Effects of Dimethyl Propranolol (UM-272; SC-27761) on Myocardial Ischemic Injury in the Canine Heart after Temporary Coronary Artery Occlusion

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SUMMARY Myocardial ischemic injury after temporary occlusion of the left anterior descending coronary artery (LAD) for 90 min followed by reperfusion was estimated from the epicardial ST-segment elevation 15 min after occlusion (ST

CARDIAC FAILURE remains the major cause of death among patients who have experienced an acute myocardial infarction. Considerable effort has been made in the past several years to devise pharmacologic approaches to protecting the ischemic heart with the hope of minimizing the ultimate extent of myocardial damage. Several pharmacologic and metabolic interventions have been reported to exert a beneficial effect in reducing the amount of cardiac tissue that would otherwise undergo irreversible damage due to myocardial ischemia.1-3 Recent studies have demonstrated that pretreatment with dimethyl propranolol, UM-272 (SC-27761), a compound which lacks beta-adrenergic receptor blocking properties, significantly reduced the extent of myocardial injury in the canine heart subjected to a period of coronary artery occlusion followed by reperfusion.4 In the latter study, the extent of irreversible myocardial injury was assessed gravimetrically from transverse ventricular sections which had been incubated in nitro-blue-tetrazolium (NBT) for the purpose of demarcating the area of ischemic damage.

A number of recent studies have made use of electrocardiographic and biochemical changes, such as epicardial ST-segment elevations, loss of R wave voltage, development of pathologic Q waves, and the loss of myocardial creatine kinase (CPK) activity as markers for determining and/or predicting the extent of myocardial ischemic injury in response to coronary artery occlusion.1,3,6,8 The present study was undertaken in an attempt to assess further the potential benefits of pretreatment with dimethyl propranolol in protecting the ischemic heart against irreversible damage by utilizing epicardial electrocardiographic recordings along with determinations of myocardial CPK activity and direct gravimetric analysis of infarct size.

The results of the present study support our previous observations2 regarding the ability of UM-272 to protect the ischemic myocardium from irreversible injury, and in addition, demonstrate that pretreatment with UM-272 significantly reduced the development of pathologic Q waves from potentially ischemic regions of the ventricle and significantly reduced the loss of myocardial CPK from the ischemic tissues.

Methods

Animal Preparation

Male, mongrel dogs weighing between 16.1 to 22.0 kg (mean of 18.6 kg) were anesthetized with pentobarbital sodium (30 mg/kg) i.v. Each dog was intubated with auffed endotracheal tube and ventilated with room air using a Harvard respirator. Sterile catheters were implanted in the left carotid artery and jugular vein and were brought to the surface of the skin at the back of the neck. Arterial blood pressure was monitored using a Statham P23DC pressure transducer connected to the carotid catheter. The jugular vein catheter was used for all subsequent drug administrations. Electrocardiograms from limb leads II and aVF were recorded on a Grass model 7 polygraph.

A lateral thoracotomy was performed through the fifth left intercostal space and the heart suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated below its first diagonal branch and, at the appropriate time, the blood flow was interrupted with a screw clamp (fig. 1). After 90 min of LAD occlusion, the blood flow was re instituted over 20 min in an incremental fashion in which 25% of the preischemic flow was re instituted at 0 time (i.e., after 90 min of occlusion), another 25% after 10 min and then the LAD was released completely after 20 min. An electromagnetic flow probe was used to continuously monitor LAD flow. The gradual re-institution of coronary flow minimized the development of a reactive hyperemic response and thus reduced the incidence of hemorrhagic infarction and ventricular fibrillation.9 Epicardial electrograms were recorded with 12 platinum
electrodes embedded in a 3 × 4 cm acrylic plaque (Perm, The Hygienic Dental Co.) in such a manner that the electrodes were 1 cm apart. The plaque was sutured to the free wall of the left ventricle so that several electrodes would be positioned outside the area perfused by the occluded vessel and thus represent normal myocardial regions and the remaining electrodes would be situated on the border of the ischemic zone and within the center of the area perfused by the occluded coronary artery. Each of the areas could be distinguished by its relationship to the distribution of the coronary artery which was to be occluded (fig. 1) as well as by a dark blue appearance of the myocardium during the period of myocardial ischemia. Each dog, therefore, served as its own control. Epicardial electrograms from the 12 sites were monitored sequentially and recorded on a Grass polygraph before and 15 minutes after complete LAD occlusion. Epicardial ST-segment elevations greater than 2 mV were considered abnormal as previously described¹ and were used to indicate areas of myocardial ischemic injury. The number of such sites was determined. This technique permitted continuous monitoring of the epicardial electrical potential changes (Q- and R-wave changes) without any manual manipulations and with minimal baseline shift which often is quite difficult to obtain with cotton wick electrodes as applied by Maroko et al.¹ The same electrode plaque was sutured back to the original position on the next day, thus allowing accurate measurements of the electrical potential changes at the identical sites 24 hours later. After the 24 hour epicardial electrograms were obtained, the animals were sacrificed with an overdose of pentobarbital sodium and transmural sections of the left ventricle under each electrode site, 1 cm × 1 cm, were excised for subsequent biochemical studies.

ST-segment analysis was not attempted at sites which showed local conduction delay, as indicated by prolongation of the interval from the onset of the QRS to the intrinsic deflection exceeding 40 msec or prolongation of the entire QRS beyond 65 msec.

The animals were divided randomly into two groups. The control group received equivalent volumes of 0.9% sodium chloride solution. In the drug-treated group, the initial dose of UM-272 (2 mg/kg) was given 30 min before LAD occlusion and was repeated in a dose of 2 mg/kg every 90 min for a total i.v. dose of 10 mg/kg.

Myocardial CPK Activity

Myocardial creatine kinase activities were assayed according to the method of Rosalki⁹ with minor modifications. Briefly, transmural specimens under each electrode, 1 cm × 1 cm, of nonischemic, intermediate, and severely ischemic regions were homogenized individually (5% w/v) using a dounce-ball type homogenizer and then subjected to centrifugation at 22,000 × g for 40 min at 4° C. The reaction was started by addition of 0.025 to 0.050 ml of diluted supernatant to the reconstituted lyophilized CPK reagent (Calbiochem). CPK activity was calculated from the appearance of NADPH at 340 nm and was generally linear for 15 min after a 5 min equilibration. The CPK activity is expressed in international units per mg of protein with correction for temperature. Protein concentration was determined by the method of Lowry et al.¹⁰ with bovine serum albumin as the standard.

Quantification of Infarct Size

The amount of infarcted tissue 24 hours after coronary occlusion was estimated from the nitro-blue-tetrazolium (NBT) staining reaction for the presence of intracellular dehydrogenases as described previously.³, ¹¹ Briefly, the transmural sections under each electrode site were divided into two equal portions, one for CPK activity assay as described above and the other for NBT staining. In addition, the remaining ventricular muscle was sliced into 1 cm thick parallel slices made in a plane perpendicular to the apex-base axis. The ventricular muscle was then incubated for 15 min at 37° C in a phosphate buffered solution of NBT. Incubation with NBT results in a deep blue staining reaction in undamaged regions of the heart (presence of intracellular dehydrogenases), whereas areas of irreversibly damaged tissue (lack of intracellular dehydrogenases) appear as pale zones. This technique permits a simple differentiation of the infarcted tissue from the normal, undamaged myocardium. The stained and unstained regions of the respective ventricular slices were dissected out and weighed. Infarct mass was determined and the percentage of infarcted tissue was expressed as percent of total left ventricular mass (left ventricular free wall plus septum).

Miscellaneous

The electrocardiographic changes were monitored at 24 hours after temporary myocardial ischemia. The severity of the ventricular rhythm disturbance was graded according to the method of Lown et al.¹⁰ as follows: 0 = no ventricular ectopic contraction; 1 = occasional isolated premature ventricular contraction (PVC); 2 = frequent PVC (> 1/min); 3 = multiform PVC; IV = repetitive PVC; and V = early PVC, i.e., R-on-T.

All data were analyzed statistically using Student’s t test. The regression lines were determined by the least squares method. P values smaller than 0.05 were considered to be statistically significant.

![Figure 1](http://circ.ahajournals.org/lookup/fig/1)

**Figure 1.** A schematic representation of the canine myocardium. The left anterior descending coronary artery (LAD) was isolated and occluded below its first diagonal branch with a screw clamp. An electromagnetic flow probe was used to monitor LAD blood flow. Epicardial electrocardiographic changes were monitored by platinum electrodes embedded in an acrylic plaque. LCX = left circumflex coronary artery.
Results

Hemodynamic and Cardiac Rhythm Studies

After a one-stage ligation of the left anterior descending (LAD) coronary artery below its first diagonal branch (fig. 1), 2 out of 17 dogs fibrillated within 15 minutes of occlusion. Two additional dogs survived the 90 min of ischemia but fibrillated 1 and 7 hours after reperfusion. In the 13 dogs surviving at least 24 hours, (7 saline-control and 6 UM-272 pretreated) occlusion of the LAD coronary artery resulted in a significant decrease in mean arterial blood pressure (−21.3 ± 6.8%) determined at 24 hours as compared to the mean preischemic blood pressure (133.1 mm Hg) and an increase in the incidence of ventricular dysrhythmias. The severity of ventricular dysrhythmias, 24 hours after myocardial ischemia in the saline-treated control animals, were graded as III (5) and II (1), suggesting that the dysrhythmias appear to originate from multifocal ectopic sites.12 Pretreatment with UM-272 (2 mg/kg), 30 min prior to LAD occlusion, resulted in a slight decrease in heart rate (−7.4 ± 2.4%) and no change in mean arterial blood pressure. The subsequent decrease in blood pressure 24 hours after LAD occlusion was not significantly different between UM-272-treated and saline-treated animals. UM-272 treatment, however, did appear to reduce markedly the severity of ventricular dysrhythmias at 24 hours. In the UM-272 pretreated animals, the ventricular dysrhythmias monitored over a one hour period were graded primarily as II (5) and III (1) according to Lown’s classification,12 indicating that unifocal ectopic rhythms were predominant in the drug-treated animals.

Despite a gradual re-introduction of coronary flow into the ischemic region after a 90 min period of coronary occlusion, patchy hemorrhagic infarction was observed to occur in five out of seven control and one out of six UM-272 pretreated animals. A minimum of eight transmural specimens from the available 12 electrodes was used for the subsequent studies, thus eliminating those areas that exhibited hemorrhagic infarction.

Electrocardiographic Studies

Figure 2 shows a typical tracing of the epicardial electrocardiographic changes during 90 min of LAD occlusion followed by reperfusion in the control and drug-treated animals. In both groups, there was an increase in ST-segment elevation after coronary occlusion which declined gradually thereafter. The decline was more marked upon reperfusion of the ischemic region. In the control animals, there was a small development of Q waves after 90 min of coronary occlusion and their appearance was greatly enhanced during reperfusion. In the UM-272-treated animals, however, despite a similar increase in ST-segment elevation 15 min after LAD occlusion, the subsequent development of Q waves was significantly less than in the control animals. The effects of UM-272 pretreatment on electrocardiographic changes after temporary LAD occlusion and reperfusion are shown in table 1. Epicardial ST-segment elevation greater than 2 mV is considered abnormal as previously suggested by Maroko et al.1 In the seven control animals, 50 out of a total of 79 (63%) electrode sites monitored exhibited ST-segment elevation greater than 2 mV 15 min after LAD occlusion with a mean of 10.7 ± 0.9 mV. Subsequently, there was a loss in R wave amplitude and development of Q waves at 24 hours. Fourteen sites among the seven control animals were excluded from the present analysis since they exhibited local conduction delay as indicated by prolongation of the entire QRS complex exceeding 65 msec.

Pretreatment with UM-272, 30 min prior to LAD occlusion, failed to markedly reduce the total number of epicardial electrode sites exhibiting ST-segment elevation greater than 2 mV 15 min after occlusion (35 out of a total 70 electrode sites, 50%). The averaged ST-segment elevation (10.4 ± 0.9 mV) was not significantly different when com-

Table 1. Effects of UM-272 Treatment on Electrocardiographic Changes after 90-min LAD Occlusion and Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of sites</th>
<th>ST1,2im (mV)</th>
<th>∆Q,AR (mV)</th>
<th>∆(Q+AR),site (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1,2im &lt;2 mV</td>
<td>15</td>
<td>0.1 ± 0.2</td>
<td>2.6 ± 1.2</td>
<td>10.3 ± 2.0</td>
</tr>
<tr>
<td>ST1,2im &gt;2 mV</td>
<td>50</td>
<td>10.7 ± 0.9</td>
<td>6.5 ± 0.5</td>
<td>18.3 ± 1.3</td>
</tr>
<tr>
<td>Conduction Defect</td>
<td>14</td>
<td>7.7 ± 0.6</td>
<td>23.4 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>UM-272 pretreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1,2im &lt;2 mV</td>
<td>27</td>
<td>0.1 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>ST1,2im &gt;2 mV</td>
<td>35</td>
<td>10.4 ± 0.9</td>
<td>2.5 ± 0.6</td>
<td>12.6 ± 1.0</td>
</tr>
<tr>
<td>Conduction Defect</td>
<td>8</td>
<td>7.9 ± 0.9</td>
<td>21.1 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>
Comparing the saline-control to the drug-treated animals. The UM-272 treated animals, however, subsequently developed smaller Q-wave voltages (2.5 ± 0.6 mV) at 24 hours as compared to the control group (6.3 ± 0.5 mV). Similarly the sum of the R wave voltage decrease and the Q wave voltage in the drug-treated group was significantly less than the values obtained in the saline treated control group.

Myocardial CPK Activity Studies

Myocardial creatine kinase (CPK) activity was measured from each electrode site used for epicardial recording in order to further determine the extent of myocardial ischemic injury and the effect of UM-272 treatment. Transmural sections, 1 cm × 1 cm, were removed from nonischemic, intermediate, and ischemic regions. Criteria for the determination of the different regions included the general anatomy of the LAD, the site of occlusion, and the relative magnitude of the ST-segment elevation 15 minutes after occlusion. The results are summarized in figure 3. In the nonischemic region (A), both saline-control and UM-272 treated animals had insignificant changes in the epicardial ST-segment recordings 15 min after coronary artery occlusion and did not develop subsequent changes in the Q and/or R wave voltages at the same electrode sites when examined 24 hours later. Myocardial creatine kinase activities measured from nonischemic regions (A) at 24 hours were not significantly different between the saline-treated and drug-treated animals. In the intermediate region (B) there was an increase in ST-segment elevation and a subsequent development of Q-waves and loss of R-waves. Myocardial CPK activity was decreased by 58%, indicating myocardial ischemic injury. Pretreatment with UM-272, while not affecting the development of ST<sub>iso</sub> elevation compared to control animals, was associated with a reduction in the subsequent development of Q and R wave changes which were less than those obtained in the control animals. In addition, the loss of myocardial CPK activity from the intermediate zone (B) of UM-272 treated animals was significantly reduced as compared to that observed in nontreated control animals, suggesting that drug treatment resulted in some salvage of cardiac tissue and the prevention of irreversible damage. In the severely ischemic regions (C), there was a greater ST<sub>iso</sub> and subsequently greater development of Q waves and combined R and Q wave changes. Myocardial CPK activity was decreased to a greater degree (<72%).

Pretreatment with UM-272 again reduced the development of Q waves and reduced the CPK depletion, but had no significant effects on ST<sub>iso</sub>. The present findings suggest that pretreatment with UM-272 can significantly protect the ischemic myocardium from undergoing irreversible damage.

Quantification of Infarct Size

In order to quantify the amount of necrotic tissue as a result of temporary LAD occlusion and reperfusion, both tissue creatine kinase (CPK) activity measurement and nitro-blue-tetrazolium (NBT) staining reaction for the presence of intracellular dehydrogenases were studied. In this series of experiments, the transmural section under each electrode site was divided into two equal portions, one for determination of CPK activity and the other for NBT staining. Both methods depend upon the presence of intracellular enzyme markers, and a significant correlation was obtained between the loss of CPK activity and the loss of NBT staining (i.e., loss of intracellular dehydrogenases) (fig. 4). Figure 5 shows the effect of UM-272 treatment on the total infarct volume as determined by NBT staining 24
hours after temporary myocardial ischemia. The saline-treated animals, subjected to 90 min of occlusion followed by reperfusion, had an infarct volume of 24.2 ± 2.8% of the left ventricular mass (septum and the free wall of the left ventricle). Pretreatment with UM-272 resulted in a significant reduction in the total infarct size. The infarct volume was 6.6 ± 3.7% of the mean left ventricular mass of 84.6 ± 5.3 g. This result is in good agreement with that obtained with tissue CPK activity measurements (fig. 3), suggesting that pretreatment with UM-272 can significantly reduce the amount of myocardial tissue undergoing irreversible damage after temporary coronary occlusion and reperfusion.

Pathologic Q Wave Development

The development of pathologic Q waves has been proposed to be a good marker for determining the extent of myocardial infarction after coronary occlusion. Figure 6 shows the time course of the development of epicardial Q waves during the 90 min LAD occlusion followed by reperfusion. There was slight evidence of Q waves after 90 min of LAD occlusion (ΣΔQ = 0.74 ± 0.11 mV) at all the electrode sites exhibiting ST_ism greater than 2 mV. The appearance of Q waves was greatly enhanced during reperfusion reaching a maximum at 60 min (ΣΔQ = 6.94 ± 0.52 mV) and was maintained for 24 hours (ΣΔQ = 6.62 ± 0.53 mV). The average Q wave amplitude at 24 hours was not statistically different from that observed at 60 min after reperfusion. Good correlations were observed between CPK activity measured at 24 hours and the development of epicardial Q waves at 24 hours, 2 hours and even as early as 1 hour after reperfusion (table 2). In the UM-272 pretreated animals, despite a similar ST-segment elevation at 15 min after LAD occlusion, there was a smaller development of Q waves both during occlusion and after reperfusion. This was correlated with less tissue CPK depletion at the same site 24 hours later (fig. 3).

Discussion

Recently it has been reported that pretreatment with UM-272, the quaternary analog of propranolol, significantly reduced the amount of myocardial tissue undergoing irreversible ischemic injury when canine hearts were subjected to 60 min of occlusion of the left circumflex coronary artery (LCX). In addition, treated animals were noted to have a decrease in the incidence of rhythm disturbances during LCX occlusion and after reperfusion. The present study has employed alterations in R and Q wave voltages as a means of assessing myocardial injury and cell death. The results provide further evidence that pretreatment with UM-272 affords protection against myocardial cell injury caused by temporary myocardial ischemia. In addition, two independent quantitative measurements of infarct size were
used in the present study. One method involved the determination of myocardial CPK activity in nonischemic and ischemic myocardial regions and the other depended upon the presence of intracellular dehydrogenase enzymes and substrate and their reaction with NBT. Each of these estimates of cell viability provided further evidence that pretreatment with UM-272 protected ischemic myocardium from irreversible cell injury and death. These observations support those of a previous study in which occlusion of the LCX for 60 min followed by reperfusion for 24 hours resulted in an infarct volume of 23.8 ± 3.2% of the left ventricle as compared to animals pretreated with UM-272 (12.5 mg/kg over 5 hours) in which the infarct volume was 7.0 ± 3.3 percent. The observations in the present study in which the LAD was occluded for 90 min resulted in an infarct volume of 24.2 ± 2.8% in controls vs. 6.6 ± 3.7% in UM-272 treated dogs are in excellent agreement with our earlier investigations showing that UM-272 can protect the ischemic myocardium against permanent cell injury and ultimate necrosis.

Although pretreatment with UM-272 did not prevent ventricular arrhythmias from occurring at 24 hours after temporary LAD occlusion and reperfusion, it reduced the severity of these arrhythmias as quantitated according to the method of Lown et al. It has been reported recently that there are limitations to Lown’s classification, but the marked differences in frequency and pattern of ventricular dysrhythmias between the saline controls and UM-272 treated animals in the present study clearly indicated an antiarrhythmic property of UM-272. In the UM-272 treated animals, ventricular arrhythmias were characterized by electrocardiographic patterns suggesting that they were of unifocal origin as compared to untreated animals in which the arrhythmias were graded as being more severe in character in that they were multifocal. This observation may be of clinical significance in view of the recent observations of Lown and Grayboys in which it was noted that one of the therapeutic objectives for the management of malignant arrhythmias and the prevention of sudden death was to suppress the more severe grades (grades 4 and 5) of ventricular arrhythmias. The results of the present study support earlier observations in which UM-272 produced a marked reduction in ventricular ectopic activity noted to occur within the first 5 hours after a 60 min period of LCX occlusion followed by reperfusion in the canine heart. Whereas UM-272 pretreatment did not produce significant effects upon epicardial ST-segment elevations measured at 15 min after LAD occlusion, the drug produced a significant reduction in the development of pathologic Q waves recorded from these same sites at 24 hours and reduced the loss of myocardial CPK from tissue specimens taken at the same electrode sites.

A number of intracellular enzyme markers have been used to quantify the amount of myocardial tissue undergoing irreversible cell injury or necrosis. In recent years, depletion of creatine kinase (CPK) from myocardial tissue has been employed as an estimate of myocardial cell death after coronary artery occlusion. An extremely useful method for experimental studies is that of Nachlas and Shnitka which involves the use of nitro-blue-tetrazolium (NBT) which stains myocardial tissue a deep blue color in the presence of intracellular dehydrogenases (undamaged tissue) and leaves irreversibly injured or infarcted areas appearing as unstained pale regions. Thus, the incubation of transverse ventricular sections in a buffered solution of NBT results in a staining pattern that permits one to differentiate easily between infarcted and normal myocardium. Previous studies from this laboratory have shown that the NBT staining myocardial tissue is normal when examined histologically on H & E stained sections and that unstained areas show histologic changes characteristic of myocardial necrosis. Furthermore, it was demonstrated that UM-272 itself did not alter the NBT staining reaction. Both the CPK activity in myocardial tissue and the NBT staining reaction are dependent upon the presence of intracellular enzymes. It is not surprising therefore that a significant correlation was found to exist between the loss of CPK activity and the loss of NBT staining (fig. 4). Furthermore, the beneficial effects of UM-272 pretreatment to reduce the extent of myocardial ischemic injury, as determined by the reduction of CPK depletion, was in good agreement with the reduction in infarct size as determined by the NBT staining reaction. It would appear therefore that the measurement of myocardial tissue CPK activity and the NBT staining reaction for intracellular dehydrogenases permit good quantitative estimates of irreversible cardiac damage due to experimental coronary artery occlusion. Because of its simplicity, the NBT method of assessing the extent of ischemic myocardial damage would appear to be most useful in studies designed to evaluate the efficacy of pharmacologic interventions for reducing infarct size.

The development of pathologic Q waves has been demonstrated to be a good estimate of the extent of the underlying cardiac damage following coronary artery occlusions. Studies in dogs subjected to coronary occlusion have shown that the number of epicardial pathologic Q waves reliably indicates the area of myocardial necrosis, as determined by biochemical (reduction of tissue CPK activity) and histologic criteria. More recently, Awan et al. have reported that cardiac function can be evaluated noninvasively by application of precordial multiple lead Q wave mapping. The development of epicardial Q waves, however, is not immediate upon coronary artery occlusion, but may be delayed for several hours. The appearance of pathologic Q waves is greatly accelerated when a previously occluded coronary artery is reperfused after a period of 60-90 min of ischemia. The mechanism by which the restoration of blood flow leads to rapid cellular injury and irreversible changes is not known. Previous experimental studies employing histologic and biochemical assessment of tissue injury demonstrated that coronary artery reperfusion after a sufficient period of ischemia would extend the area of irreversible cardiac damage. However, the extent of cardiac damage as determined by NBT staining at 24 hours resulting from 90 min of LAD occlusion followed by reperfusion was not statistically different from the volume of tissue infarcted after 24 hours of permanent LAD occlusion (24.2 ± 2.8% vs. 18.9 ± 2.5%, respectively; unpublished data). This is not an unexpected observation in view of the fact that previous studies by other investigators have shown that myocardial ischemia in excess of 60 min produced irreversible damage to almost 100% of the cells in
the previously ischemic region. Disruption of sarcolemmal membranes and washout of ions from the interstitial space during reperfusion might also alter the electrophysiologic properties of the ischemic myocardial cells in a manner that could accelerate the development of pathologic Q waves.

The early appearance of pathologic Q waves and their persistence at 24 hours at each of the same electrode sites provided an accurate assessment of the degree of ischemic myocardial damage in the control and UM-272 treated animals. Pretreatment with UM-272 reduced the rate and magnitude of epicardial Q wave development. Furthermore, the use of epicardial Q wave mapping permitted one to assess the benefits of the therapeutic intervention as early as 60 min after reperfusion since Q wave development was nearly maximal at this time and had not changed significantly when determined at the same electrode sites 24 hours later. Furthermore, in UM-272 treated animals, the decrease in pathologic Q wave development correlated with the reduction in CPK loss from myocardial tissue at each of the electrode sites. As shown by previous investigators, pathologic Q waves are a reliable index of myocardial damage as determined by biochemical determinations. The results of the present investigation are in agreement with our previous studies showing that UM-272 could reduce the mass of ventricular myocardium undergoing irreversible damage as a result of ischemia followed by reperfusion. The present studies provide additional evidence for the protective effects of UM-272 by demonstrating that CPK activity of the previous ischemic region is preserved and pathologic Q wave development is not as marked in the presence of drug treatment as compared to saline-treated controls.

The present study adds further evidence to support the concept that pharmacologic interventions might save the jeopardized, but still viable ischemic myocardial cells. The mechanism by which UM-272 protects the ischemic heart is not addressed in this present study. Previous studies, however, have demonstrated that UM-272 has the ability to reduce myocardial oxygen consumption by reducing myocardial oxygen demands and these changes were associated with a reduction in coronary blood flow. The reduction in myocardial oxygen demand was related to a decrease in heart rate and contractility, resulting in a decrease in cardiac output and tension-time index. The decrease in contractility alone was sufficiently pronounced to decrease myocardial oxygen demands since myocardial oxygen consumption remained below the control value even when heart rate and blood pressure were restored to at least control levels by electrical pacing and aortic obstruction. Thus, it is reasonable to assume that UM-272 might bring about a more favorable balance between oxygen supply and oxygen demand particularly in the postocclusive period when the “no-reflow” phenomenon may result in limited flow to large areas of the myocardium so as to render them relatively ischemic for a variable time period following reperfusion. In the absence of drug treatment, such areas of relative ischemia would be in jeopardy and subjected to the dynamic processes leading to irreversible cell injury and ultimate cell death and necrosis. Whereas UM-272 may not alter the changes that occur within the microcirculation and some degree of ischemia may persist beyond the time of reperfusion, the ability of the drug to reduce myocardial oxygen demands may account for its protective effect upon the jeopardized myocardial cells. Still another mechanism by which one could explain the protective effects of UM-272 upon the ischemic heart is provided by the data of Warltier et al. and Gross et al. who showed that the drug produced a redistribution of coronary blood flow from epicardial to endocardial regions in hearts subjected to a critical stenosis of the LAD. This latter effect together with a reduction in myocardial oxygen consumption would be sufficient to explain the beneficial effects of dimethyl propranolol upon the ischemic myocardium that would result in a reduction in the volume of ventricular muscle undergoing necrosis, the preservation of myocardial CPK activity, and the prevention of pathologic Q wave development.

From a clinical standpoint, the need to preserve jeopardized, but still viable myocardial tissue, is evident in view of the fact that the degree of morbidity and mortality subsequent to myocardial infarction are related to the amount of myocardial tissue undergoing irreversible damage. The dimethyl-quaternary analog of propranolol, UM-272, which lacks beta-adrenergic receptor blocking properties, should provide an interesting potential therapeutic intervention for the purpose of reducing infarct size. The studies with UM-272 to date suggest it possesses all of the beneficial properties of propranolol with respect to restoring the balance between oxygen supply and oxygen demand in the ischemic heart. In addition, UM-272 brings about a favorable improvement in the endocardial-epicardial blood flow ratio in the ischemic heart. Thus, UM-272, like propranolol, might be of value clinically in preventing the extension of ischemic injury in patients with acute myocardial infarction. UM-272 would have a therapeutic advantage over propranolol in that it would not produce beta-receptor blockade and thus deprive the heart of needed inotropic and chronotropic support via the autonomic nervous system. Furthermore, the ability of UM-272 to protect the ischemic heart during the period of reperfusion might suggest a potential use of the drug in patients with unstable angina undergoing bypass vein graft surgery in whom the rate of perioperative infarction associated with the appearance of new significant Q waves may be as high as 15% and may be associated with the reflow of arterial blood to a previously ischemic region.

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