The Wavefront Phenomenon of Ischemic Cell Death

1. Myocardial Infarct Size vs Duration of Coronary Occlusion in Dogs

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SUMMARY Irreversible ischemic myocardial cell injury develops in an increasing number of cells as the duration of coronary occlusion is prolonged. The present study quantitates myocardial necrosis produced by 40 minutes, 3 hours, or 6 hours of temporary circumflex coronary occlusion (CO) followed by 2 to 4 days of reperfusion, or by 24 or 96 hours of permanent circumflex ligation in pentobarbital anesthetized open chest dogs. After 40 minutes of ischemia, myocyte necrosis was subendocardial but with increasing duration of coronary occlusion, irreversible injury progressed as a wavefront toward the subepicardium. Transmural necrosis was 38 ± 4% after 40 min, 57 ± 7% after 3 hours, 71 ± 7% after 6 hours and 85 ± 2% after 24 hours of ischemic injury. These results document the presence of a subepicardial zone of ischemic but viable myocardium which is available for pharmacologic or surgical salvage for at least three and perhaps six hours following circumflex occlusion in the dog.

IN RECENT YEARS, there has been much interest in the idea that it is possible to salvage acutely ischemic, but still reversibly injured, myocardium, by either pharmacologic or surgical intervention.1-9 This concept is based on the belief that not all cells die simultaneously in an area of acute myocardial infarction in man. The evidence supporting this belief is largely intuitive but is suggested by a variety of permanent and temporary coronary artery occlusion experiments in dogs. Although subendocardial necrosis results from even a brief period (20-40 min) of temporary coronary occlusion, much of the subepicardial myocardium survives if blood flow is restored.10, 11 Temporary occlusion studies employing a variety of models and techniques, have generally supported the idea that salvageable myocardium is present for 2-3 hours after coronary artery occlusion.1, 10, 12-18 On the other hand, permanent occlusion of the vessel for 24 or more hours causes necrosis which is essentially transmural.9 These observations indicate that not all cells in the ischemic myocardium die simultaneously and that, in the dog, ischemic cells in the mid- or subepicardial myocardium are available for salvage for a period of hours.

The present study was done to assess the progression of cell death during acute myocardial ischemic injury in dogs and to determine the amount of ischemic but viable myocardium available for salvage at various times following occlusion of a major coronary artery. The results show that irreversible injury develops at variable times. A wave of cell death begins in the subendocardial myocardium and progresses toward the subepicardial myocardium. The subendocardial myocardium dies early, while many cells in the subepicardial myocardium survive for up to six hours following coronary occlusion. These results provide an anatomic baseline in dogs for evaluating pharmacologic or surgical attempts to modify infarct size.

Materials and Methods

Mongrel dogs weighing 9-20 kg were assigned to one of five groups as described below. Each dog was anesthetized with intravenous sodium pentobarbital (30 mg/kg), underwent endotracheal intubation, and was ventilated with a Harvard 1063 respirator. Sterile instruments, sponges and drapes were used during the surgical procedure. A catheter was placed in the right femoral artery via its saphenous branch to monitor blood pressure. Lead II of the standard ECG and peripheral blood pressure were recorded on a Brush model 440 recorder at standard intervals throughout the experimental procedure. The left chest was opened through the fourth intercostal space. The lung was retracted and the pericardium was opened. The circumflex artery was isolated 10-15 mm from the aorta and one #1 silk suture was placed around it.

Three groups of dogs underwent temporary left circumflex coronary artery occlusion followed by two-four days of reperfusion: Group 1: 40 minute occlusions; Group 2: three hour occlusions; and Group 3: six hour occlusions. In these dogs, occlusion was produced by pulling both ends of the suture through a small glass tube and fastening the suture ends with the tips of a mosquito hemostat, thereby kinking the artery within the end of the tube. In two groups, occlusion was permanent (Group 4: 24 hours; Group 5: 96 hours) and was produced by double ligation of the artery. Subsequent to reperfusion in groups 1-3 and subsequent to ligation in groups 4 and 5, incisions were closed, air was removed from the chest and dogs were allowed to recover from anesthesia.

Determination of Extent of Necrosis

After the appropriate period of occlusion with or without reperfusion, each dog was re-anesthetized, the chest reopened and the heart excised. The circumflex artery was opened to establish the point of occlusion and ensure that arterial thrombi had not developed in the reperfused groups. The left ventricle was opened and three transmural slices were cut longitudinally through the posterior papillary muscle which is centrally located within the area made ischemic by circumflex coronary artery occlusion.20 The slices were photographed and fixed in 10% phosphate buffered formalin for preparation of histologic sections. Myocardial necrosis was identified in sections stained with hematoxylin and eosin (H&E) and was quantitated from photographic enlargements of the sections stained with Heidenhain's variant of Mallory's connective tissue stain (CT) (fig. 1). (The latter stain accentuates contraction bands and cytoplasmic

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granularity which are prominent features of necrosis especially following temporary coronary occlusion.) Necrosis was expressed as the overall percentage for the three transmural slices cut from each heart. Statistical comparison between groups was done using the Student's non-paired t-test.

Results

Gross and Microscopic Features of Ischemic Injury

Temporary Occlusions

Necrosis was observed in every dog following either temporary or permanent coronary occlusion. Following 40 min of temporary circumflex occlusion, necrosis occurred throughout the region supplied by the circumflex artery but typically was restricted to the subendocardial myocardium. Longitudinal sections through the posterior papillary muscle (which is in the approximate center of the region supplied by the circumflex artery) always demonstrated subendocardial necrosis (fig. 2). Microscopically necrotic myocytes showed loss of nuclei and frequently showed prominent myofilibrillar contraction bands (fig. 3). By two days an acute inflammatory response was present and by three to four days, the process of phagocytosis and repair had begun around the edge of the necrotic areas.

Transmural sections from hearts reperfused after three and six hours of coronary occlusion showed, on the average, considerably larger areas of necrosis. Necrosis was uniform throughout the subendocardial myocardium and typically extended irregularly into the midmyocardium and (especially in group 3) subepicardial myocardium. Subendocardial hemorrhage was prominent. In addition, there were often small, pale gray foci, usually in the center of the papillary muscle, which grossly appeared to contain no blood (fig. 4). These pale gray foci, surrounded by hemorrhage, were present in two of eight dogs with three hour occlusions and in six of eight dogs with six hour occlusions.

Microscopically, little hemorrhage or inflammatory
vascular damage and resultant hemorrhage around these central pale foci suggested that microvascular perfusion was impaired and the myocardium within these small foci most closely resembled myocardium undergoing autolysis in the absence of perfusion.\(^{36,38}\) Myocytes showed widely spaced cross striations indicating myofibrillar relaxation and nuclei which were very faintly stained but still appeared to be intact. In the hemorrhagic midzone (fig. 5b), necrotic myocytes showed myofibrillar contraction bands, increased cytoplasmic granularity, and nuclear karyolysis.

In the subepicardial myocardium, necrosis was occasionally confluent but typically islands of necrotic myocytes were interspersed with islands of viable cells often seen adjacent to small penetrating arteries. Hemorrhage was absent, but the processes of inflammation and repair had begun, suggesting that the vasculature was preserved in this zone.

**Permanent Infarcts**

Infarcts produced by permanent coronary occlusions were similar in many respects to those resulting from six hours of temporary coronary occlusion but differed according to their age at sacrifice. At both 24 (fig. 6) and 96 (fig. 7) hours, three distinct zones could be identified. The subendocardial myo-

Figure 4. Transmural slice through the posterior papillary muscle obtained two days after a three hour temporary occlusion. The inner two thirds of the wall is necrotic but the outer third of the wall (bottom) is viable myocardium salvaged by reperfusion. Hemorrhage is prominent in the subendocardial region but does not extend into the midmyocardium. A small pale central core is present just below and to the right of the chordae tendinae. Viable muscle at the apex of the heart (right) is in the distribution of the nonoccluded LAD artery.

Response was observed within the central pale areas but they were always surrounded by hemorrhage and an infiltrate of segmented neutrophils (fig. 5a). Necrotic arterioles obstructed by swollen endothelial cells were frequent. The vasculature was preserved in this zone.
cardiomyocytes were characterized by a subendocardial central core, a central hemorrhagic zone and a peripheral zone.

A hemorrhagic midzone was invariably present at 24 hours but hemorrhage was relatively mild. By 96 hours of permanent coronary occlusion (fig. 7), hemorrhage was prominent and appeared equivalent to hemorrhage in infarcts produced by 3 or 6 hours of temporary coronary occlusion with 96 hours of reperfusion.

The peripheral zone at 24 hours was characterized by coagulation necrosis and acute inflammation. Myocytes showed nuclear pallor or karyolysis and glassy hyper-eosinophilic cytoplasm. Contraction bands, which were absent from subendocardial areas of necrosis, were present in a few scattered cells on the periphery of the area of necrosis. However, they were much less frequent following permanent than following temporary coronary occlusion. By 96 hours, phagocytosis of dead cells and replacement by granulation tissue had begun on the periphery of the areas of necrosis (fig. 8b).

### Transmural Progression of Ischemic Cell Death

Summarizing the morphologic observations, myocardial infarcts showed three distinct zones of necrosis which developed sequentially with increasing duration of coronary occlusion. These were 1) a central zone in which hemorrhage and inflammation were absent; 2) an hemorrhagic midzone; and 3) a peripheral non-hemorrhagic zone in which inflammation, phagocytosis and infarct repair occurred relatively quickly.

These zones and the transmural progression of ischemic injury with respect to duration of occlusion are summarized diagrammatically in figure 9. Irreversible injury of myocardium was typically subendocardial at 40 min whereas subepicardial myocardium was preserved by re-establishing blood flow at this time. As the ischemic period was prolonged, irreversible injury moved progressively toward the subepicardial myocardium and by 24 hours necrosis typically was transmural. Microvascular injury, as evidenced by hemorrhage and delayed repair of necrotic areas, also progressed from subendocardial to subepicardial myocardium. However, the time scale of microvascular injury appeared to be slower since hemorrhage did not extend out to the edge of necrotic areas. Rather, there was always a peripheral zone of myocyte necrosis in the presence of an apparently intact vasculature.

### Infarct Size vs. Duration of Occlusion

The transmural extent of necrosis, calculated as a percent of transmural sections through the posterior papillary muscle, is summarized in table 1 and figure 10. The progression of irreversible injury from subendocardium to subepicardium with increasing duration of occlusion described above resulted in significantly ($P < 0.05$) more necrosis at three hours ($57 \pm 7\%$) compared with 40 min ($38 \pm 4\%$) and at 24 hours ($85 \pm 3\%$) compared with three hours ($P < 0.01$). Mean infarct size at six hours ($71 \pm 7\%$) was intermediate between the three hour and 24 hour means but did not differ significantly from either.

The average relative proportion of the transmural myocardium at risk but still salvageable after various durations of coronary occlusion can be estimated (fig. 11) as follows: Since permanent ligation resulted in necrosis of 85% of myocardium in the transmural sections studied and 40 min of TCO caused 38% necrosis, 38/85 x 100 = 45% of the myocardium at risk was already irreversibly injured by 40 min. Nevertheless, an average of 55% of the myocardium at risk was reversibly injured at 40 min and was salvaged by reperfusion. By three hours, about 100 - (57/85 x 100) = 33% of myocardium at risk was still potentially salvageable and by six hours only 16% remained salvageable.

### Hemodynamics, Electrocardiographic Changes and Survival

Acute occlusion of the circumflex coronary artery in the open chest dog, while producing a large area of ischemia, caused little acute change in heart rate and a mild reduction of mean blood pressure. No differences were observed between groups. Maximum ST-segment elevation occurred in lead II between 5 and 10 min after occlusion and was similar in the five groups.

During the first 30 min of occlusion, premature ven-
tricular contractions often were frequent. Between 30 and 120 min, arrhythmias temporarily subsided, but with occlusions continued beyond two hours, intermittent ventricular tachycardia usually developed. Reperfusion after 40 min of coronary occlusion was associated with a high incidence of ventricular fibrillation (41% of dogs surviving occlusion). However, six of 14 of these dogs were resuscitated by direct current countershock. In contrast to the 40 min period of ischemia, reperfusion after three or six hours of occlusion did not precipitate VF. Deaths occurred during the first few minutes after occlusion in all groups, and at reflow in the 40 min group. Deaths subsequent to the acute experiment were infrequent in dogs that were reper-

**Figure 8.** Four day permanent infarct. a) Central core: Myocytes are relatively well preserved but show pale or absent nuclei and pale cytoplasm with loss of sarcomere cross striations. Capillaries appear obstructed by swollen endothelial cells. There is a prominent neutrophilic infiltrate around the edge of this core. H & E X 325. b) Peripheral edge: Necrotic myocytes have been removed from the edge of the infarct and have been replaced by capillaries and fibroblasts which have begun to grow into the infarct from surrounding viable muscle. Viable muscle is present in the subepicardial myocardium around a small penetrating artery (low left corner).

**Figure 9.** Diagrammatic summary of the progression of the wavefront of ischemic cell death with respect to duration of coronary occlusion. Necrosis (all shaded areas) occurs first in the subendocardial myocardium and with longer durations of coronary occlusion involves progressively more of the transmural thickness of the ischemic zone. (Dashed line indicates the anatomic boundary between ischemic circumflex and nonischemic LAD coronary beds.) Microvascular injury, evidenced by interstitial hemorrhage (horizontal cross hatching), also progresses from subendocardial to subepicardial zones but the time scale is slower than that for myocyte necrosis. Complete cessation of microvascular perfusion may result in a central core (dotted areas) of necrotic muscle devoid of either hemorrhage or inflammatory response. Thus following either temporary or permanent coronary occlusions, the infarct may demonstrate, grossly and by light microscopy, a central core of relatively preserved necrotic muscle, a hemorrhagic midzone, and a peripheral zone of organizing necrosis. At three hours after occlusion there is typically a significant amount of subepicardial myocardium which is viable and can be salvaged by reperfusion.
wavefront which gradually moves toward the subepicardium during a period of six hours (figs. 9-11). Previous studies have shown that cell death does not develop immediately or simultaneously even in areas of severe ischemia.\textsuperscript{19} Circumflex occlusion for 15 min is completely reversible in that reperfusion completely prevents necrosis, but occasional small foci of necrosis result from 20 min occlusions.\textsuperscript{18} Reperfusion after 40 min of circumflex occlusion always results in focal or confluent subendocardial necrosis. Three hours of occlusion produces, on the average, significantly more necrosis which is typically confluent in the subendocardial myocardium with focal involvement of the mid- or subepicardial myocardium. By 6 to 24 hours, necrosis becomes nearly transmural. Residual viable myocardium in these sections is observed primarily in subepicardial foci, which are often adjacent to blood vessels, and at the base of the papillary muscle near the apex of the heart, i.e., at the junction of the beds perfused by the circumflex and the anterior descending coronary arteries. A transmural progression of cell death has previously been suggested by histochemical and electrophysiologic techniques. Cox has shown irreversible loss of dehydrogenase enzyme activity\textsuperscript{21} and of electromotive force\textsuperscript{22} gradually progressing from subendocardial to subepicardial myocardium following coronary occlusion.

The mechanism of this subendocardial to subepicardial progression of injury probably is related primarily to the transmural distribution of coronary collateral flow. In dogs, collateral flow occurs largely via epicardial anastomoses and reaches the myocardium via penetrating muscular arteries which are functionally end arteries. During systole, intramyocardial tension is greatest in,\textsuperscript{23} and limits perfusion of the subendocardial myocardium.\textsuperscript{24} Increased subendocardial flow must occur during diastole in order to attain the uniform transmural distribution of flow normally observed in nonischemic myocardium\textsuperscript{25, 26}. This autoregulatory mechanism cannot compensate for total coronary artery occlusion and a transmural gradient of collateral flow develops. Flow in the central subendocardial zone is reduced to less than 15% of flow in nonischemic areas (severe ischemia). In the mid- and subepicardial myocardium, flow may be up to 15–30% of normal (moderate ischemia).\textsuperscript{27}

In addition, recent studies by Dunn and Griggs\textsuperscript{28} suggest that, in vivo, there may be a transmural gradient of metabolic rate with the greatest energy utilization occurring in the subendocardial myocardium. Thus transmural differ-

### Table 1. Percent Necrosis in the Posterior Papillary Muscle

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Mean: 38 ± 4; 57 ± 7; 71 ± 7; 85 ± 2; 79 ± 6

Number of dogs: 23 8 8 16 3

\*Overall percent necrosis in three transmural sections of the PP (see methods and fig. 1).

TCO = temporary coronary occlusion; PCO = permanent coronary artery occlusion

### Discussion

This study demonstrates that in dogs with circumflex coronary artery occlusions, irreversible ischemic injury occurs first in the subendocardial myocardium and extends as a wavefront which gradually moves toward the subepicardium during a period of six hours (figs. 9-11). Previous studies have shown that cell death does not develop immediately or simultaneously even in areas of severe ischemia.\textsuperscript{19} Circumflex occlusion for 15 min is completely reversible in that reperfusion completely prevents necrosis, but occasional small foci of necrosis result from 20 min occlusions.\textsuperscript{18} Reperfusion after 40 min of circumflex occlusion always results in focal or confluent subendocardial necrosis. Three hours of occlusion produces, on the average, significantly more necrosis which is typically confluent in the subendocardial myocardium with focal involvement of the mid- or subepicardial myocardium. By 6 to 24 hours, necrosis becomes nearly transmural. Residual viable myocardium in these sections is observed primarily in subepicardial foci, which are often adjacent to blood vessels, and at the base of the papillary muscle near the apex of the heart, i.e., at the junction of the beds perfused by the circumflex and the anterior descending coronary arteries. A transmural progression of cell death has previously been suggested by histochemical and electrophysiologic techniques. Cox has shown irreversible loss of dehydrogenase enzyme activity\textsuperscript{21} and of electromotive force\textsuperscript{22} gradually progressing from subendocardial to subepicardial myocardium following coronary occlusion.

The mechanism of this subendocardial to subepicardial progression of injury probably is related primarily to the transmural distribution of coronary collateral flow. In dogs, collateral flow occurs largely via epicardial anastomoses and reaches the myocardium via penetrating muscular arteries which are functionally end arteries. During systole, intramyocardial tension is greatest in,\textsuperscript{23} and limits perfusion of the subendocardial myocardium.\textsuperscript{24} Increased subendocardial flow must occur during diastole in order to attain the uniform transmural distribution of flow normally observed in nonischemic myocardium\textsuperscript{25, 26}. This autoregulatory mechanism cannot compensate for total coronary artery occlusion and a transmural gradient of collateral flow develops. Flow in the central subendocardial zone is reduced to less than 15% of flow in nonischemic areas (severe ischemia). In the mid- and subepicardial myocardium, flow may be up to 15–30% of normal (moderate ischemia).\textsuperscript{27}

In addition, recent studies by Dunn and Griggs\textsuperscript{28} suggest that, in vivo, there may be a transmural gradient of metabolic rate with the greatest energy utilization occurring in the subendocardial myocardium. Thus transmural differ-

### Figure 10. Transmural necrosis, calculated as described in the text and legend to figure 1, is plotted with respect to duration of occlusion. Brackets indicate the SEM. Infarct size increased progressively with time. Reperfusion at 40 min and 3 hours resulted in significantly less necrosis compared with 24 hour infarcts. Six hour infarcts were intermediate between but not significantly different from either three or 24 hour infarcts.

### Figure 11. Proportion of ischemic muscle which is viable and potentially salvageable as a function of time after coronary occlusion. Data are plotted as a percent of 24 hour infarct size.
ences in metabolic demand may also contribute to the wavefront phenomenon of cell death.

Effects of Reperfusion

Reperfusion of the area of ischemia had one or more of several effects in the present study which can be summarized as follows:

1) Reperfusion preserved viable myocytes.
2) Reperfusion, where not impeded by vascular damage (peripheral zone), accelerated the disruption of irreversibly injured myocytes and permitted the process of inflammation, phagocytosis and infarct repair to begin quickly.
3) Reperfusion permitted interstitial hemorrhage from vessels which had been injured by ischemia but were still perfusible at the time of reflow.
4) Reperfusion had no effect on areas which apparently had become nonperfusible (central pale zone).

Necrosis following reperfusion of irreversibly injured myocardium has been extensively studied at both the light and electron microscopic level. Reperfusion after 40 min of coronary occlusion results in the rapid development of prominent myofibrillar contraction bands (fig. 3) and tissue edema (by light microscopy). The ultrastructural features of accelerated necrosis following reperfusion of irreversibly injured ischemic myocardium include explosive cell swelling with formation of large subsarcolemmal blebs, breaks in the sarcolemma, and the development of myofibrillar contraction bands and mitochondrial calcification. Interstitial hemorrhage was prominent after reperfusion of 3 and 6 hour occlusions. It seems likely that this hemorrhage reflects ischemic injury of the vasculature and that hemorrhage may have contributed to increased interstitial pressure resulting in production of the no reflow phenomenon in the central core and of the decreased compliance and the further deterioration of local contractile function which has been observed by Lang et al. and others. It is unlikely, however, that vascular damage and the resultant hemorrhage following reperfusion, caused infarct extension because a) reperfusion resulted in a smaller average transmural extent of necrosis at 3 hours and in no change at 6 hours compared with permanent infarcts, and b) the transmural progression of myocyte necrosis appeared to precede the development of vascular damage such that a midzone of hemorrhage was always surrounded by a peripheral zone of necrosis with an intact vasculature.

The relative morphologic preservation of the central core in permanent occlusions has been emphasized by Bishop et al., who implied that myocytes in this core might still be viable. The latter hypothesis is untenable in light of the severe early ultrastructural damage which has been demonstrated in this central zone. Rather, the slow progression of histopathologic changes in the central zone is equivalent to the changes associated with postmortem autolysis in the complete absence of blood perfusion. The presence of hemorrhage and vascular obstruction around these central core areas suggest that little or no perfusion is penetrating such foci.

Estimation of Salvageable Myocardium with Respect to Duration of Ischemia

Although much research effort has been devoted to the concept of limiting infarct size by pharmacologic or surgical means, the rate of progression of ischemic injury and the size of the ischemic but still viable "border zone" with respect to the duration of ischemia has received relatively little attention in experimental animals. Also, at the present time, it is impossible to estimate, during life, the amount of salvageable myocardium following onset of myocardial infarction in man.

In the present study, necrosis within transmural sections of the posterior papillary muscle was used as a quantitative estimate of the transmural extent of cell death. Although total left ventricular infarct size was not measured in this study, the transmural progression of injury described occurred throughout the circumflex bed. Necrosis within transmural sections of the posterior papillary muscle has been shown to be a reliable index of overall left ventricular necrosis in this model (fig. 12).

The present study re-emphasizes the fact that cell death occurs early in the subendocardial zone of severe ischemia and that about half of the ischemic myocardium that is necrotic at 24 hours has already died by 40 min. However, cell death usually occurs more slowly in the mid- and subepicardial myocardium such that on the average, about a third of the ischemic myocardium at risk is still salvageable at three hours. By six hours, there is little salvageable myocardium in most dogs.

These conclusions are in general agreement with other reperfusion studies. Estimated or anatomically measured infarct size has been reduced by reperfusion after 30 min to three hours but not after four or more hours of temporary coronary occlusion. Functional reduction of ST segment elevation or CPK depletion has been observed with a variety of pharmacologic or mechanical interventions begun 30 min or more after coronary occlusion and some salvage of ischemic myocardium has been documented anatomically with propranolol therapy instituted three hours after circumflex occlusion in our model. Functional studies have shown that reperfusion as early as one hour after coronary occlusion does not result in immediate hemodynamic improvement or in reversal of contractile abnormalities. However, Constantini has shown that reperfusion after three hours of coronary occlusion, while providing no immediate improvement in cardiac function, produces a notable improvement in chamber size.

The necrosis which occurred in the subendocardial zone of reperfused dogs occurred before the reperfusion was terminated. In a previous study, necrosis within the subendocardial zone of dogs reperfused for one hour after 20 min of occlusion was limited to the "border zone" and was not considered to be a reliable index of overall left ventricular necrosis. In the present study, however, the extent of necrosis within the subendocardial zone of reperfused dogs was always greater than the extent of necrosis within the subendocardial zone of permanently occluded dogs. Necrosis in the subendocardial zone of dogs reperfused for three hours after 30 min of occlusion was greater than the extent of necrosis in the subendocardial zone of permanently occluded dogs, and the necrosis in the subendocardial zone of dogs reperfused for six hours after 40 min of occlusion was greater than the extent of necrosis in the subendocardial zone of permanently occluded dogs. These differences indicate that reperfusion induces necrosis which extends beyond the "border zone", and that reperfusion is associated with a greater extent of necrosis than occurs with permanent occlusion.
function, decreased mortality and improved cardiac function measured seven days later. Bresnahan et al. observed increased CPK release following reperfusion and concluded that reperfusion causes infarct extension which they related to the development of hemorrhage. It has subsequently been shown, however, that reperfusion facilitates washout of CPK from ischemic areas and results in overestimation of true infarct size.18

Conclusions and Clinical Implications

In conclusion, this study demonstrates that irreversible injury of ischemic myocardium develops as a transmural wavefront which begins in the subendocardial myocardium and moves progressively toward the subepicardial myocardium (fig. 9). In dogs with circumflex occlusions there is a subendocardial zone of severe ischemia which dies relatively quickly. Subepicardial myocardium, which is moderately ischemic, dies more slowly and survives if the ischemic insult is terminated by reperfusion. The timing of the transmural progression of cell death (figs. 10, 11) provides a basis for evaluating pharmacologic interventions in this model.

A similar wavefront of cell death may occur in man. If we assume that the ischemic cells do not die simultaneously, and that a similar transmural wavefront of necrosis develops in man, the rate of progression of cell death in man may proceed at a rate different from that we have observed in the open chest anesthetized dog. The transmural progression of injury in man may be influenced by the fact that the chest is closed and the patient is conscious. Several important additional variables in man include a) degree of coronary stenosis or occlusion, b) anatomic size of the ischemic bed, c) size and number of pre-existing collateral vessels, and d) presence or absence of hemodynamic complications. In man, as in the dog, it seems likely that severe ischemia results in relatively rapid cell death. Such severely ischemic cells would be difficult to salvage both because of the short time available and because blood flow would be inadequate to sustain delivery of therapeutic doses of drugs. On the other hand, areas of moderate ischemia die relatively slowly. Such areas are potentially salvageable for up to 3 to 6 hours in the dog and possibly for longer periods in man. Since the amount of potentially salvageable myocardium progressively decreases as the period of ischemia is prolonged, time is the most critical variable involved in delaying or preventing ischemic myocardial cell death.

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References


34. Lang T-W, Corday E, Gold H, Meerbaua S, Rubins S, Constantin C,
Infarct Size Reduction by Propranolol before and after Coronary Ligation in Dogs

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SUMMARY Coronary occlusion in the dog results in irreversible myocardial cell injury which develops first in subendocardial areas of severe ischemia and subsequently spreads into mid and subepicardial areas of moderate ischemia. The effect of propranolol on this progression of ischemic injury was evaluated. Three groups of dogs were studied: 1) untreated, 2) treated with propranolol before and throughout coronary ligation, and 3) treated with propranolol beginning three hours after ligation. Dogs were sacrificed 24 hours after coronary ligation and necrosis was quantitated from histologic sections of transmural slices through the posterior papillary muscle. Propranolol reduced infarct size by preventing necrosis in peripheral (subepicardial) areas of moderately ischemic myocardium. Propranolol treatment with propranolol reduced necrosis from 85 ± 3% (untreated) to 52 ± 4% (P < 0.05). Delayed propranolol therapy was about half as effective as pre-treatment and reduced necrosis to 71 ± 3% (P < 0.05). Propranolol also limited microvascular injury so that perfusion defects, detected with the dye thioflavin S, were smaller in treated dogs.

MYOCARDIAL CELL DEATH following acute coronary occlusion, develops first in severely ischemic subendocardial myocardium, and progressively spreads to involve areas of moderately ischemic mid- and subepicardial myocardium.1-4 Many studies have supported the idea that this process can potentially be limited by early pharmacologic, mechanical or surgical intervention.1-4

Previous studies have demonstrated that β blockade with propranolol or practolol reduces epicardial or precordial ST segment elevation following experimental coronary occlusion or following myocardial infarction in man.6-10 Histologic studies of necrosis following temporary coronary occlusions have provided direct demonstration that propranolol delays cell death in areas of severe ischemia.11-13

The present study was done to determine whether necrosis within the peripheral, moderately ischemic zone of infarcts produced by permanent coronary ligation in dogs could be limited by propranolol. Infarct size was significantly reduced by propranolol therapy instituted prior to, and maintained throughout coronary occlusion. Intervention with propranolol three hours after coronary ligation was less effective but still produced a significant reduction in infarct size.

Materials and Methods

Mongrel dogs of both sexes weighing 9–28 kg were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and ventilated with a Harvard (Model 1063) respirator. Aseptic surgical techniques were used. Lead II of the standard electrocardiogram (ECG) and peripheral blood pressure, via a catheter in the right femoral artery, attached to a Statham (P23AC) pressure transducer, were monitored on a Grass (Model 5) polygraph. The right saphenous vein was catheterized for propranolol or saline infusion and both catheters were kept open with dilute heparinized saline.

The chest was opened in the fourth left intercostal space, the pericardium was incised, and the circumflex coronary artery was isolated 1–2 cm from the aorta. Dogs in a pretreated group (group B) were given 5 mg/kg propranolol (intra-arterially) 10 min prior to coronary occlusion and an additional 1.0 mg/kg/hr in heparinized saline was infused intravenously at 0.7 cc/min beginning 30 min after occlusion.
The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs.
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