Cardioprotective Effects of Lidoflazine During 1-hour Normothermic Global Ischemia

W. Flameng, M.D., W. Daenen, M.D., M. Borgers, Dr. Sc., F. Thone, R. Xhonneux, A. Van de Water, and H. Van Belle

SUMMARY The cardioprotective effects of lidoflazine, a drug with calcium homeostatic properties, were investigated in dogs subjected to 1 hour of normothermic global ischemia, followed by reperfusion. None of the eight control dogs could be weaned from the extracorporeal bypass, confirming the severity of the ischemic model. All eight acutely pretreated dogs showed rapid recovery from the prolonged ischemic arrest and could support their own circulation. Recovery of preischemic values was 95% for systolic arterial pressure, 71% for diastolic arterial pressure, 99% for left ventricular dP/dt max and 80% for cardiac output. Light and electron microscopy and calcium cytochemistry were performed on left ventricular biopsies taken before, during and after ischemic arrest. In the control dogs, loss of structural integrity of the sarcolemma and mitochondria was prominent at the end of the ischemic period. Intracellular edema, hypercontraction of sarcomeres and great accumulation of calcium in severely damaged mitochondria occurred after 5 and 30 minutes of reperfusion. In the lidoflazine-treated dogs, such lesions were largely prevented during the ischemic period and completely reversed after reperfusion. These observations suggest that the tolerance to ischemia is markedly augmented by lidoflazine.

Nayler suggested that lidoflazine exerts a protective effect on the ischemic and reperfused heart muscle. In the experiments on the isolated rabbit heart perfused by the Langendorff method, the drug was found to suppress the steep rise in end-diastolic contracture that takes place upon reoxygenation of the ischemic myocardium. The recovery of developed tension was found to be strongly enhanced in pretreated hearts. Mitochondrial functions, determined after measurements of oxidative phosphorylation, ATP production and calcium content, which were drastically altered after prolonged ischemia and reperfusion, remained close to control values in the lidoflazine-pretreated hearts.

Lidoflazine (Clinium), 4-[4,4-bis(4, fluorophenyl)butyl]-N-(2,6-dimethyl-phenyl)-1-piperazine acetamide, is known to be beneficial in the long-term treatment of ischemic heart disease and to enhance work tolerance in patients with angina pectoris. It has

From the Department of Cardiovascular Surgery, University of Leuven, Leuven, and Janssen Pharmaceutica Research Laboratories, Beerse, Belgium.

Supported in part by a grant from IWONL.

Address for correspondence: W. Flameng, M.D., Department of Cardiovascular Surgery, University Clinic St. Rafael, Kapucijnenvoer 35, B-3000 Leuven, Belgium.

Received July 9, 1980; revision accepted February 6, 1981.

Circulation 64, No. 4, 1981.
pharmacologically been characterized as a drug that favors cellular calcium homeostasis. 8-10

We report the cardioprotective effects of lidoflazine in prolonged normothermic global ischemia of the dog heart. Measurements of cardiac performance, hemodynamics, histology, electron microscopy and calcium cytochemistry served as criteria of myocardial structure and function.

Materials and Methods

Experimental Procedure

The experiments were carried out on 16 purebred beagles of either sex. Body weight ranged from 10-17 kg.

After premedication with 5 mg/kg piritramide and 0.05 mg/kg scopolamine subcutaneously11 and induction with pentobarbital 5 mg/kg intravenously, anesthesia was maintained with N2O/O2 (50/50%) using a Siemens-Elema respirator. ECG lead II was continuously recorded.

A Millar tip manometer was placed in the ascending aorta via the left femoral artery to measure aortic pressure. A Swan-Ganz thermodilution catheter was introduced through the femoral vein and placed in the pulmonary artery. Central venous pressure, pulmonary artery pressure and cardiac output were monitored. A right thoracotomy was then performed through the fourth intercostal space, the pericardium was opened and the heart was suspended in a pericardial cradle. A second Millar tip manometer was placed in the left ventricle through a pulmonary vein. All pressure measurements and the ECG-triggered thermodilution system were on-line, connected with a PDP 11/20 computer system as described earlier.12 All hemodynamic variables (heart rate, systolic and diastolic aortic pressures, central venous pressure, pulmonary artery pressure, left ventricular systolic and end-diastolic pressures, and left ventricular dp/dt max and dp/dt min) were printed out every 30 seconds with a teletype writer. Cardiac output, stroke volume and systemic vascular resistance were estimated every 5 minutes. Data were analyzed according to standard statistical procedures. In the compound group and the solvent group, the values after 1 hour of ischemia were evaluated for statistical significance by applying a t test on related samples. Intergroup comparison was made with a t test on unrelated samples.13 In the decision-making procedure, a two-tailed region of rejection of \( \alpha \) [p (type I error)] \( \leq 0.05 \) was applied.

In the drug-treated group \( n = 8 \), 1.25 mg/kg body weight of lidoflazine, and in the control group \( n = 8 \) the solvent in a comparable amount were given intravenously 20 minutes before starting the experiment. Thereafter, hemodynamics were followed up for 10 minutes. Then the dogs were placed on cardiopulmonary bypass. The arterial line was connected by cannulating the right femoral artery. The right atrium was cannulated for venous drainage. The left ventricle was vented through the left atrium. Two Sarns roller pumps, a heat exchanger and a bubble oxygenator were used. The heart-lung machine was primed with 2 l of fresh blood from a donor dog. Five milligrams of lidoflazine was added in the drug-treated group. The aorta was cross-clamped for 60 minutes. The myocardial temperature was monitored with a thermistor needle inserted in the interventricular septum. The temperature was kept constant at 37°C. Left ventricular pressure was carefully monitored to ensure that the ventricle remained empty. After 60 minutes of cross-clamping, the clamp was removed and the heart reperfused. The left ventricle remained empty during the 30 minutes of reperfusion. When ventricular fibrillation persisted beyond the tenth minute, the heart was defibrillated electrically. After 30 minutes of reperfusion the dogs were weaned from bypass. If this was possible, recovery of hemodynamics was followed up for another 30 minutes.

Light and Electron Microscopy

Transmural biopsies were taken from the left ventricular anterior free wall approximately 2 cm from the apex before the onset of ischemia, at the end of the 60-minute ischemic period, and after 5 minutes and 30 minutes of reperfusion. The biopsies were divided in a subepicardial and mid- and subendocardial part and immediately fixed with cold 3% glutaraldehyde in 0.09 M potassium oxalate, brought to pH 7.4 with N potassium hydroxide. After a 24-hour fixation at 4°C, the biopsies were washed in potassium oxalate 0.09 M supplemented with 0.22 M sucrose and again divided in a sample for ultrastructural examination and a sample for calcium localization studies.

The former was postfixed for 1 hour in 1% osmium tetroxide, buffered to pH 7.4 with 0.05 M veronal acetate, dehydrated in graded series of ethanol and routinely embedded in epon.

Vibratome sections 100 \( \mu \)m thick were prepared from the samples destined for calcium distribution according to a combined oxylate-pyroantimonate method.14-16 The 100-\( \mu \)m sections were postfixed in a solution containing 1% osmium tetroxide and 2% potassium pyroantimonate for 2 hours at 4°C. They were then routinely prepared for electron microscopy as described above. Semithick sections for light microscopy stained with toluidine blue served for topographic localization of the lesions and for estimation of the involved areas. In an attempt to quantify the degree of cell degeneration in the different layers of the myocardium after 60 minutes of ischemia and 30 minutes of reperfusion, three semithin sections of each part of the biopsy were examined and a total of 500 nucleated cells per part was evaluated. The criteria used for cell degeneration were nuclear chromatin clumping and pyknosis, intracellular edema, vacuolization and absence of darkly stained mitochondria. Intergroup comparison was made with Mann-Whitney U test. Ultrathin sections were examined, either unstained or briefly stained with uranium acetate to lead citrate, in a Philips EM 300 microscope.

To control for the specificity of the precipitate in the calcium localization attempts, chelation of calcium
with ethylene glycol bis (β-aminoethyl ether)N,N',N'-tetraacetic acid (EDTA)) was accomplished on thin sections. All sections became devoid of precipitate after this treatment, so the precipitated cation was considered to be calcium.

**Results**

**Hemodynamic Study**

The mean ± SEM of all hemodynamic variables during the control period are presented in Table 1. Systemic blood pressure was significantly lower in the lidoflazine-treated group, 98 mm Hg systolic and 64 mm Hg diastolic vs 124 mm Hg systolic and 88 mm Hg diastolic (p < 0.05). Left ventricular end-diastolic pressure was significantly lower in the drug-treated group, 4.2 vs 8.3 mm Hg (p < 0.05). The other variables were not significantly different (p > 0.05).

After cross-clamping of the aorta, the hearts in the control group continued to beat for 5 minutes (range 4–6.5 minutes). In the drug-treated group, cardiac arrest or fibrillation occurred after 9 minutes (range 5–20 minutes). The difference was not statistically significant.

After 60 minutes of normothermic global ischemia, three of the eight dogs in the control group developed a "stone heart" and could not be weaned from cardiopulmonary bypass. In the other five dogs in this group, hemodynamics deteriorated dramatically after weaning from the bypass and the dogs could not support their own circulation longer than 5 minutes (Table 2 and 3, Fig. 1). Table 3 is a summary of the values compared with the preischemic values in these five dogs.

**Table 2. Survival After Termination of Cardiopulmonary Bypass**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Treated</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 minute</td>
<td>5 minutes</td>
<td>0.2000</td>
</tr>
<tr>
<td></td>
<td>5 minutes</td>
<td>8/8</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>30 minutes</td>
<td>0/8</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

All of the lidoflazine-treated dogs could be weaned from the bypass, and hemodynamic recovery was rapid (Table 2 and 4, Fig. 1). Ten minutes after the heart-lung machine was stopped, recovery of systolic pressure was 95%, diastolic pressure 71%, cardiac output 80% and left ventricular dP/dt max 99%.

Thirty minutes after weaning from the bypass, recovery was 90% for systolic pressure, 66% for diastolic pressure, 77% for cardiac output and 95% for left ventricular dP/dt max. Heart rate and left ventricular end-diastolic pressure significantly increased (p < 0.05) and diastolic aortic blood pressure and stroke volume significantly decreased (p < 0.05) during the whole recovery period. The tendency of systemic pressure, cardiac output and contractility to decrease after 20 minutes is probably due to loss of volume with low filling, because no attempts were made to support the hearts with infusions.

**Table 3. Recovery of Hemodynamics in Five Control Dogs Without Stone Heart After Reperfusion After 1 Hour Normothermic Global Ischemia**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before CBP (control)</th>
<th>After CBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>103 ± 9 NS</td>
<td>122 ± 16 104 ± 16</td>
</tr>
<tr>
<td>Syst Ao BP (mm Hg)</td>
<td>113 ± 6 44*</td>
<td>32* ± 7</td>
</tr>
<tr>
<td>Diast Ao BP (mm Hg)</td>
<td>80.6 ± 5.6 28*</td>
<td>21* ± 4.1</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>1.65 ± 0.22 0.19*</td>
<td></td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>16.5 ± 2.7 3.9*</td>
<td>1.4* ± 2.5</td>
</tr>
<tr>
<td>LV dP/dt max (mm Hg/sec)</td>
<td>2212 ± 199 647*</td>
<td>303* ± 169</td>
</tr>
<tr>
<td>LV dP/dt min (mm Hg/sec)</td>
<td>2238 ± 236 479*</td>
<td>289* ± 125</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>7.6 ± 2.3 16.4*</td>
<td>16.2* ± 1.5</td>
</tr>
<tr>
<td>SVR (dyn/sec/cm²)</td>
<td>4734 ± 600 1041*</td>
<td>628* ± 638</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Abbreviations: Syst Ao BP = systolic aortic blood pressure; Diast Ao BP = diastolic aortic BP; LV = left ventricular; LVEDP = left ventricular end-diastolic pressure; SVR = systemic vascular resistance. *Two-tailed t-test between related samples, p < 0.05.

Abbreviations: CPB = cardiopulmonary bypass; Syst Ao BP = systolic aortic blood pressure; Diast Ao BP = diastolic aortic BP; LV = left ventricular; LVEDP = LV end-diastolic pressure; SVR = systemic vascular resistance.
CARDIOPROTECTIVE EFFECTS OF LIDOFLAZINE/Flameng et al.

SYSTOLIC (0) and DIASTOLIC (4) Ao BLOOD PRESSURE

mmHg

120
100
80
60
40
20

Pre-ischemic value (PIV)

1
5
10
15
20
25
30 min after CPB

LEFT VENTRICULAR dP/dt max

mmHg/sec

3000
2000
1000

Pre-ischemic value (PIV)

1
5
10
15
20
25
30 min after CPB

CARDIAC OUTPUT

LITRE/min

2
1

60 min.

ischemia

30 min.
reperfusion

FIGURE 1. Recovery after termination of
cardiopulmonary bypass (CPB) in the eight
lidoflazine-treated dogs and the five control
dogs that did not develop a "stone heart"
during the reperfusion phase. Data are
mean ± SEM.

made to support the circulation with either drugs or
volume loading.

Morphologic Findings

In the biopsies taken before ischemia, the subcellular morphology was well preserved. The semi-thick light microscopic sections revealed regularly arranged sarcomeres and uniformly stained rows of mitochondria. The extracellular spaces were widened during glutaraldehyde fixation (fig. 2A). In the electron microscope, most cells showed sarcomeres in a slightly contracted state. The slight dilatation of the sarcoplasmic reticulum is most probably a result of the high concentration of potassium in the fixative. Mitochondria were uniformly preserved and possessed a large number of matrix granules, typical of well-oxygenated cells (fig. 3). Precipitated calcium was confined to few elements of the sarcoplasmic reticulum, the subsarcolemmal cisternae and in weak amounts to the mitochondrial matrices (fig. 4).

There were no obvious differences in subcellular preservation between the subendocardial and mid- and subepicardial parts.

In untreated dogs, the myocardial cells had changed dramatically by the end of the 60-minute ischemic period. These were most pronounced in the subendocardial part and primarily concerned with the mitochondria and cell periphery. The basement membrane, which normally is closely apposed to the plasmalemma, broadened and focally lost its intimate contact with the altered plasmalemma. Intracellular edema occurred focally around the mitochondria. Swollen mitochondria were common. They were devoid of their typical granules and many already presented broken cristae (figs. 5 and 6). Some of them possessed flocculent densities in their matrices (Jennings' granules). All showed a substantial increase in
TABLE 4. Recovery of Hemodynamics in Eight Lidoflazine-treated Dogs After 1 Hour of Normothermic Global Ischemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before CPB (control)</th>
<th>1 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>88</td>
<td>129*</td>
<td>131*</td>
<td>136*</td>
<td>140*</td>
<td>136*</td>
<td>137*</td>
<td>139*</td>
</tr>
<tr>
<td></td>
<td>± 5</td>
<td>± 8</td>
<td>± 10</td>
<td>± 10</td>
<td>± 9</td>
<td>± 12</td>
<td>± 10</td>
<td>± 11</td>
</tr>
<tr>
<td>Syst Ao BP (mm Hg)</td>
<td>97.8</td>
<td>79.9*</td>
<td>87.4</td>
<td>93.1</td>
<td>92.6</td>
<td>90.9</td>
<td>89.5</td>
<td>85.0*</td>
</tr>
<tr>
<td></td>
<td>± 3.0</td>
<td>± 4.3</td>
<td>± 5</td>
<td>± 5</td>
<td>± 5</td>
<td>± 4.3</td>
<td>± 5</td>
<td>± 5.6</td>
</tr>
<tr>
<td>Diast Ao BP (mm Hg)</td>
<td>64.1</td>
<td>44.6*</td>
<td>43.9*</td>
<td>45.4*</td>
<td>46.0*</td>
<td>43.8*</td>
<td>44.1*</td>
<td>42.3*</td>
</tr>
<tr>
<td></td>
<td>± 2.6</td>
<td>± 2.4</td>
<td>± 3.3</td>
<td>± 3.3</td>
<td>± 4.2</td>
<td>± 4.1</td>
<td>± 4.7</td>
<td>± 5.1</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>1.64</td>
<td>1.12</td>
<td>1.18*</td>
<td>1.32</td>
<td>1.36</td>
<td>1.38</td>
<td>1.29</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>± 0.25</td>
<td>± 0.14</td>
<td>± 0.18</td>
<td>± 0.19</td>
<td>± 0.17</td>
<td>± 0.17</td>
<td>± 0.17</td>
<td>± 0.17</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>18.4</td>
<td>8.7*</td>
<td>9.2*</td>
<td>9.9*</td>
<td>9.9*</td>
<td>10.7*</td>
<td>9.4*</td>
<td>9.1*</td>
</tr>
<tr>
<td></td>
<td>± 2.4</td>
<td>± 0.8</td>
<td>± 1.0</td>
<td>± 1.3</td>
<td>± 1.3</td>
<td>± 1.6</td>
<td>± 1.0</td>
<td>± 1.0</td>
</tr>
<tr>
<td>LV dP/dt max (mm Hg/sec)</td>
<td>2001</td>
<td>1487*</td>
<td>1659</td>
<td>1974</td>
<td>1993</td>
<td>1960</td>
<td>1954</td>
<td>1901</td>
</tr>
<tr>
<td></td>
<td>± 158</td>
<td>± 145</td>
<td>± 161</td>
<td>± 187</td>
<td>± 210</td>
<td>± 181</td>
<td>± 170</td>
<td>± 219</td>
</tr>
<tr>
<td>LV dP/dt min (mm Hg/sec)</td>
<td>2198</td>
<td>1362*</td>
<td>1662</td>
<td>1979</td>
<td>1971</td>
<td>1851</td>
<td>1779</td>
<td>1628</td>
</tr>
<tr>
<td></td>
<td>± 61</td>
<td>± 182</td>
<td>± 197</td>
<td>± 213</td>
<td>± 226</td>
<td>± 188</td>
<td>± 216</td>
<td>± 171</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.2</td>
<td>6.7</td>
<td>9.3*</td>
<td>9.2*</td>
<td>8.8*</td>
<td>8.4*</td>
<td>8.3*</td>
<td>7.6*</td>
</tr>
<tr>
<td></td>
<td>± 0.4</td>
<td>± 1.0</td>
<td>± 1.7</td>
<td>± 1.9</td>
<td>± 1.4</td>
<td>± 1.3</td>
<td>± 1.1</td>
<td>± 0.9</td>
</tr>
<tr>
<td>SVR (dyn/sec/cm²)</td>
<td>4465</td>
<td>4591</td>
<td>4522</td>
<td>4480</td>
<td>4400</td>
<td>4183</td>
<td>4272</td>
<td>4123</td>
</tr>
<tr>
<td></td>
<td>± 625</td>
<td>± 448</td>
<td>± 518</td>
<td>± 684</td>
<td>± 696</td>
<td>± 719</td>
<td>± 527</td>
<td>± 520</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
*Two-tailed t test between related samples, \( p < 0.05 \).

Abbreviations: CPB = cardiopulmonary bypass; Syst Ao BP = systolic aortic blood pressure; Diast Ao BP = diastolic aortic BP; LV = left ventricular; LVEDP = LV end-diastolic pressure; SVR = systemic vascular resistance.
the amount of precipitated calcium in the clarified matrices. The amount of glycogen was markedly decreased. Similar lesions occurred in the subepicardial regions, but to a lesser degree. No flocculent densities appeared in subepicardial mitochondria. The lesions in the midportion of the biopsies were very much like those in the subendocardial region.

Further involuntary changes were expressed in biopsies taken after 5 minutes of reperfusion. At the light microscopic level, the damage was uniform throughout the endocardial portion and the midportion of the left ventricles (fig. 2B), whereas the subepicardial areas were less affected. Ultrastructurally, the lesion consisted of edematous swelling of the cytosol, chewing up of the sarcolemmal lipid bilayer, hypercontraction of the sarcomeres, margination and clumping of nuclear chromatin and tremendous swelling of mitochondria, which became heavily loaded with precipitated calcium (fig. 7). Many mitochondria exhibited flocculent densities, characteristic of irreversible damage (fig. 7). Similar changes could be observed in the midportion of the biopsies and in part of the cells of the subepicardial region. The other part of the cells showed no further involution or recovered from the ischemic damage. In such cells, the mitochondria contained much less precipitated calcium.

After 30 minutes of reperfusion, the subendocardial cells and part of the cells derived from the midportion uniformly deteriorated (table 5). At the light-

---

**Figure 2.** Light microscopic survey pictures of toluidine blue-stained semithick sections of subendocardial parts of the left ventricular biopsies. Magnification × 400. (A) Control dog, preischemic sample showing regular contracted sarcomeres and uniformly dense-stained rows of mitochondria in all cells. (B) Control dog, after 60 minutes of ischemia and 5 minutes of reperfusion. Intracellular clarification indicates the development of edema and swelling of mitochondria. (C) Control dog, after 60 minutes of ischemia and 30 minutes of reperfusion. The transversely cut cells all show huge intracellular vacuolization and no densely stained mitochondria are seen. Nuclei are pyknotic. (D) Lidoflazine-treated dog after 60 minutes of ischemia and 5 minutes of reperfusion. Note the absence of edema and of mitochondrial swelling.

**Figure 3.** Preischemic subendocardial left ventricular biopsy from a control dog. Ultrastructural details of the cell periphery show the intact sarcolemma (s), T tubules (t), sarcoplasmic reticulum (sr) and mitochondria (m), which have typical osmiophilic granules (arrows). Magnification × 22,600.

**Figure 4.** Preischemic left ventricular biopsy from a control dog. Localization of calcium with a combined oxalate-pyroantimonate procedure. Fine deposits of precipitated calcium (arrows) are seen inside the mitochondria and in the sarcoplasmic reticulum (arrowheads). Magnification × 34,500.
microscopic level, darkly stained mitochondria were absent and the cytoplasm was filled with holes (fig. 2C). Indeed, only mitochondria remnants remained in irreversibly altered cells, in which pyknotic nuclei, fragments of sarcolemma and contracted myofilaments predominated. Inside of the mitochondrial remnants, which were often composed of the outer membrane only, were huge amounts of clumped calcium precipitate (fig. 8). Myocardial capillaries often contained degenerated cell material, which strongly suggests no reflow in these areas. As noticed early after reperfusion, part of the subepicardial cells apparently recovered and possessed a nearly normal ultrastructure.

Lidoflazine-pretreated dogs showed a different structural and cytochemical pattern. The samples taken after 60 minutes of ischemia showed limited ultrastructural deterioration and swelling of mitochondrial matrices in which the typical granules were totally absent. Disruption of part of the cristae occurred regularly. Other organelles appeared well preserved; in particular, the sarcolemma and the basement membrane looked intact (fig. 9). Calcium precipitate in mitochondria was moderately increased compared with preischemic samples. The amount of glycogen was decreased. The cells of the subendocardial areas were more susceptible to the ischemic arrest than the subepicardial cells. Cells from the midportion were well-preserved and resembled those of the subepicardial region. Recovery from the damage was already obvious in the biopsies after 5 minutes of reperfusion, except for a few cells situated close to the fibrous endocardium. By light microscopy, the structural organization of the cells resembled that of the preischemic biopsies. Intracellular edema was absent and the sarcomeres presented a usual pattern of contraction (fig. 2D). Ultrastructurally, mitochondria resumed their normal shape and size but often presented twisted cristae. Typical granules were still absent in mitochondria from subendocardial cells. Calcium precipitate which was present in the form of small singular dots in the mitochondrial matrices had only slightly increased when compared with preischemic counterparts. Cells of the subepicardial region recovered very fast and possessed mitochondria with typical matrix granules. After 30 minutes of reperfusion, just before the dogs were weaned from the bypass, most cells from epicardial and mid- and endocardial regions showed full recovery (table 5), both in the structure of the subcellular

**FIGURE 5.** Left ventricular biopsy from a control dog after 60 minutes of ischemia, subepicardial area. Note the relatively well-preserved sarcolemma (s) and mitochondria (m), which possess regularly spaced cristae but lack osmiophilic granules. Magnification × 18,600.

**FIGURE 6.** Left ventricular biopsy in a control dog after 60 minutes of ischemia, subendocardial region. Mitochondria (m) are greatly swollen and present broken cristae. The sarcolemma becomes vesiculated (arrows) and the apposed glycocalyx focally appears less dense and fragmented. Magnification × 18,600.
CARDIOPROTECTIVE EFFECTS OF LIDOFLAZINE/Flameng et al.

**FIGURE 7.** Left ventricular biopsy from a control dog after 60 minutes of ischemia and 5 minutes of reperfusion, subendocardial region. The edematous cytoplasm contains hypercontracted sarcomeres (sm) and heavily damaged mitochondria that contain clusters of precipitated calcium (arrows.) At the periphery the sarcolemma is totally fragmented and the glycocalyx (arrowhead) has lost its close contact with the triple-layered membrane. Magnification × 11,700. The inset shows accumulations of calcium deposits (arrows) and flocculent densities (arrowheads) in which similar calcium precipitates are found. Magnification × 24,200.

**TABLE 5.** The Frequency (Percent) of Severely Degenerated Myocardial Cells Found in the Subepicardial, Intermediate and Subendocardial Parts of the Biopsy Taken After 60 Minutes of Ischemia and 30 Minutes of Reperfusion of Eight Control and Eight Lidoflazine-treated Dogs

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control dogs</th>
<th>Lidoflazine-treated dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Mid</td>
</tr>
<tr>
<td>1587</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1588</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>1591</td>
<td>43</td>
<td>98</td>
</tr>
<tr>
<td>1596</td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td>1606</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1611</td>
<td>0</td>
<td>96</td>
</tr>
<tr>
<td>1642</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1645</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median</td>
<td>0</td>
<td>21.5</td>
</tr>
<tr>
<td>95% conf. lower limit</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>95% conf. upper limit</td>
<td>43</td>
<td>98</td>
</tr>
</tbody>
</table>

*Mann-Whitney U-test vs control group, two-tailed probability ≤ 0.05.
The criteria for severe degeneration were clumping of nuclear chromatin and pyknosis, intracellular edema, vacuolization and the absence of darkly stained mitochondria (fig. 2c). The percentage is derived from scorings of at least 500 nucleated cells.
Abbreviations: Epi = epicardial; Mid = intermediate; Endo = endocardial.
organelles, including mitochondria, and in the amount and distribution of subcellular calcium.

**Discussion**

Lidoflazine clearly protects the myocardium against the detrimental effects of 1 hour of global ischemia at normothermia. Functional recovery after 30 minutes of reperfusion of the empty heart was nearly complete and in sharp contrast to the control hearts, which could not support their own circulation. The slight slope in the recovery curves 20 minutes after termination of the cardiopulmonary bypass must be interpreted as the result of uncompensated loss of volume by bleeding from the operative field. No attempt was made to correct this volume loss.

The ultrastructural observations fully support the observed functional evolution of the hearts after the prolonged period of global ischemia. In view of the possible nonrepresentative character of the limited number of examined cells, the evaluation of the damage by ultrastructural examination is hazardous. Therefore, our detailed subcellular data are backed up by gross structural observations on semithick sections. The degeneration of the myocardium that occurs after 60 minutes of normothermic arrest, followed by 30 minutes of reperfusion, is so pronounced that the affected areas could be easily quantified by light microscopy. This allowed us to obtain a good idea about the uniformity of the damage in the control series and the recovery over the total length of the transmural biopsies in the lidoflazine series. Still, the biopsy technique allows only a very small sampling of the entire heart. This is reflected in the wide scatter of the data in the semiquantitative analysis, especially in those obtained from the midzone. However, prevelation of larger areas of myocardium or multiple biopsies will interfere with the function of the heart. The severity of this normothermic ischemic model is already demonstrated by the ultrastructural findings in biopsies taken at the end of the ischemic arrest, which could often be classified morphologically as irreversible, especially in the subendocardial area.

Further degeneration of the myocardium during the reperfusion phase is possibly due to the phenomenon described as the "oxygen paradox;" however, it might also be a mere progression of the lesion with time. The left ventricle was, in most of the nonmedicated animals, so susceptible to ischemic contraction that it is doubtful that the subendocardial coronaries were perfused at all. If this is so, we can hardly speak of an exaggeration of the lesions due to reperfusion with well-oxygenized blood.

The difference in susceptibility after prolonged...
FIGURE 9. Left ventricular biopsy from a lidoflazine-treated dog, subendocardial area. After 60 minutes ischemia, mitochondria (m) are swollen and present ruptured cristae, whereas other subcellular entities, including the sarcolemma and glycocalyx (arrows), are well preserved. Magnification × 22,700.

FIGURE 10. Left ventricular biopsy from a lidoflazine-treated dog, subendocardial area. After 60 minutes of ischemia and 5 minutes of reperfusion, recovery of mitochondria is noticed, although some display distorted cristae (arrows). The cell periphery (arrowheads) is intact. Magnification × 25,400.

global ischemia of subendocardial vs subepicardial cells is striking. The fact that the differences are already pronounced before reperfusion might be related either to a different energy reserve between epicardial vs subendocardial cells or to a difference in cell metabolism during ischemia. On the other hand, the hearts continued beating for several minutes after interruption of coronary blood flow. During this period, subendocardial tension might be higher than subepicardial and might make the subendocardial layer more susceptible to ischemic damage.

The cytochemical results on precipitated calcium in mitochondria of the ischemic myocardium fully subscribe Nayler's ideas on the involvement of calcium in the process of cell degeneration.19 The inverse relationships between the ATP-producing capacity of cardiac mitochondria and their calcium content is well established.19-23

Nayler et al.19 clearly demonstrated that mitochondrial calcium content increases markedly after ischemia and 24 that simultaneously mitochondrial functions deteriorate. In our study, we found a direct relationship between the degree of damage in mitochondria and the amount of cytochemically detectable calcium. These observations suggest that the ischemic damage to the energy-depleted myocardium is caused by an excessive inward transfer of calcium through the altered sarcolemma and possibly also by shifts of intracellular calcium. Thus, mitochondria scavenge large amounts of calcium in order to keep the cytosol calcium as low as possible. However, when mitochondria become overloaded with calcium, they compromise their own structure and function and eventually become irreversibly injured.

Morphologic and cytochemical observations substantiate that lidoflazine offers considerable protection against the structural damage provoked by ischemia and/or reperfusion. The effect of the drug is already clearly noticed in the biopsies taken at the end of the ischemic period, suggesting that if there is an effect on coronary circulation during the reperfusion phase, it is unlikely to be the primary effect. Nevertheless, we cannot exclude the possibility that the coronary vasodilatory properties of the drug have some beneficial influence on the final outcome. Most striking is the observation that in lidoflazine-pretreated...
animals the cellular periphery (sarcolemma and basement membrane) retained their normal structure after a 60-minute ischemic period, whereas in untreated dogs, severe damage to the sarcolemma and internal organelles was common. Activation of phospholipases by calcium might be responsible for the observed alterations in the sarcolemmal lipid bilayer structure. Furthermore, the condition of the basement membrane might play an important role in at least partly controlling the balance of calcium. The limited degree of damage in the lidoflazine group, which is restricted to mitochondria, allows fast recovery, as seen after 5 minutes of reperfusion. How lidoflazine exerts its protective effect is unknown.

The drug has well-established calcium transport inhibitory properties in stimulated vascular smooth muscle, i.e., counteracts experimentally induced coronary spasm,

Therefore, it varies considerably from drugs reported to alleviate ischemic damage, such as DL-propranolol, verapamil and nifedipine.

According to Nayler, lidoflazine maintains the myocardial stores of ATP above the critical levels needed to ensure that sufficient energy is available to support the calcium-accumulating activity of the sarcoplasmic reticulum, and possibly for extruding calcium from the cell. However, considering its known inhibitory properties of stimulated calcium influx through the cell membrane, it is possible that through this mechanism the cellular calcium is kept well beneath the threshold that causes irreversible damage to the myocardial cell.

In conclusion, calcium is at least one of the factors responsible for irreversible damage to the myocardium. The involutional changes caused by prolonged normothermic ischemia, which become fully expressed during reoxygenation, are paralleled by an excessive accumulation of calcium inside the mitochondria. Cardiac performance and hemodynamic measurements show that cells altered in this way and that uniformly present sarcomeres in a severe state of contracture cannot exert any contractile function.

Lidoflazine appears to alleviate myocardial damage characterized by preservation of the cellular structural integrity and by preventing mitochondrial calcium overload, allowing recovery of cardiac contractility. Although this drug does not affect myocardial calcium...
influx in normal heart muscle, it appears to favorably influence the normal homeostasis of calcium during ischemic aggression followed by reperfusion.

Acknowledgment

We are indebted to A. Van Glabbeek and M. Van Reusel for technical assistance; L. Wouters for statistical analysis; L. Leijssen for preparing the micrographs; H. Vanhove for reviewing; and L. Geentjens for typing the manuscript.

References

12. Snoeckx LH, Verheyen JL, van de Water A, Lewi P, Reneman RS: On-line computation of cardiac output with the thermo-
Cardioprotective effects of lidoflazine during 1-hour normothermic global ischemia.
W Flameng, W Daenen, M Borgers, F Thone, R Xhonneux, A van de Water and H van Belle

Circulation. 1981;64:796-807
doi: 10.1161/01.CIR.64.4.796

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/64/4/796

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/