Effects of Mibefradil, a Novel Calcium Channel Blocking Agent With T-Type Activity, in Acute Experimental Myocardial Ischemia

Maintenance of Ventricular Fibrillation Threshold Without Inotropic Compromise

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Objectives. We tested whether mibefradil, a selective T-type calcium channel blocking agent, could differentially inhibit experimental ventricular arrhythmogenesis more than contractility during acute regional ischemia and reperfusion compared with that during L-channel blockade by verapamil.

Background. T-type calcium channels are found in nodal and conduction tissue and in vascular smooth muscle, but in much lower density in contractile myocardium. The potential role of mibefradil in ventricular arrhythmogenesis remains unclear.

Methods. Mibefradil (Ro 40-5967, 1 mg/kg body weight intravenously [IV]) was given as a bolus 30 min before anterior descending coronary artery ligation, followed by 2 mg/kg per h IV during 20 min of ischemia and 25 min of reperfusion in open chest pigs. In a second group, mibefradil was given in a dose twice as high. A third group received verapamil (0.3 mg/kg IV), followed by an infusion of 0.6 mg/kg per h.

Results. During the ischemic period, the low (clinically relevant) dose of mibefradil prevented the fall of the ventricular fibrillation threshold, without depressing the maximal rate of pressure development of the left ventricle (LVmax dP/dt). This low dose increased left ventricular blood flow, whereas peripheral arterial pressure remained unchanged. The higher dose of both mibefradil and verapamil was antiarrhythmic during ischemia, at the cost of depressed contractile activity. During reperfusion, only the higher dose of mibefradil and verapamil was antiarrhythmic but both depressed contractile activity.

Conclusions. Mibefradil is antiarrhythmic, without inotropic compromise. Speculatively, both T-type and L-type calcium channel blockade are involved in these effects.

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Despite the continuous advances in the therapy of acute myocardial infarction, sudden cardiac death largely caused by ventricular arrhythmias remains important in the spectrum of mortality in coronary heart disease (1). Such deaths typically occur out of the hospital, outside the reach of thrombolytic therapy. Because the incidence of ischemic heart disease is likely to rise very substantially in the developing world early in the next millennium (2), the global issue of sudden cardiac death will become even more important.

Hypothetically, excess cytosolic calcium, as found in myocardial ischemia, may play an important role in ischemic ventricular arrhythmogenesis (3–6). From the therapeutic point of view, there is no direct manner of modification of cytosolic calcium in ischemic cells, but working from first principles, two indirect pharmacologic procedures are possible: 1) Beta-adrenergic blockade should, by virtue of inhibition of the formation of the second-messenger cyclic adenosine monophosphate, lessen the probability of opening of the calcium channel and hence be antiarrhythmic (4). 2) Calcium channel blocking agents should achieve a similar effect by directly binding to the calcium channel and lessening calcium ion influx, which might be important in the initiation and maintenance of ventricular fibrillation (7). These proposals could have clinical relevance in postinfarction patients, as shown by the decrease in sudden cardiac death when beta-blockers are given prophylactically. Verapamil, a classic L-type calcium channel blocker, reduced mortality only in a predetermined subgroup without previous heart failure (8), suggesting that its protection could be limited by its negative inotropic action, as in the case of diltiazem (9). Experimentally, verapamil prevents ventricular fibrillation in variety of models (10–14), but at the cost of depressed myocardial contractile function (14).

Mibefradil is a structurally novel calcium channel blocker
Mibefradil is a new class of benzimidazoly-substituted tetraine derivatives (15,16). Mibefradil inhibits calcium influx through T-type low voltage–activated calcium channels (17) ten times more powerfully than influx through L-type channels (18). T-type channels are present in very low densities in adult, nondiseased ventricular tissue of various species (19,20), but there appear to be no data on humans (21). In contrast, T-channels are well expressed in pacemaker tissue, such as the sinus node (22). A T-channel blocker such as mibefradil could therefore be expected to decrease heart rate, with a lesser effect on myocardial contractility. In addition mibefradil has relatively weak L-channel blocking activity (23). Mibefradil increased survival in a rat model of heart failure (24). When given to patients with varying degrees of left ventricular dysfunction, intravenous mibefradil is well tolerated. Ejection fraction increases, but a decrease in the maximal rate of pressure development of the left ventricle (max dP/dt) occurs in some patients with previous heart failure (25). Mibefradil has been reported (26) to stimulate the release of endothelial-derived relaxing factor. Mibefradil may also be relatively selective, causing coronary versus peripheral vasodilation (27). We therefore hypothesized that T-channel blockade by mibefradil would be able to inhibit ischemic ventricular arrhythmias without major depression of left ventricular contractile function.

We used the open chest porcine model, subject to acute coronary artery ligation (CAL), followed by reperfusion. The vulnerability of the heart to ventricular arrhythmias was monitored by repetitive measurements of the ventricular fibrillation threshold (VFT). The coronary and myocardial effects of mibefradil were assessed by measurements of regional myocardial blood flow and left ventricular contractile activity. Comparisons were made with verapamil, using a dose known to have antiarrhythmic effects in this model (14).

**Methods**

The ethics and research committee of the University of Cape Town approved this study. Animals were handled in accordance with the “Principles of Laboratory Animal Care” of the National Society for Medical Research.

**Study protocol.** Male pigs (Large White crossed with Landrace) weighing 27 to 30 kg were sedated by intramuscular ketamine (10 mg/kg body weight). Anesthesia was induced by intravenous thiopentone sodium (10 mg/kg). Approximately 5 min later, a bolus dose of 55 mg/kg of alpha-chloralose was given, followed by a constant infusion of alpha-chloralose (60 mg/kg) over ~60 min. In addition, the neuromuscular blocker alcuronium chloride (0.02 mg/kg per min) was given as an intravenous infusion for the duration of the experiment. Animals were ventilated with room air; pO2 was controlled between 95 and 105 mm Hg and pH between 7.43 and 7.48. Standard limb lead electrocardiograms and arterial pressure were monitored continuously. After a midsternal thoracotomy, the left anterior descending coronary artery was dissected free for ~5 mm at a point approximately one-half the distance from its origin to its apical termination. It was abruptly ligated by tightening thin polyvinyl tubing around a 2-cm length of rigid tubing placed alongside the artery.

We measured the VFT by using the “train” method. An electrical stimulus consisting of a train of 10 square wave pulses was triggered by the R wave and distributed over 210 ms during the T wave. The stimulus was passed between two platinum electrodes sutured onto the anterior wall of the left and right ventricles, 2.5 cm apart. The anode was placed across the border of the expected ischemic zone in the left ventricle and the cathode within the nonischemic zone of the right ventricle. Pulses were generated using a Grass S88 stimulator. The stimulus current was progressively increased in 2 mA steps starting at 4 mA until ventricular fibrillation (VF) occurred (28). The VFT was taken as the lowest current required to produce VF. Care was taken not to use a longer train of pulses, which may spill over into the refractory phase or late portion of the T wave (29). A single VFT measurement required ~1 min. Epicardial direct current shock was then applied usually within 4 s of the onset of VF and repeated if necessary. A return to sinus rhythm could almost always be achieved within 30 s. After 30 s, we alternated cardiac massage with defibrillation. However, if defibrillation could not be accomplished within 90 s, the experiment was terminated. The VFT was measured before CAL, 5 min after CAL and 10 min after reperfusion. In addition to measurement of VFT, spontaneously occurring ventricular arrhythmias were also monitored. Ventricular tachycardia (VT) was defined as more than four consecutive uniformly or multiform ventricular premature systoles. Runs of VF or VT were considered terminated when they were followed by at least three normally conducted sinus beats. Twenty minutes after ligation, the ligature was released to reperfuse.

**Left ventricular pressures** and LVmax dP/dt were measured using a Cardiomax computerized system (Columbus Instruments). This system gives real-time displays of digital and analog signals. Each variable is measured over at least three consecutive cardiac cycles at a time when no arrhythmias are evident. Each data point represents the mean of two readings.

**Left ventricular blood flow** was measured in the placebo group, the low dose mibefradil group and the verapamil group but not in the higher dose mibefradil group. Microspheres were given before drug administration and CAL, 15 min after CAL as well as 15 min after reperfusion using the sample
reference method. The radionuclides utilized were gadolinium-153, strontium-85 and tin-113 (New England Nuclear). The microspheres (mean size 15 μm) were prepared in a 0.9% saline solution containing Tween-80 0.01% and administered according to previously described details and precautions (30,31). Samples for gamma-spectrometry were dissected from the midischemic and remote nonischemic zones of the left ventricle.

Size of underperfused zone. At the end of the experiment, the coronary artery was again ligated at the same site as before, and the underperfused zone was delineated by injection of 5 ml of patent blue 7.5% solution (May and Baker, UK) in saline into the left atrium. The heart was then excised and arrested. The size of this zone was expressed as a percent of total left ventricular mass. When the underperfused zone was <26% or >34%, the results were not processed. This predetermined exclusion criterion was used because of links between eventual infarct size and arrhythmias (32,33).

Study groups. The prime aim of the present study was to compare the effects of antiarrhythmic doses of mibefradil and verapamil on contractile function.

Selection of doses. In a pilot study, mibefradil, 1 mg/kg bolus followed by 2 mg/kg per h, was the lowest dose that showed antiarrhythmic activity during ischemia. In a previous study, verapamil at a clinically relevant dose (0.2 mg/kg) was not antiarrhythmic in our model. A supraclinical dose of 0.6 mg/kg did show antiarrhythmic activity (14). We therefore used a comparable supraclinical dose of verapamil in the present study.

Placebo group (n = 13). Each pig received 8 ml of saline 0.9% starting 30 min before CAL, followed by an infusion of saline 0.9% for the remainder of the experiment.

Mibefradil, 1-mg/kg bolus followed by 2-mg/kg per h infusion over 90 min (n = 17): “low dose.” Each pig received mibefradil (Hoffman-La Roche, Basel, Switzerland), 1 mg/kg intravenously (IV) (powder dissolved in 8 ml of saline 0.9%), as a bolus dose over 5 min starting at 30 min before CAL, followed by 2 mg/kg per h IV for the remainder of the experiment.

Mibefradil, 2-mg/kg bolus followed by 4-mg/kg per h infusion over 90 min (n = 9): “higher dose.” Each pig received mibefradil (Hoffman-La Roche), 2 mg/kg powder dissolved in 8 ml of saline 0.9%, as a bolus dose over 5 min starting at 30 min before CAL, followed by 4 mg/kg per h IV for the remainder of the experiment.

Verapamil, 0.3-mg/kg bolus followed by 0.6-mg/kg per h infusion over 90 min (n = 11). Verapamil was obtained as a 5-mg/2-ml solution (Lennon, South Africa). Each pig received verapamil (0.3-mg/kg bolus dose over 5 min) starting 30 min before CAL, followed by an infusion of 0.6 mg/kg per hour IV for the remainder of the experiment.

Statistical procedures. Results are expressed as mean value ± SEM. One-way analyses of variance and the Student t test (two-tailed) were applied using paired analyses where appropriate. The Fisher exact test was used for comparing the incidence of spontaneous arrhythmias. The Bonferroni correc-

### Table 1. Occurrence of Spontaneous Tachyarrhythmias and Ventricular Fibrillation Threshold During Ischemia and Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>VT</th>
<th>VF</th>
<th>VFT (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0/13</td>
<td>0/13</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>Mibefradil (1-mg/kg bolus)</td>
<td>0/15</td>
<td>0/15</td>
<td>17.6 ± 1.0*</td>
</tr>
<tr>
<td>Mibefradil (2-mg/kg bolus)</td>
<td>1/9</td>
<td>0/9</td>
<td>17.6 ± 1.9†</td>
</tr>
<tr>
<td>Verapamil (0.3-mg/kg bolus)</td>
<td>0/9</td>
<td>0/9</td>
<td>16.2 ± 0.8†</td>
</tr>
<tr>
<td>Placebo</td>
<td>9/13</td>
<td>10/13</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>Mibefradil (1-mg/kg bolus)</td>
<td>15/15</td>
<td>8/15</td>
<td>16.9 ± 4.0</td>
</tr>
<tr>
<td>Mibefradil (2-mg/kg bolus)</td>
<td>0/9†</td>
<td>1/9†</td>
<td>19.6 ± 2.3*</td>
</tr>
<tr>
<td>Verapamil (0.3-mg/kg bolus)</td>
<td>1/9‡</td>
<td>1/9‡</td>
<td>15.1 ± 2.6†</td>
</tr>
</tbody>
</table>

*p < 0.001, †p < 0.02, ‡p < 0.05, versus placebo. VF = ventricular fibrillation; VFT = ventricular fibrillation threshold; VT = ventricular tachycardia.

A p value of <0.05 was considered significant.

Results

A total of 50 pigs were used in the main study. Two pigs in the mibefradil low dose group and two in the verapamil group were excluded because the size of the underperfused zone was <26% of total left ventricular mass. For various practical reasons, all measurements could not always be made in all pigs. Furthermore, certain procedures, for example, blood flow measurements by costly radioactive microspheres, were confined to a limited number of experiments. These two factors explain the differing number of observations within each group.

Spontaneous ventricular arrhythmias and VFT during ischemia and reperfusion (Table 1). Ischemia. VFT before CAL was 17.5 ± 2.7 mA (placebo group), 21.7 ± 2.5 mA (mibefradil low dose group), 19.8 ± 2.0 (mibefradil higher dose group) and 18 ± 2.1 mA (verapamil group). In the placebo group, CAL caused a marked fall in VFT. In both mibefradil groups as well as in the verapamil group, VFT was maintained at higher levels than in the placebo group, indicating antiarrhythmic activity. The incidence of spontaneous ventricular tachyarrhythmias was low in all groups.

Reperfusion. All runs of VT and VF occurred within the first 10 min of reperfusion. At the time of the VFT measurement, no rhythm disturbances were evident. Only the higher dose of mibefradil and verapamil decreased the incidence of VT and VF. Similarly, mibefradil (higher dose) and verapamil
Table 2.  Left Ventricular Pressures, Mean Arterial Pressure and Heart Rate

<table>
<thead>
<tr>
<th></th>
<th>Before Drug (mean ± SEM)</th>
<th>Before CAL (mean ± SEM)</th>
<th>During Ischemia (mean ± SEM)</th>
<th>During Reperfusion (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 11–12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSP</td>
<td>100 ± 5</td>
<td>105 ± 3</td>
<td>95 ± 4</td>
<td>85 ± 6*</td>
</tr>
<tr>
<td>LV max dp/dt</td>
<td>2,003 ± 68</td>
<td>1,884 ± 85</td>
<td>1,645 ± 51†</td>
<td>1,332 ± 122†</td>
</tr>
<tr>
<td>MAP</td>
<td>93 ± 3</td>
<td>86 ± 3</td>
<td>83 ± 4†</td>
<td>73 ± 5†</td>
</tr>
<tr>
<td>HR</td>
<td>122 ± 4</td>
<td>111 ± 6</td>
<td>113 ± 8</td>
<td>124 ± 8</td>
</tr>
<tr>
<td>Mibefradil (1-mg/kg bolus followed by 2-mg/kg per h infusion) (n = 11–12)</td>
<td>100 ± 5</td>
<td>105 ± 3</td>
<td>95 ± 4</td>
<td>85 ± 6*</td>
</tr>
<tr>
<td>LVSP</td>
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<tr>
<td>MAP</td>
<td>122 ± 4</td>
<td>111 ± 6</td>
<td>113 ± 8</td>
<td>124 ± 8</td>
</tr>
<tr>
<td>Mibefradil (2-mg/kg bolus followed by 4-mg/kg per h infusion) (n = 6–9)</td>
<td>111 ± 8</td>
<td>80 ± 7†‡§</td>
<td>61 ± 6†‡§</td>
<td>62 ± 6†‡§</td>
</tr>
<tr>
<td>LVSP</td>
<td>1,844 ± 96</td>
<td>1,429 ± 102†‡§</td>
<td>766 ± 86†‡§</td>
<td>758 ± 67†‡§</td>
</tr>
<tr>
<td>LV max dp/dt</td>
<td>87 ± 6</td>
<td>59 ± 5†‡§</td>
<td>45 ± 4†‡§</td>
<td>44 ± 4†‡§</td>
</tr>
<tr>
<td>MAP</td>
<td>110 ± 6</td>
<td>90 ± 8</td>
<td>90 ± 7†§</td>
<td>86 ± 9†§</td>
</tr>
<tr>
<td>Verapamil (0.3-mg/kg bolus-followed by 0.6-mg/kg per h infusion) (n = 6–9)</td>
<td>97 ± 5</td>
<td>87 ± 5†‡§</td>
<td>83 ± 4</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>LVSP</td>
<td>1,831 ± 106</td>
<td>1,215 ± 109†‡§</td>
<td>1,195 ± 100†‡§</td>
<td>910 ± 87†‡§</td>
</tr>
<tr>
<td>LV max dp/dt</td>
<td>90 ± 3</td>
<td>73 ± 3†‡§</td>
<td>65 ± 24†‡§</td>
<td>69 ± 2‡§</td>
</tr>
<tr>
<td>MAP</td>
<td>116 ± 4</td>
<td>107 ± 6</td>
<td>113 ± 5</td>
<td>106 ± 6§</td>
</tr>
</tbody>
</table>

*p < 0.05, †p < 0.02 versus before drug (or coronary artery ligation [CAL] in placebo group). §p < 0.05, ¶p < 0.001 versus mibefradil low dose. ¶p < 0.05, ¶p < 0.001 versus placebo. HR = heart rate (beats/min); LV max dp/dt = maximal rate of left ventricular pressure development (mm Hg/s); LVSP = left ventricular systolic pressure (mm Hg); MAP = mean arterial pressure (mm Hg).

maintained VFT at higher levels than in the placebo group, whereas the low dose of mibefradil did not.

Left ventricular pressures, arterial pressure and heart rate (Table 2). CAL in the placebo group resulted in a decrease in LV max dp/dt, mean arterial pressure (MAP) and left ventricular systolic pressure (LVSP). All three of these variables remained low throughout the periods of ischemia and reperfusion. The administration of low dose mibefradil did not change left ventricular or arterial pressures before CAL. All values of LV max dp/dt, LVSP and MAP during ischemia and reperfusion in this group were comparable to those in the placebo group. The higher dose of mibefradil as well as verapamil markedly decreased LV max dp/dt, LVSP and MAP before CAL. These lower values were maintained during ischemia and reperfusion. Heart rate was decreased during ischemia and reperfusion in the higher dose mibefradil group and during reperfusion in the verapamil group.

Regional left ventricular blood flow (Fig. 1). Left ventricular blood flow before CAL in the placebo group was 185 ± 18.6 ml/100 g per min, which did not differ from the predrug and pre-CAL levels in the low dose mibefradil and verapamil groups. Blood flow in each tissue sample during ischemia and reperfusion was expressed as a percent of the predrug and pre-CAL values for that specific sample. Blood flow was not measured in the mibefradil higher dose group.

Blood flow during ischemic period. Midischemic zone: In all three groups, blood flow fell to <10% of pre-CAL values. Nonischemic zone: Blood flow in the mibefradil low dose group was higher than that in the placebo group (129 ± 14% vs. 93 ± 5%, p < 0.05). Blood flow in the verapamil group did not differ from that in the placebo group.

Blood flow during reperfusion period. Midischemic zone: There was a marked increase in blood flow in the mibefradil low dose group (175 ± 19% vs. 125 ± 9% in the placebo group, 19%).
Blood flow in the verapamil group was lower than that in the mibefradil group and did not differ from that in the placebo group. Nonischemic zone: Both the mibefradil low dose and verapamil increased blood flow (126 ± 14% [p < 0.05] and 135 ± 12% [p < 0.01], respectively, vs. 89 ± 5% in the placebo group).

Size of underperfused zone. The zone sizes were 31 ± 2% (placebo group), 28 ± 1% (mibefradil low dose group), 29 ± 2% (mibefradil higher dose group), and 30 ± 2% (verapamil group).

Discussion

We tested the hypothesis that mibefradil, a proposed selective T-type calcium channel blocker, could have antiarhythmic activity without depressing contractile activity. During ischemia, the lower dose of mibefradil decreased the vulnerability of the heart to VF, whereas changes in contractile activity were comparable to those in the placebo group. Because of the excellent bioavailability of mibefradil (>90%) (23), intravenous doses are comparable to oral doses. Thus, the lower intravenous dose used in our study is clinically relevant because it more or less equals the oral antianginal dose of ~2 mg/kg in patients (35).

The finding that mibefradil reduced experimental arrhythmias at a clinically relevant dose without depressing contractile activity may render the agent useful in the setting of acute myocardial infarction. In contrast, no dose of verapamil in our model offered antiarhythmic activity during ischemia without a concomitant depression of left ventricular contractile activity. In a previous study (14), a clinically relevant dose of verapamil (0.2 mg/kg) was not antiarhythmic. A higher (supraclinical) dose of verapamil (0.6 mg/kg) was antiarhythmic but at the cost of depressing contractile activity (14). A comparable dose of verapamil (0.3-mg/kg bolus followed by 0.6 mg/kg per h) in the present study again depressed contractile activity.

Antiarhythmic mechanisms of mibefradil during ischemia. A recent report (36) proposed that when intracellular coupling is reduced, L-type calcium currents could cause microreentry, thereby predisposing to arrhythmias in conditions such as ischemia. L-type calcium current blockade should therefore be particularly effective as antiarrhythmic agents under these conditions. Moreover, mibefradil is a more effective blocker of L-type calcium channels in depolarized myocardial cells than verapamil (37,38). Mibefradil may therefore be particularly active in blocking L-channels in myocardial cells that are depolarized by ischemia (39), with a greater effect as cells become more depolarized. It is in such depolarized cells that calcium overload may lead to severe ventricular arrhythmias (3,40). We therefore speculate that mibefradil is “ischemia selective,” by virtue of these effects on L-channels of depolarized cells. However, in the absence of the appropriate electrophysiologic studies on isolated ventricular myocytes subject to simulated ischemia, this proposal remains hypothetical.

In addition to L-channel effects, we speculate that mibe-
prvoke VF (49). We took suitable precautions, as described in Methods (29).

**Mibefradil and left ventricular contractile activity.** An important observation in the present study is that the lower (clinically relevant) dose of mibefradil, given during ischemia, did not change contractile activity, as measured antiarrhythmically by $LV_{\text{max}}$ dP/dt, compared with placebo group. When myocardial ischemia was introduced in dogs with a previous infarction (6), mibefradil in a comparable dose (1 mg/kg) protected against VF induced by programmed electrical stimulation, without adverse effects on $LV_{\text{max}}$ dP/dt.

In contrast, in our model (14) a clinically relevant dose of verapamil neither depressed contractile activity nor had antiarrhythmic effects. Antiarrhythmic effects were obtained with a supraclinical dose of verapamil in the present study, but at the cost of depression of contractile activity. These results suggest that a mibefradil-like agent may be promising for further assessment in acute myocardial infarction.

**Conclusions.** In the present study the T-type calcium channel blocker mibefradil, used in a clinically relevant dose, had ischemic antiarrhythmic activity and increased coronary blood flow, without depression of left ventricular contractile activity. These data suggest, but do not prove, that T-channel blockade could differentially influence arrhythmogenesis while maintaining contractile activity. An additional antiarrhythmic property of mibefradil may lie in its selective inhibition of L-channels in depolarized tissue. Further testing is warranted when pure T-type channel blockers become available.

These proposals may lead to a better understanding of the full therapeutic spectrum of mibefradil. Although this drug has recently been withdrawn from the market because of potentially adverse drug interactions, the concept of combined T-type and L-type calcium channel blockade remains novel and of potential clinical interest.

**References**