). With glibenclamide infusion, mibefradil no longer reduced IS (13.1\(\pm\)4.3\% versus 17.8\(\pm\)5.6\% with glibenclamide alone, \(P=NS\)).

Conclusions—The IS-limiting effect of mibefradil, in contrast to that of verapamil, was not dependent on favorable hemodynamics but was abolished by glibenclamide, suggesting a direct cardioprotective action of mibefradil. (Circulation. 1999;99:305-311.)

Key Words: calcium \textbullet{} mibefradil \textbullet{} myocardial infarction

Controversy continues about the ability of calcium antagonists to reduce infarct size.\(^1,2\) Diltiazem consistently reduced infarct size in ischemia/reperfusion models\(^3-7\) as well as in a permanent occlusion model.\(^8\) Verapamil reduced infarct size in some\(^9-13\) but not all\(^12,10\) ischemia/reperfusion models; results in permanent occlusion models are also controversial (no reduction\(^9,14,15\) versus reduction of infarct size\(^16,17\)). Except for one study,\(^18\) dihydropyridines did not alter infarct size in ischemia/reperfusion models\(^12,13,19-21\); once again, results in permanent occlusion models are controversial (no reduction\(^19,22,23\) versus reduction of infarct size\(^14,22,24-26\)). Part of these controversial findings may be explained by the timing of treatment, ie, pre versus early versus late treatment.

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Apart from these divergent effects of calcium antagonists on experimental infarct size, their clinical use in the acute phase of myocardial infarction is limited, because they reduce perfusion pressure and exert negative inotropic effects, which are of particular concern in patients with left ventricular (LV) dysfunction.\(^27\)

The benzimidazolyl-substituted teraline derivative mibefradil selectively inhibits T-type calcium channels in vitro at concentrations that only partially block L-type calcium channels.\(^28\) In patients with chronic stable angina pectoris, mibefradil reduced the number of ischemic episodes by 70\%\(^29\) and increased exercise duration, time to onset of angina, and time to persistent 1-mm ST-segment depression,\(^30\) with few negative inotropic effects.\(^31\) In the anesthetized dog,\(^32\) mibefradil reduced infarct size to an extent comparable to that of verapamil but to a lesser extent than ischemic preconditioning. In this study, however, both calcium antagonists also reduced heart rate and blood pressure.

Therefore, in the present study in anesthetized pigs, the effect of mibefradil on infarct size in the presence and absence of favorably altered systemic hemodynamics was compared with that of placebo and 2 other calcium antagonists, amlodipine and verapamil, at equipotent coronary dilator doses. To further elucidate the underlying mechanism of cardioprotection, mibefradil was infused in the presence of glibenclamide.

Methods
The experimental protocols used in this study were approved by the Bioethical Committee of the district of Düsseldorf.

Experimental Model
The experimental model has been described elsewhere\(^33\); in brief, 64 Göttinger miniswine (20 to 40 kg) of either sex were initially sedated...
with ketamine hydrochloride and anesthetized with sodium thiopental; anesthesia was then maintained with enflurane with an oxygen/nitrous oxide mixture. Rectal temperature was monitored, and body temperature was kept between 37°C and 38°C with heating pads. Both common carotid arteries were cannulated, one for the measurement of arterial pressure and the insertion of an intra-aortic balloon (5F Fogarty, Baxter Deutschland GmbH) and the other to supply blood to the extracorporeal circuit. A left lateral thoracotomy was performed, and a micromanometer was placed in the left ventricle through the apex.

The proximal left anterior descending coronary artery (LAD) was cannulated and perfused from an extracorporeal circuit. Before coronary cannulation, the pigs were anticoagulated with sodium heparin. The system included a roller pump, Windkessel, and 2 side ports for the injection of radiolabeled microspheres and the infusion of drugs. Coronary arterial pressure was measured from the sidearm of a polyethylene T connector. Heart rate was controlled by left atrial pacing.

Regional Myocardial Blood Flow

Radionuclide microspheres (15-μm diameter; 113mCe, 114mIn, 55mCr, 119mSn, 103mRu, 69mNb, or 44mSc; NEN–Du Pont Co) were injected into the coronary perfusion circuit (1×10^7 to 2×10^7 suspended in 1 mL saline) to determine the regional myocardial blood flow in the LAD perfusion bed (model 5912, Gammaszint BF 5300, Packard). The averaged subendocardial blood flow to the entire LAD-perfused territory was measured and related to myocardial infarct size.

Morphology

The heart was sectioned from base to apex into 5 transverse slices in a plane parallel to the atrioventricular groove. The tissue slices were immersed in a 0.09 mol/L sodium phosphate buffer (pH 7.4) containing 1.0% triphenyl tetrazolium chloride (TTC, Sigma) and 8% dextran (molecular weight, 77 800) for 20 minutes at 37°C to identify infarcted tissue. Reductions of blood flow during ischemia by >85% were taken to indicate myocardium at risk. Infarct size is expressed as a percentage of the LV area at risk.

Experimental Protocols

In a first step, the effect of mibefradil on infarct size was compared with that of amlodipine and verapamil. Because only mibefradil and verapamil significantly reduced infarct size, the importance of decreases in heart rate and LV pressure for such cardioprotection was investigated in a second step. Because mibefradil in the presence of matched heart rate and LV pressure still reduced infarct size, an effect similar to that of ischemic preconditioning, the importance of activation of ATP-dependent potassium channels (KATP) for the effect of mibefradil was assessed in the third step. Activation of KATP is the most likely end effector of ischemic preconditioning.33

A total of 8 groups of pigs were studied (Figure 1). In each group, systemic hemodynamics and regional myocardial blood flow were measured under control conditions and at 5 and 90 minutes after the reduction in coronary blood flow, which was set to decrease mean coronary arterial pressure to ≈30 mm Hg. After 90 minutes of ischemia, the myocardium was reperfused for 120 minutes to facilitate the identification of necrotic tissue.

Step 1

Placebo (Group 1, n=9). After control measurements, 1 mL/min saline solution was infused intravenously over 30 minutes.

Mibefradil (Group 2, n=9) or Amlodipine (Group 3, n=9), or Verapamil (Group 4, n=8). After control measurements, either mibefradil (0.60 mg/kg) [(15S)-2-[(3-2(benzimidazolyl)propyl)methylamino]ethyl]-(6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naph- thyl-methoxy-acetate dihydrochloride], or amloidipine (0.20 mg/kg), or verapamil (0.15 mg/kg) was infused intravenously. The peak free plasma concentration with 0.6 mg/kg mibefradil averaged 348±44 (SD) ng/mL in 4 additional pigs. At constant coronary arterial pressure, each of the calcium antagonists doubled coronary blood flow within 20 to 25 minutes after its administration. Measurements were performed no earlier than 30 minutes after administration of the calcium antagonist before coronary blood flow was reduced.

Step 2

Mibefradil (Group 5, n=9) or Verapamil (Group 6, n=6) Plus Matched Heart Rate and LV Pressure. After control measurements, either mibe- fradil or verapamil was infused intravenously. Measurements were repeated 30 minutes after administration of either drug and once again in the presence of matched heart rate and LV pressure before coronary blood flow was reduced.

Step 3

Glibenclamide (Group 7, n=7). After control measurements, gliben- clamide was infused intravenously (0.5 mg/kg bolus followed by 0.5 mg/min). This dose regimen has previously been demonstrated to abolish ischemic preconditioning in the same porcine preparation.33 Thirty minutes after the bolus injection of glibenclamide, measurements were repeated before coronary blood flow was reduced.

Glibenclamide Plus Mibefradil Plus Matched Heart Rate and LV Pressure (Group 8, n=7). After control measurements, glibenclamide was infused intravenously. Thirty minutes after the bolus injection of glibenclamide, mibefradil was infused intravenously. Measurements were repeated in the presence of matched heart rate and LV pressure before coronary blood flow was reduced.

Data Analysis and Statistics

Hemodynamic parameters were continuously digitized and recorded. A 20-second period during each microsphere injection (~33 consecutive beats over ≥2 complete respiratory cycles) was analyzed with CORDAT II software.33 Hemodynamic parameters were calculated on a beat-to-beat basis, and data were then averaged. The incidence of ventricular extrasystoles was determined from a surface ECG lead and counted in 5-minute intervals, starting 10 minutes before ischemia in group 1 and 10 minutes before drug infusion in groups 2 through 8.
Statistical analysis was performed with SYSTAT software. Hemodynamic data were compared by 2-way ANOVA for repeated measures. Area at risk and infarct size were compared by 1-way ANOVA. When significant differences were detected, individual measures. Area at risk and infarct size were performed in all groups and compared by ANCOVA.

### Results

There were no significant differences in any measured parameter between groups under control conditions (Table).

Thirty minutes after administration of either mibefradil (group 2) or amlodipine (group 3) or verapamil (group 4), coronary blood flow was almost doubled. LV pressure decreased in all groups, but heart rate was reduced only with mibefradil and verapamil. Glibenclamide (groups 7 and 8) slightly increased LV pressure. In the presence of glibenclamide, at matched heart rate and LV pressure, mibefradil had no effect on systemic hemodynamics during control conditions.

In all groups, by a decrease in the pump speed, coronary blood flow was reduced. In groups 1 through 6, LV pressure was significantly decreased at 5 minutes of ischemia. During the remainder of the 90-minute ischemic period, systemic hemodynamics did not change further.

### Arrhythmias

In all groups, the incidence of arrhythmias was <2 within a 5-minute interval before ischemia. During ischemia, the incidence of extrasystoles increased, with a peak between 40 and 60 minutes of ischemia. The peak rate of extrasystoles averaged 14±25, 10±25, 10±21, 5±9, 5±7, 4±5, 12±15, and 4±4 (P=NS) per 5-minute interval in groups 1 through 8, respectively.

<table>
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<tr>
<th>Systemic Hemodynamics</th>
<th>Control</th>
<th>Drug</th>
<th>HR Match, LVP Match</th>
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<td>102.0±38.3*</td>
<td>6.4±2.0*†</td>
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</table>

HR indicates heart rate; LVP, LV pressure; CBF, mean coronary blood flow; NA, not available; Group 1, placebo; Group 2, mibefradil; Group 3, amlodipine; Group 4, verapamil; Group 5, mibefradil at matched HR and LVP; Group 6, verapamil at matched HR and LV pressure; Group 7, glibenclamide; and Group 8, glibenclamide and mibefradil at matched HR and LV pressure.

*P<0.05 vs control; †P<0.05 vs preceding value; ‡P<0.05 vs placebo; and §P<0.05 vs drug.
Mibefradil and Infarct Size

Infarct Size

The area at risk was comparable among all groups (Figures 2, 4, and 6). Infarct size tended to be decreased with amiodipine but was reduced significantly only with mibefradil ($P<0.05$ versus placebo and amiodipine) and verapamil ($P<0.05$ versus placebo) (Figure 2). Subendocardial blood flow in the area at risk at 5 minutes of ischemia correlated inversely with infarct size (Figure 3). The relationships between infarct size and subendocardial blood flow were shifted downward significantly with mibefradil ($P<0.05$ versus placebo and amiodipine) and verapamil ($P<0.05$ versus placebo) (Figure 2). At matched heart rate and LV pressure, decrease in infarct size with verapamil was no longer significant (Figure 4). The relationships between subendocardial blood flow and infarct size in the placebo and verapamil groups were now superimposable, whereas the relationship was still shifted downward significantly with mibefradil ($P<0.05$ versus placebo and verapamil, Figure 5). Glibenclamide prevented the significant decrease in infarct size achieved by mibefradil (Figure 6), and the relationships between subendocardial blood flow and infarct size in the glibenclamide and glibenclamide+mibefradil groups were superimposable (Figure 7).

Discussion

In the present study, both mibefradil and verapamil, in contrast to amiodipine, reduced infarct size. The infarct size–limiting effect of mibefradil, in contrast to that of verapamil, was not dependent on decreases in heart rate and LV pressure. Infarct size reduction by mibefradil was abolished by glibenclamide, suggesting that mibefradil and ischemic preconditioning may converge to the same effector, the activation of $K_{ATP}$.

Critique of Methods

Infarct size as determined by TTC staining after 90 minutes of ischemia and 2 hours of reperfusion was one major end point of the present study. Studies from different laboratories have used TTC staining to delineate myocardial necroses within such a time frame of ischemia/reperfusion.

In pigs, complete occlusion of the LAD results in a high incidence of ventricular fibrillation and extensive infarction of the left ventricle, with subsequent pump failure. Therefore, the LAD perfusion territory was hypoperfused at low but maintained flow, resulting in a large area at risk (45% of the LV mass) but a small infarct size when expressed as a percentage of the area at risk (15.3±10.8% in group 1). However, infarct size expressed as a percentage of total LV mass in the present study averaged 8% in the placebo group and was thus comparable to that in a previous study using pigs with total occlusion of only one distal LAD branch (7%). Many patients suffering from coronary artery disease over prolonged periods of time used TTC staining to delineate myocardial necroses within such a time frame of ischemia/reperfusion.

Heart rate and LV peak pressure matched

Infarct Size for any given subendocardial blood flow was reduced in mibefradil group ($y = -39.2x + 4.9$, $n = 9$, $r = -0.38$, $P<0.05$ vs placebo and amiodipine) and verapamil group ($y = -91.8x + 13.6$, $n = 9$, $r = -0.83$, $P<0.05$ vs placebo). Amiodipine did not significantly shift relationship ($y = -107.0x + 16.4$, $n = 9$, $r = -0.86$) between infarct size and subendocardial blood flow vs placebo group ($y = -383.3x + 37.2$, $n = 9$, $r = -0.93$).

Heart rate and LV peak pressure matched

Infarct size for any given subendocardial blood flow was reduced in mibefradil group ($y = -383.3x + 37.2$, $n = 9$, $r = -0.93$) and verapamil group ($y = -263.6x + 30.8$, $n = 6$, $r = -0.84$) were nearly superimposable. In contrast, relationship with mibefradil ($y = -22.5x + 4.7$, $n = 8$, $r = -0.19$) was shifted downward ($P<0.05$ vs placebo and verapamil), such that infarct size was decreased at any given subendocardial blood flow.
concentration of mibefradil averaged 348 ± 6 ng/mL with oral application of 50 to 100 mg and thus was close to the peak free plasma concentration of mibefradil in patients, respectively. Although the mibefradil dose in the present study thus appears to be clinically relevant, the doses of amlodipine and verapamil were chosen with reference to their equipotent coronary dilation.

In the present study, a dose dependency of the effect of mibefradil was not established. The peak free plasma concentration of mibefradil averaged 348 ± 44 (SD) ng/mL and thus was close to the peak free plasma concentration of 200 to 700 ng/mL with oral application of 50 to 100 mg mibefradil in patients, respectively. Although the mibefradil dose in the present study thus appears to be clinically relevant, the doses of amloidine and verapamil were chosen with reference to their equipotent coronary dilation.

### Mibefradil and Infarct Size

The extent of infarct size reduction with mibefradil was similar to that achieved by ischemic preconditioning in the same animal model. Activation of K\textsubscript{ATP} is the most likely end effector of ischemic preconditioning. In the present study, blockade of K\textsubscript{ATP} by glibenclamide prevented the infarct size reduction achieved by mibefradil. Activation of K\textsubscript{ATP} results in shortening of action potential duration, and indeed, mibefradil, like ischemic preconditioning, also reduces action potential duration. However, glibenclamide also blocks chloride channels at higher concentrations, and blockade of chloride channels has been demonstrated to contribute to protection from ischemia/reperfusion injury. Classic K\textsubscript{ATP} openers, such as cromakalim, increase the incidence of ventricular arrhythmias and fibrillation in the scenario of ischemia/reperfusion. Although mibefradil may interact with K\textsubscript{ATP}, the incidence of arrhythmias with mibefradil was not different from that with placebo. In the anesthetized dog, mibefradil reduced infarct size to a lesser extent than ischemic preconditioning. One potential explanation for the observed difference in the extent of cardioprotection between this and the present study is the mibefradil concentration used. In the anesthetized dog, mibefradil was infused at a high concentration of up to 40 \( \mu \)mol/L, whereas in the present study, the concentration was <10 \( \mu \)mol/L. In cultured cardiac fibroblasts, mibefradil at concentrations <10 \( \mu \)mol/L reduces the intracellular calcium content, whereas at higher concentrations (10 to 100 \( \mu \)mol/L), the intracellular calcium content is significantly elevated, possibly as a result of calcium release from intracellular stores. Such intracellular calcium release at high mibefradil concentrations might partially offset its otherwise infarct-size-reducing effect. In anesthetized rats, the infarct size reduction after mibefradil infusion was also independent of changes in the rate-pressure product. However, the extent of infarct-size reduction was less than that observed in the present study.

### Clinical Implications

The experimental model used in the present study can be extrapolated only to the situation of patients pretreated with mibefradil who suffer an acute myocardial infarction and are reperfused within 90 minutes after the onset of ischemic symptoms. Whether the results of the present study can also be extrapolated to a broader spectrum of coronary artery disease, ie, whether patients will also have less irreversible tissue damage and better prognosis if reperfusion is lacking or treatment is started after the onset of ischemic symptoms, remains to be determined.

In 1992, Opie introduced, entirely on experimental grounds, the concept of ischemia selectivity of calcium antagonists, which was later expanded by Heusch. Mibefradil—interestingly, as initially hypothesized by Opie—might indeed be classified as an ischemia-selective agent with few negative inotropic effects but a marked cardioprotective effect on ischemic myocardium.

### Addendum

Since this article was written, the manufacturer has withdrawn mibefradil from the market because of serious drug interactions but not because of its lack of cardioprotection.
Acknowledgments

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