The Association of Increased Plasma MB CPK Activity and Irreversible Ischemic Myocardial Injury in the Dog

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SUMMARY To evaluate the concordance between elevated plasma MB CPK and irreversible myocardial ischemic injury, coronary occlusion was induced for 10 minutes to 48 hours in 21 open chest dogs and 13 conscious animals. Results of plasma CPK and MB CPK assayed in samples obtained serially for 24 hours were compared to microscopic changes in hearts from the same animals examined 48 hours after occlusion. Twelve of the 34 dogs died within two hours after coronary occlusion. Among the surviving 22 dogs, one failed to exhibit gross or electrocardiographic evidence of ischemia and was therefore excluded. Twelve had coronary occlusion maintained for 30 minutes or longer and in 11 of these peak plasma MB CPK activity exceeded the normal range (mean ± 2 SD) and baseline values by at least 100%. Necrosis was present in the hearts from each manifested by nuclear pyknosis, eosinophilia, shrinkage of cytoplasm, and leukocytic infiltration. In the remaining nine dogs with occlusion for less than 30 minutes, peak plasma MB CPK activity was not elevated and necrosis was not detected. The close concordance between plasma MB CPK elevations and myocardial necrosis was significant ($\chi^2 = 14.5, P < 0.001$), and thus, increased plasma MB CPK activity reflected irreversible myocardial ischemic injury.

THE DIAGNOSIS of acute myocardial infarction often depends upon the demonstration of elevated plasma enzyme activity. Enzymatic estimation of infarct size depends on several premises, one of which is that release of enzyme from the ischemic heart reflects cell death rather than reversible injury. However, it is possible that release of myocardial enzymes into the circulation may not reflect necrosis but may occur when ischemic cells are not irreversibly damaged. Elevated activity of MB CPK in plasma, an isoenzyme of creatine phosphokinase, has been found to be a sensitive index of myocardial injury. In man, the MB CPK isoenzyme is confined almost exclusively to myocardium and is virtually absent from skeletal muscle, although this point is controversial. In dogs, MB CPK appears to be found in the gastrointestinal tract as well as the heart.

Despite substantial circumstantial evidence, it is not clear whether release of MB CPK from the ischemic heart is tantamount to myocardial cell death. This issue deserves consideration particularly because under some experimental conditions, such as exposure of myocardium to calcium-free media, enzyme is released from the heart in the absence of irreversible contractile failure. Furthermore, although changes in activity of enzymes in plasma such as glutamic oxaloacetic transaminase (GOT) have been associated with experimental myocardial ischemic injury for many years, release resulting from ischemia insufficient to produce cell death has not been completely excluded. Many experimental studies concerned with these issues employed open chest animal preparations in which release of enzyme from traumatized skeletal muscle may have occurred.

The recent recognition of MB CPK as a marker of myocardium in contrast to skeletal muscle and the development of techniques permitting quantitative estimation of MB CPK activity in plasma samples encouraged us to examine the relationship between plasma MB CPK elevations and myocardial necrosis. The present study was designed to determine whether release of MB CPK from myocardium into peripheral blood after myocardial ischemia maintained for selected intervals in dogs was concordant with the presence of myocardial cell death in animals surviving sufficiently long to allow the development of morphological criteria of necrosis.

Materials and Methods

Myocardial ischemia was produced in anesthetized and conscious mongrel dogs by occluding the left anterior descending coronary artery with a snare or an exteriorized inflatable balloon cuff for selected intervals ranging from 10 minutes to 48 hours. Total CPK and MB CPK activity in plasma were assayed before, during, and after coronary occlusion. Blood samples (2 to 4 ml) from the external jugular vein were obtained at 30 minute intervals for 6 hours beginning 30 minutes prior to occlusion and hourly for 18 hours thereafter. After each sample had been obtained, an equivalent volume of normal saline was given intravenously in order to maintain blood volume. Since enzyme release may not be evident for several hours after the onset of an ischemic insult, sampling was continued for a minimum of 24 hours in each case. Forty-eight hours after occlusion of the coronary artery, hearts were examined by a blinded observer (JRW) for gross and microscopic evidence of myocardial necrosis. The 48 hour interval provides sufficient time for the evolution of conventional morphological criteria of necrosis.

Animal Preparations

Thirty-eight conditioned mongrel dogs underwent surgery. Animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg, i.v.); ventilation was maintained with room air with the use of a Harvard pump. The electrocardiogram and femoral arterial blood pressure were monitored and recorded at selected intervals throughout the operative procedure. A left thoracotomy was performed under sterile conditions. The left anterior descending coronary artery...
artery was exposed and separated from the adjacent vein in a region approximately 0.5 cm distal to the point of its emergence from beneath the left atrial appendage. The subsequent surgical procedure depended upon whether the dog was assigned to Group I, II, or III.

**Group I**

Sham operated dogs (N = 4) were used as controls. In two dogs, the surgical procedure was performed as in Group II (described below) but the coronary artery was not occluded after the silk ligature was placed around it. In the remaining two dogs the surgical procedure was performed as in animals in Group III (described below) except that the coronary artery was not occluded after an inflatable balloon cuff had been placed around it. Total CPK and MB CPK were measured in serial plasma samples and the electrocardiogram and systemic arterial blood pressure were recorded in all dogs.

**Group II**

Open chest preparations (N = 21) were utilized for experimental studies in this group. An O silk ligature was placed around the coronary artery and passed through rubber tubing. Coronary artery occlusion was produced by exerting traction on the silk suture and countertraction on the rubber tubing. Occlusion was maintained for selected intervals ranging from 10 to 60 minutes in 20 dogs. In one additional dog the occlusion was maintained for 48 hours. Lidocaine, 3 mg/kg, i.v., was administered immediately before occlusion and immediately prior to release to reduce the incidence of malignant ventricular dysrhythmia. After restoration of flow, the chest was closed and the dogs were allowed to recover for 48 hours after the occlusion.

**Group III**

Closed chest preparations (N = 13) were used to verify results and obviate CPK release from surgical trauma during experiments after the time required for MB release had been defined in open chest animals. A commercially available inflatable balloon cuff was placed around the left anterior descending coronary artery and exteriorized. One week after the operation when the dogs had recovered sufficiently so that total plasma CPK activity had returned to normal, the left anterior descending coronary artery was occluded by inflating the balloon with normal saline. An electrocardiographic recording was obtained simultaneously to verify the presence of acute ST-segment changes consistent with ischemia. Coronary occlusion was maintained for selected intervals from 10 to 60 minutes prior to deflation of the balloon and restoration of coronary flow. As with the dogs in Group II, lidocaine, 3 mg/kg, i.v., was administered intravenously immediately prior to occlusion and prior to release.

Forty-eight hours after restoration of coronary flow, dogs in both groups were killed with intravenous sodium pentobarbital. The chest was opened through the same incision and the heart was removed, washed with cold saline, and preserved in 10% buffered formalin solution for subsequent gross and microscopic examination. Patency of the coronary artery at the site of the ligature or deflated cuff was verified in each case.

**Biochemical Procedures**

Whole blood samples (4 ml) were collected in .01 M neutralized EGTA and the plasma separated by centrifugation. Plasma samples were then diluted with .01 M Tris, pH 7.4 containing 0.2% bovine serum albumin such that total CPK activity was approximately 0.1 IU/ml and thus within the limits of linearity of the assay. Total CPK activity was measured spectrophotometrically with and without creatine phosphate as substrate in the reaction medium to exclude spurious contributions to apparent enzyme activity from moieties such as myokinase or ATP. MB CPK isoenzyme activity was assayed with a recently described kinetic method. In brief, samples were electrophoresed on cellulose acetate and regions of the strips encompassing each CPK isoenzyme (MM, MB, and BB) were cut out and immersed in 0.5 to 3 ml of assay medium formulated such that reduced nicotinamide adenine dinucleotide phosphate (NADPH) was generated in proportion to activity of the isoenzyme incubated. NADPH was detected by serial determination of fluorescence in the aqueous medium. Definite elevations of MB CPK activity in samples from each dog were considered to be present only if two criteria were met: 1) activity exceeded the mean + 2 SD (19 + 12 mIU/ml) of activity detectable in plasma from normal dogs (N = 8); and 2) activity was at least double that in the baseline sample from the same dog.

**Morphological Examination of the Hearts**

The unopened hearts were rinsed in saline and fixed in buffered formalin for at least 24 hours prior to sectioning in 1 cm thick slices from the apex to the mitral annulus. Both the unopened hearts and the sections were examined carefully for gross evidence of hemorrhage, fatty degeneration, and necrosis. Suspicious looking regions were blocked for subsequent microscopic studies. At least two 4 to 5 cm long transmural blocks were obtained from the left ventricle of each heart. One was taken approximately 3 cm distal to the coronary occlusion and the other (control) from the posterior wall of the left ventricle at the same level. Each block was subdivided into 1 cm segments with three frozen sections obtained from each segment. One section was stained with Oil Red O to detect fatty degeneration, and all the remaining sections from each block were stained with hematoxylin and eosin for routine microscopic examination. The same number of sections was utilized for each heart. The observer (JRW) evaluating gross or microscopic morphology had no knowledge of the duration of coronary occlusion or results of MB CPK determinations. In addition, when evaluating histology he was unaware of the gross findings in the heart from which the sections were obtained.

Shrinkage of cytoplasm, increased cytoplasmic eosinophilia, and nuclear pyknosis were taken as criteria of necrosis after ischemic injury. An infiltrate of polymorphonuclear leukocytes was considered to be confirmatory but not independently sufficient evidence of necrosis.

**Results**

**Surgical Mortality**

Thirty-four dogs were subjected to coronary artery occlusion. Of the 21 dogs in Group II (open chest), three died with ventricular fibrillation within 30 minutes after coronary
occlusion (while the occlusion persisted) and five died with ventricular fibrillation immediately (within 1 minute) after restoration of coronary flow. One surviving dog failed to develop cyanotic changes in the myocardium distal to the occluded vessel or ischemic changes on the electrocardiogram, presumably because of a rich collateral circulation distal to the occlusion. Data from this dog were excluded. Among the surviving 12 dogs in Group II from which data were analyzed, 11 had left anterior descending coronary artery flow interrupted for an interval ranging from 10 to 60 minutes. In the remaining animal, the occlusion was maintained for 48 hours. The gross changes in myocardium after abrupt occlusion of the left anterior descending coronary artery of dogs in Group II were similar to those described by Jennings et al.\textsuperscript{16, 17} with cyanosis and loss of contractility apparent within seconds. Immediately after restoration of flow, the cyanotic area exhibited hyperemia but within a few minutes, the region became indistinguishable from normal adjacent myocardium.

Among the 13 dogs in Group III (closed chest), two died of ventricular fibrillation within minutes after occlusion and two immediately after restoration of flow. In the nine surviving dogs, the left anterior descending coronary artery was occluded for intervals ranging from 10 to 60 minutes. Thus, overall mortality in Groups II and III combined was 35%, comparable to results by others.\textsuperscript{13} Mortality due to ventricular fibrillation during occlusion was 14% and 15% and after reflow was 24% and 25% in Groups II and III, respectively.

**Total and MB CPK Activity in Plasma from Animals without Myocardial Ischemia**

Among four sham operated control dogs, definite elevations of plasma MB CPK did not occur in any case. However, total plasma CPK was markedly elevated in all cases presumably as a result of surgical trauma to skeletal muscles. An example of enzyme changes observed is shown in figure 1. The electrocardiogram did not exhibit Q waves or ST-segment abnormalities in any control animal and no sustained ventricular dysrhythmias were noted.

**Total and MB CPK Activity in Plasma from Surviving Animals with Myocardial Ischemia**

Changes in plasma MB CPK activity as a function of the duration of ischemia are shown in table 1. Seven of the 12 dogs in Group II (open chest) surviving after ischemia had coronary occlusions maintained for 30 minutes or more and five had occlusions maintained for less than 30 minutes. In all the dogs with occlusion of 30 minutes or more, plasma MB CPK activity rose at least 250% above baseline and peak values exceeded the normal range in all animals. In contrast, plasma MB CPK activity did not increase by more than 60% above baseline in any of the five dogs with coronary occlusion maintained for less than 30 minutes and peak values remained within the normal range in all cases. It should be recognized that a 60% increase of MB CPK above baseline represents values of the order of 10 mIU/ml and hence a total change (assuming blood volume is approximately 4 L) of the order of 40 IU. This amount is equivalent to the amount of MB CPK in less than 500 mg of myocardium. On the other hand, the modest increase of MB seen occasionally could have come from enzyme released from the gastrointestinal tract or other tissues besides the heart as a result of changes associated with surgery. Increases of plasma MB CPK in man would not be anticipated or explicable on this basis because of the absence of MB from human gastrointestinal tissue.\textsuperscript{8} To minimize the likelihood of MB release from gastrointestinal tissues in the dog, we performed additional studies in closed chest dogs.

In four of the five surviving dogs in Group III (closed chest) with coronary occlusion maintained for 30 minutes or longer, MB CPK activity exceeded baseline values by at least 250%. Total plasma CPK activity was also elevated in each of the four dogs exhibiting increased MB CPK activity. An example of MB and total CPK changes in an animal with ischemia of 60 minutes duration is shown in figure 2. None of the surviving four dogs in Group III with coronary occlusion maintained for less than 30 minutes demonstrated increases in total or MB CPK activity exceeding 25% above baseline values. In all cases but one, MB CPK activity was 30 mIU/ml and peak MB CPK was 35 mIU/ml. We have recently shown that lidocaine does not influence CPK when injected intravenously in conscious dogs and that it does not liberate CPK from skeletal muscle or myocardium in normal animals (unpublished observations, R. Roberts and B. E. Sobel). It is possible that lidocaine could alter the pattern of release of MB CPK from myocardium undergoing infarc-
Table 1. Plasma MB CPK Activity and Morphological Changes in Myocardium after Coronary Artery Occlusion for Selected Intervals

<table>
<thead>
<tr>
<th>Dog</th>
<th>Duration of coronary occlusion (min)</th>
<th>Plasma MB CPK activity compared to baseline</th>
<th>Necrosis</th>
<th>Fatty degeneration</th>
<th>Pericarditis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>4 hr 8 hr 12 hr 16 hr 20 hr 24 hr Gross Microscopic†</td>
<td></td>
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<tr>
<td>1</td>
<td>(O)*</td>
<td>E E E E E E + + + + + + + + + + + + + +</td>
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<tr>
<td>2</td>
<td>(O)</td>
<td>E E E E E E + + + + + + + + + + + + + +</td>
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<tr>
<td>3</td>
<td>(C)</td>
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<tr>
<td>4</td>
<td>(O)</td>
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<td>11</td>
<td>(C)</td>
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<tr>
<td>12</td>
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<tr>
<td>13</td>
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<td>16</td>
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<td>17</td>
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<td>18</td>
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<tr>
<td>19</td>
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<td>20</td>
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<tr>
<td>21</td>
<td>(O)</td>
<td>E E E E E E + + + + + + + + + + + + + +</td>
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</table>

*O = open chest; C = closed chest preparation.
†E = definite elevation of plasma MB CPK defined as follows: 1) exceeding baseline by at least 100%; 2) exceeding the mean 2 SD in samples from normal, unoperated dogs. Nonelevated values are indicated as -.
†Nuclear pyknosis, cytoplasmic eosinophilia, shrinkage of cytoplasm and leukocytic infiltration combined are indicated as +.
§Fatty degeneration demonstrated by Oil Red O stain or pericarditis are indicated as +.

Figure 2. As illustrated in this example from one dog, after coronary occlusion in a closed chest preparation changes in plasma CPK and in MB CPK activity are parallel indicating release of enzyme from the heart in contrast to the case after sham operation (fig. 1). In this case coronary occlusion was maintained for 60 minutes.

tion, but this seems unlikely since intermittent administration of the drug does not appreciably alter the relationship between observed and projected plasma CPK values in patients during the evolution of infarction. Since lidocaine was administered to all dogs in the present study, it appears unlikely that it would account for the observed patterns of relationships between MB CPK release and the duration of coronary occlusion.

Morphological Changes in Hearts from Animals with Definite Plasma MB CPK Elevations

Microscopic evidence of necrosis was apparent in the hearts of all 11 surviving dogs in which coronary occlusion was of sufficient duration to lead to an elevation of plasma MB CPK activity exceeding 100% of baseline and falling outside the normal range (table 1; fig. 3). In six hearts, necrosis was evident by gross inspection as well as by light microscopy. Generally, necrosis was multifocal. In no instance was necrosis observed in control blocks from the posterior surface of the heart.

Morphological Changes in Hearts from Animals without Definite Plasma MB CPK Elevations

No necrosis was detected in the hearts of any of the nine animals with transitory coronary occlusion maintained for less than 30 minutes, an interval of ischemia not associated with definite elevations of plasma MB CPK activity. In one dog with occlusion for 30 minutes and normal plasma MB CPK, no evidence of necrosis was detected microscopically. Fatty degeneration was noted in all but one of the hearts in which necrosis was present (table 1). An example is shown in figure 3. None of the hearts with coronary artery occlusion of 15 minutes duration showed evidence of fatty degeneration. Pericarditis was observed in nine of 11 hearts with
Table 3. The Relationship between the Duration of Coronary Occlusion and Necrosis

<table>
<thead>
<tr>
<th>Duration of ligation in minutes</th>
<th>Evidence of necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
</tr>
</tbody>
</table>

\( \chi^2 = 14.1, P < 0.001 \)

Heart muscle cells were shrunken, vessels contained numerous red blood cells, and occasional polymorphonuclear leukocytes were present in the right upper portion of the micrograph. Cells in the lower left portion of the micrograph are normal. (H & E stain, \( \times 250 \).) Bottom: Light photomicrograph demonstrating characteristic appearance of patchy fatty degeneration of myocardium 48 hours after ligation of the left anterior descending coronary artery for 20 minutes. Numerous small black granules in cells in the central portion of the micrograph represent Oil Red O positive lipid droplets in otherwise normal appearing cells. (Oil Red O stain, \( \times 400 \).)

TABLE 2. The Concordance between Definite Elevations of Plasma MB CPK Activity and Necrosis after Coronary Artery Occlusion

<table>
<thead>
<tr>
<th>Criteria of necrosis</th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal MB CPK</td>
<td>10†</td>
<td>0</td>
</tr>
<tr>
<td>Elevated MB CPK*</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

\( \chi^2 = 14.5, P < 0.001 \)

Table 4. Plasma MB CPK Changes in Dogs with or without Myocardial Necrosis

<table>
<thead>
<tr>
<th>Peak change in plasma MB CPK activity compared to baseline (mIU/ml)</th>
<th>Evidence of necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ± 4 (SD)</td>
<td>Absent (10)</td>
</tr>
<tr>
<td>45 ± 15 (SD)</td>
<td>Present (11)</td>
</tr>
</tbody>
</table>

Discussion

Experimental coronary occlusion in dogs for selected intervals has been employed in a number of studies designed to investigate the effects of ischemia on the heart. A relationship between elevation of transaminase activity in serum and the presence or absence of irreversible myocardial ischemic injury in the heart has been defined in several studies. In one study utilizing a closed chest preparation elevation of transaminase appeared to be an accurate index of the extent of myocardial infarction in dogs and the transaminase content of infarct was significantly less than that in normal myocardium. These findings suggested that increased serum transaminase activity reflected irreversible myocardial injury. Subsequently, others utilizing open chest preparations and employing transaminase as a biochemical marker of myocardial cell death reported conflicting results, suggesting that the rise of transaminase activity in serum did not necessarily reflect irreversible myocardial damage. Thus, among eight dogs with a substantial rise in transaminase following occlusion of the coronary artery, only one exhibited morphological evidence of myocardial necrosis. The discrepancy between these and earlier findings may have been due to release of transaminase from skeletal muscle traumatized by thoracotomy in the open chest preparations.
Because we have sought to utilize myocardial depletion of CPK22,23 and more recently MB CPK24 and their counterparts in plasma as indices of cell death, we undertook the present study to examine the relationship between the rise of MB CPK in peripheral blood and the presence or absence of irreversible myocardial ischemic injury detectable morphologically in dogs after ischemia of graded duration. Because of the virtual absence of MB CPK from skeletal muscle,2 we anticipated that results in closed and open chest preparations would be comparable but utilized open chest preparations first for convenience to define the relationship between the duration of coronary occlusion and the appearance of elevated MB CPK activity in plasma.

Sham operated dogs were used to determine whether the surgical procedure per se influenced plasma MB CPK activity. In open chest control dogs, total plasma CPK levels after thoracotomy increased markedly. However, MB CPK activity did not rise substantially above baseline values. Similarly in closed chest sham operated dogs, no rise in MB CPK followed the surgical procedure. Thus, the surgical procedure alone did not lead to increased plasma MB CPK.

It was not the purpose of the present study to assess the quantitative relationship of MB CPK released and the amount of myocardial necrosis in the experimental preparation utilized. To do so with the dog model is difficult because MB CPK activity represents only approximately 1% of total myocardial CPK.7 We have shown previously that estimates of infarct size based on MB CPK in five conscious dogs correlated with estimates based on total CPK despite these difficulties.8 In the present study, the relationship between release of MB CPK and necrosis was evaluated qualitatively.

After complete interruption of antegrade flow in the left anterior descending coronary artery for 30 minutes or more, microscopic evidence of irreversible ischemic injury developed in 11 of 12 dogs in the area of the distribution of the occluded coronary artery. On the other hand, of nine dogs that had left anterior descending coronary flow interrupted for 20 minutes or less, none showed evidence of necrosis microscopically. These results are similar to those of several previous studies.13,17,18 Blumgart et al. who produced small infarcts9 noted that experimental occlusion of the canine left circumflex coronary artery lasting for 25 to 45 minutes generally resulted in grossly detectable myocardial lesions. In contrast, occlusions of 20 minutes or less were followed rarely by gross myocardial lesions. Jennings et al. have studied the posterior papillary muscles of dogs after occlusion of the left circumflex artery for selected intervals.17 Small (1 to 2%) regions of necrosis were observed after occlusions of 22 minutes and the area of necrosis increased with occlusions of longer duration. After occlusions of 60 minutes, 85% of the mass of the papillary muscle was generally necrotic. No necrosis was detected in the papillary muscles when the blood supply was interrupted for less than 20 minutes.

Whether definite increases in plasma MB CPK occurring after ischemic insults to the heart are tantamount to irreversible injury has not been established. Enzymes are released from viable cells in some conditions unrelated to ischemia such as perfusion of isolated hearts with calcium-free media10 or as a result of exercise in the intact organism.24 On the other hand, the bulk of clinical and experimental data suggest that when enzyme is released from myocardium subjected to ischemia, the insult is associated with irreversible injury.1

The design of the present study included an interval of reperfusion after coronary occlusion — which could affect the rate of appearance or cumulative amount of MB CPK recoverable in blood. Thus, if brief intervals of ischemia, insufficient to cause cell death, were associated with myocardial enzyme release, one would anticipate that elevated plasma MB CPK would be even more readily detected than in the setting of sustained interruption of coronary flow. However, the present results obtained with both open chest and closed chest preparations demonstrate that increased plasma MB CPK activity and morphological evidence of irreversible cell injury are concordant after coronary occlusion of graded duration. Thus, among 11 dogs with definite plasma MB CPK elevations, unequivocal evidence of myocardial necrosis was present consistently. On the other hand, among ten dogs without definite plasma MB CPK elevations, none showed evidence of myocardial necrosis.

The present findings indicate that definite plasma MB CPK elevations, arbitrarily defined as exceeding baseline by 100% and exceeding the normal range + 2 SD, occurring after coronary occlusion of graded duration followed by reperfusion in the dog are closely associated with myocardial necrosis demonstrable microscopically. Brief occlusion failed to lead to necrosis, in conformity with reports by others, and also failed to produce definite plasma MB CPK elevations. Conversely, occlusion with a duration sufficiently long to produce necrosis led consistently to definite elevations of plasma MB CPK activity.

Acknowledgment

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References

Cellular Protection during Myocardial Ischemia

The Development and Characterization of a Procedure for the Induction of Reversible Ischemic Arrest

DAVID J. HEARSE, PH.D., DAVID A. STEWART, M.SC.,
AND MARK V. BRAIMBRIDGE, B. CHR., M.A.

SUMMARY An isolated perfused working rat heart model was used to investigate the extent to which various protective agents, used either singly or in combination, were able to increase the resistance of the heart to periods of transient ischemia. The aim of the studies was to develop a solution which, if infused into the coronary vessels just prior to the onset of ischemia, would rapidly induce arrest and would also counteract several of the deleterious cellular changes known to occur during myocardial ischemia. Agents which induce cardiac arrest, modify cellular ion loss, affect substrate utilization, energy production and energy stores, affect coronary vessel diameter and cell swelling, prevent dysrhythmias, and affect metabolic rate were investigated. The additive effects of these agents were evaluated. An aqueous solution was formulated which contained high concentrations of potassium and magnesium, in combination with adenosine triphosphate, creatine phosphate and procaine. This solution increased the recovery of the ischemic (37°C for 30 min) rat heart from 0% to 93%. The safe period of ischemia could be further increased by the use of hypothermia.

OPEN HEART SURGERY requires ideally a still and relaxed heart. Cardiac arrest (cardioplegia) in diastole can be induced by several procedures10-16 which may or may not involve coronary perfusion. While few workers would question the metabolic and morphological advantages of maintaining coronary perfusion throughout the period of arrest, the simplicity and practical advantages of nonperfusion methods has resulted in the widespread use and adoption10-16 of ischemic arrest. However, the use of ischemic arrest has been criticized17-20 because associated with its prolonged use is the onset of irreversible metabolic and ultrastructural damage. Two important questions have therefore arisen. First, what is the maximum duration of ischemia that can be tolerated by the myocardium before the onset of major irreversible damage? Second, is there any way in which this period can be extended or the onset of irreversible damage be reduced or delayed?

Immediately following the onset of ischemia a number of functional, metabolic, and morphological changes occur.14 These changes are initially of a reversible nature and if blood flow is restored to the ischemic tissue during this phase of reversible damage there is a complete resumption of normal metabolism and function. If ischemia is maintained for longer periods of time, irreversible damage occurs, the restoration of blood flow no longer consistently reverses injury, and a permanent impairment of functional capacity occurs. The time taken for the onset of irreversible damage is determined by a number of factors such as the severity of ischemia, the nutritional and hormonal status of the tissue, the availability of energy supplies such as glycogen, adenosine triphosphate (ATP), and creatine phosphate (CP), the metabolic capacity for anaerobic energy production, the contractile state of the tissue, the age and temperature of the tissue, and the composition of the coronary blood in the tissue at the onset of ischemia.

The temperature of the myocardium and the composition of the extracellular fluid during ischemia provide an effective means of modifying the rate at which ischemic tissue deteriorates. The use of topical hypothermia and the consequent reduction of metabolic rate affords considerable protection to the ischemic myocardium.14, 15, 18, 19 Similarly,
The association of increased plasma MB CPK activity and irreversible ischemic myocardial injury in the dog.
S A Ahmed, J R Williamson, R Roberts, R E Clark and B E Sobel

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