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Graded Experimental Myocardial Contusion in Rabbits: Antemortem and Postmortem Diagnosis

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Abstract

A Controlled animal model was used to clarify the role of serum total creatine phosphokinase (CPK), and creatine phosphokinase-MB (CPK-MB) fraction in the diagnosis of myocardial contusion in patients, as well as, the role of cardiac tissue levels of creatine phosphate (CP) and adenosine triphosphate (ATP) in the diagnosis of fatal myocardial injury on autopsy. Graded myocardial contusion was produced by a single blow with a weighed pendulum in two groups of rabbits (group I received a moderate impact, 78 ± 5m Joules/g; Group II received a major impact, 87 ± 7 m Joules/g). Gross and microscopic examinations on autopsy showed a minor pathologic entity in Group I, while in Group II a distinct pathologic entity could be identified. Twenty minutes after the impact, a very highly significant ($p < 0.001$) increase in the serum total CPK appeared in both Groups. CPK-MB fraction was not detected in the serum of control animals (uninjuried group), but its level and percentage were 10.9 ± 0.80 IU/L, 6.98 % in group I and 29.7 ± 1.23 IU/L, 8.18% in Group II. Cardiac tissue levels of CP and ATP, 25 minutes after impact, revealed a very highly significant ($P<0.001$) reduction in both groups. It is clear that, the increase in the serum enzyme and the decrease in the cardiac tissue levels of high-energy phosphates were proportional to the severity of the blow. These results suggest that the serum total CPK and CPK -MB fraction are most reliable indicators of myocardial contusion following blunt chest trauma. The cardiac tissue CP and ATP levels also can be used as diagnostic criteria for a suspected fatal cardiac injury and not clearly apparent on autopsy.

Introduction

MYOCARDIAL contusion can occur with any direct blow to the chest or from rapid deceleration [1]. The sudden appearance of lifethreatening arrhythmia, cardiogenic shock, or cardiac arrest following chest injury has been reported frequently, and the capricious nature of this condition has been stressed [2]. Through the years various tests have been advocated as the most accurate means of diagnosing myocardial contusion, but till now there is lack of universally accepted means [3]. The electrocardiogram (ECG) has intermittently been called the best screening tool [2, 4]. However, the ECG is not a sensitive indicator of blunt cardiac injury [5, 6, 7] Doty et al. [8] and Kumar et al. [9] recommended technetium scanning in patients with suspected myocardial contusion; but other authors [10, 11] have found the technetium Scan to be of no value in the diagnosis of this condition. Lindsey et al., [12] championed the use of serum creatine phosphokinase-MB (CPK-MB) determinations to identify patients with myocardial contusion. Although Michelson [13] also reported that CPK -MB was a sensitive indicator of blunt myocardial injury, other authors [14,15] have failed to demonstrate strong correlations between serum CPK-MB levels and clinical or autopsy evidence of myocardial contusion.

It would be desirable to have a reliable indicator of the presence or absence of myocardial injury in patients with blunt multisystem trauma [3]; since untreated and unrecognized myocardial contusion may lead to disability and death [9], and the pathological findings used to diagnose fatal myocardial injury at autopsy may not be apparent [15]. Therefore, the aim of this study was to clarify the potential utility of serum CPK-MB assays in the diagnosis of blunt myocardial injury using an open-chest model in rabbits subjected to graded myocardial contusion; in addition to, estimation of high-energy phosphate levels creatine phosphate (CP) and adenosine triphosphate (ATP), as well histopathological findings, in the cardiac tissue to evaluate their usage as postmortem diagnostic criteria for myocardial contusion.

Materials and Methods

Animals: Thirty male rabbits with a body weight ranging from 2.5-3 kg were used in the study. They were divided into three equal groups; one group served as control and each of the other two groups received a blunt cardiac trauma with different severity.

Experimental Preparation: It is the same as that of Lau [16, 17]. All animals were anesthetized with 30mg/kg intraperitoneal (IP) sodium pentobarbital. Each animal was restrained in a supine position on a V-shaped support and artificially ventilated through an endotracheal tube. A midsternal thoracotomy was performed exposing the pericardium for impact.

Mechanical Impact: Myocardial contusion was produced by a single blow from a pendulum weighing 44g according to the method of Baxter, et al. [18]. Energy delivered was calculated as kinetic energy and was varied by changing the height from which the pendulum was released. Energy delivered to Group I and Group II hearts was 78 ± 5 m Joules / g and 87 ± 7 m Joules/g, respectively (height of release was 18 ± 1 cm and 20 ± 2 cm, respectively).

The animals of control group were subjected to the above mentioned sequence without pericardial impact.

Blood samples were obtained from the hearts 20 minutes postinjury [18] and the serum was separated by centrifugation and stored at-20°C until used for measurements of serum total creatine phosphokinase (CPK) by the method of Szasz et al. [19], and serum CPK-MB fraction by the method of Wurzburg et al. [20].

Hearts were freeze-clamped 25 minutes after the injury [18] for creatine phosphate (CP) and adenosine triphosphate (ATP) assays which were done according to the methods outlined by Lowry and Passonneau [21].

For histopathological study, specimens of fresh cardiac tissue were fixed in 10% neutral buffered formaline, dehydrated, cleared and embedded in paraffin Wax. Sections of 5μm were prepared and stained by Haematoxylin and Eosin and Verhoff Van Gieson's stains. Sections were examined and photographed using the light microscope.

Statistical Analyses: Data are expressed as mean with standard error. Student's t-test was used for comparisons between the means of two groups. A P value of less than 0.05 was chosen to be statistically significant.

Results

Enzyme Release and High-energy Phosphate Levels:

The effects of blunt myocardial injury on the serum total CPK and CPK-MB fraction (20 minutes after the injury) are compiled in table (1). A very highly significant ($p < 0.001$) increase is seen in the serum total CPK in both Groups I and II, in comparison with control. CPK-MB fraction was not detected in the serum of control animals (uninjured group), while its level in both Groups I and II was 10.9 ± 0.08 and 29.7 ± 1.23 IU/L, respectively. The percentage of CPK-MB fraction was 6.98% in Group I and 8.18% in Group II.

The results of CP and ATP assays performed on frozen hearts are summarized in table (1). Both CP and ATP levels were very highly significantly ($P < 0.001$) diminished in both Groups I and II, compared with control. ATP, however was reduced more than CP, this increased CP/ATP ratio (CP/ATP ratio was very highly significantly ($P < 0.001$) raised than that of control in Group I and Group II).

Table (1): Serum Total Creatine Phosphokinase (CPK) and CPK-MB Fraction, 20 Minutes after Injury, and Cardiac Tissue Levels of Creatine Phosphate (CP), Adenosine Triphosphate (ATP), and CP / ATP Ratio, 25 Minutes Postinjury. Values Express the Mean ($\overline{X}$) ± SE of Ten Rabbits (n = 10).

Group	Total CPK	CPK-MB		CP	ATP	CP / ATP Ratio
	(IU / L)	IU / L	MB%	(μ moles / g)	(μ moles / g)	(μ moles / g)
Control	18.0 ± 1.11			30.18 ± 1.01	17.95 ± 0.44	1.68 ± 0.02
Group I	156.2 ± 3.37***	10.9 ± 0.80	6.98%	24.42 ± 0.97***	12.06 ± 0.55***	2.03 ± 0.02***
Group II	363.0 ± 5.39***	29.7 ± 1.23	8.18%	22.91 ± 0.68***	10.48 ± 0.59***	2.19 ± 0.06***

*** Singificant difference from the corresponding control at $P < 0.001$.

Postmortem Examination:

Grossly: The only evidence of injury was a slight myocardial contusion in Group I and moderate myocardial contusion in Group II.

Histopathologically: The main histopathological lesions in the animals of Group I were congestion of coronary vessels. Small areas of extravasated blood were noticed. This was sometimes accompanied by mononuclear cell infiltration. Muscle laceration was minimal and limited to few foci in the wall of left ventricle, which was observed only in three animals (Figs. 2,3).

In Group II the hearts showed variable degrees of damage. Dilatation and congestion were common features of coronary vessels. The wall of some of these vessels was damaged leading to leakage of blood in between the myocardial muscle fibres. There were also variable degrees of selective muscle lacerations (necrosis) and mononuclear cell infiltration (Figs. 4, 5, 6, 7, 8). The right ventricular wall, by nature of its proximity to the impact, was commonly more affected; however in some animals the left ventricular wall was also damaged, and these injuries well most likely occur if the impact occurs coincident with diastole, when the left ventricle is distended. Some collections of blood were seen to be bounded by endothelium and could be considered as giant capillary sinusoids to form arteriovenous (A-V) communications (Fig. 8).

Fig. (1): Section in the heart of a control rabbit shows the structure of the atrium (A), ventricle (V) with branches of a coronary artery and vein (ca, cv) in between (H & E Orig. Mag. X 200).

Discussion

The data of the present study clearly showed a relationship between the myocardial contusion and the increasing serum enzyme concentration. Total CPK and CPK-MB fraction were very highly significantly elevated after blunt cardiac trauma and paralleled the severity of blow. Although the MB fraction of total serum CPK was not detectable in the sera of control uninjured animals, the percentage of MB concentration was 6.98% in Group I and 8.18% in Group II. These results corroborate the findings in other studies that serum total CPK and CPK-MB fraction were elevated in the sera of patients with myocardial contusion **[1, 2, 3, 9, 22, 23]**. In experimental animals, Baxter et al., **[18]** who performed graded myocardial contusions,

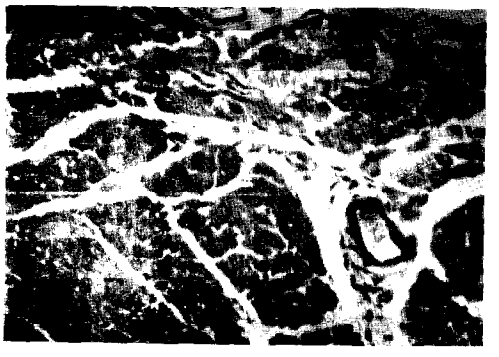

Fig. (2): Section in the heart of a rabbit in Group I shows the left atrioventricular region with congestion of coronary vessels and a tear in the subpericardial one (arrow) (H & E Orig. Mag. X 400).

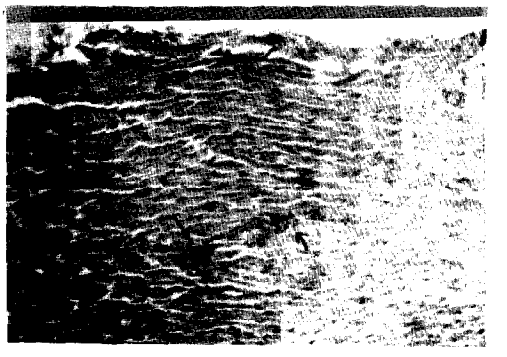

Fig. (3): Left ventricular wall from a rabbit in Group I shows small collections of extravasated blood together with mononuclear cellular infiltration (arrow) (H & E Orig. Mag. X 200).

Fig, (4): Right ventricular wall from an animal in Group II shows muscle laceration with mononuclear cell infiltration (arrow). A teatr can be observed in the endocardium (arrow). Congestion of coronary blood vessels is noted (Van Gieson's stain Orig. Mag. X 400).

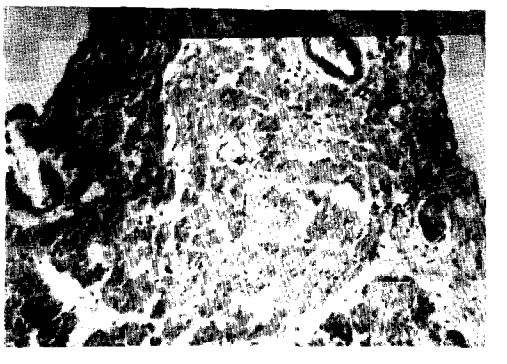

Fig. (5): Section in the heart (right atrioventricular region) of a rabbit in Group II shows tear in the wall of a coronary artery (arrow) with extravasation of blood (H & E Orig. Mag. X 200).

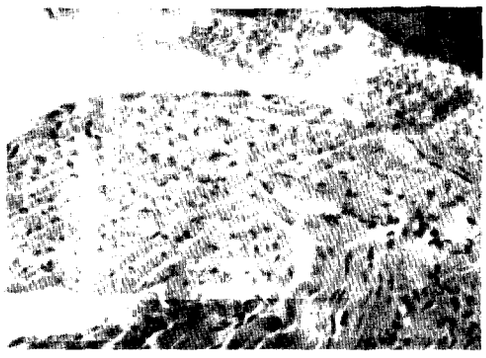

Fig. (6): Section in the base of the interventricular septum from a rabbit in Group II shows congestion of coronary vessels with extravasation of blood between the muscle fibres (H & E Orig. Mag. X 400).

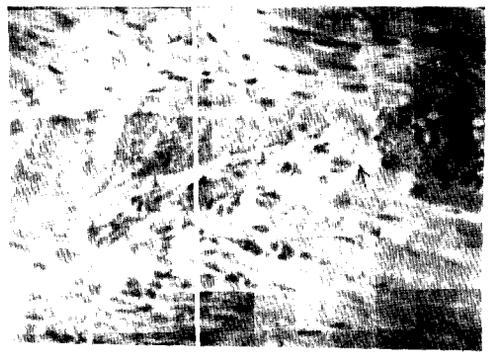

Fig. (7): Left ventricular wall from a rabbit in group II shows marked congestion of a longitudinally cut coronary artery together with lacer ation of its wall (arrow). Areas of muscle laceration (M) with extravasation of blood and mononuclear cell infiltration are observed (H & E Orig. Mag. X 400).

Fig. (8): Section in the heart of a rabbit in Group II shows part of the right ventricle. Marked congestion of coronary vessels, in addition to dilatation and congestion of intramural capillary channels (arrow) are observed. Areas of muscle laceration and extravasated blood in the myocardium are also noticed (H & E Orig. Mag. X 100).

similar to those used in the present study, on isolated rats' hearts demonstrated that serum total CPK increased significantly after blunt myocardial trauma and were approximately twice as high in Group II as in Group I. It is clear that, these finidings are in harmony with those of our study.

CPK isoenzymes have evolved as the most commonly applied measure for determining myocardial contusion [2, 7, 22]. They are felt to be both sensitive and specific for myocardial damage [7]. The level of CPK-MB considered diagnostic for contusion has been somewhat varied, since there is no consensus concerning the isoenzyme level which is diagnostic of myocardial contusion. Potkin et al. [15], reported that a serum CPK-MB concentration of

6 IU was used as the diagnostic threshold for myocardial contusion. Other studies contended that a CPK-MB fraction above 5% is indicative of myocardial injury [1, 2, 7, 9, 24, 25]. On the other hand, keller and Shatney [3] mentioned that, serum CPK-MB/CPK ratio > 2.2% is abnormal and diagnostic for myocardial contusion.

Frazee et al. [22] have employed CPK-MB as a screening tool and employed two-dimensional echocardiography (2-DE) to differentiate cardiac concussion from cardiac contusion. Patients with any elevation of CPK-MB with normal 2-DE were classified as having cardiac concussion, and those with abnormal 2-DE were classified as having cardiac contusion. In their hands 2-DE was abnormal in 40% of 58 patients with any CPK-BM elevation and these were diagnosed as having myocardial contusion, with the remaining 60% diagnosed as concussion. Based on these criteria, Fabian et al. [1] who found some elevation of CPK-MB in 119 patients, classified 21% of these patients as contusion (abnormal 2-DE) and with 79% classified as concussion. Other authors [13, 26] mentioned that, the differentiation between myocardial contusion and myocardial concussion can be based on the magnitude of the serum CPK-MB concentration. This issue of concussion and contusion is likely more than semantic [1]. These authors said that, liberation of CPK from muscle probably indicates some element of anatomic cellular damage. Contusion is noted histologically by extravasation of red blood cells into and between myocardial cells, and necrosis of myocardial muscle fibres [7]. Cellular dysfunction might be suspected to occur without destruction, and this can be considered concussion, similar to the distinction between cerebral concussion and contusion [1].

Important point for cardiac injury diagnosis relates to the timing of CPK-MB measurement in trauma patient. Previous studies [7, 13, 14, 22] have reported that in patients with blunt cardiac injury the serum CPK-MB concentration peakes within 24 hours of hospital admission and is no longer detectable by 72 hours. Frazee et al. [22] mentioned that, Kumar et al. [9] reported two cases of myocardial contusion diagnosed by technetium - 99m pyrophosphate scanning in which CPK-MB was not detected, and this absence of CPK-MB in cases of documented myocardial contusion may reflect improper timing of sampling; where in our experience in patients with documented cardiac contusion, CPK-MB was identified at admission or by the morning thereafter (< 24 hours after injury). In another study, Fabian et al, [1] also reported that, of considerable interest is the time at which serum CPK-MB elevations occured in the contusion patients, whereas elevations occured only at admission and 6 hours later in 53%, and on admission only in 21%, and therefore, if these sampling times are missed, up to three fourths of patients with myocardial contusion might not be diagnosed. These

authors also said that, by 24 hours after admission 74% of these patients had no further CPK-MB elevation. CPK consists of two subunites, M and B, which form three isoenzymes: MM which predominates in muscle; BB which predominates in brain; MB which has the highest concentration in heart muscle (15-20% of total cardiac CPK) [27]. Noncardiac tissues have absent or trace amounts (1%) of MB [28, 29]. CPK-MB disappears from the circulation more rapidly than MM and is more labile [30].

Regarding the high-energy phosphate levels in the cardiac tissue in our study, creatine phosphate (CP) and adenosine triphosphate (ATP) showed very highly significant decreases in both Groups I and II, in proportion to the severity of impact, 25 minutes after trauma. This decrease in the high-energy phosphate levels in the cardaic tissue may be helpful in the diagnosis of fatal blunt cardiac trauma in which the signs of cardiac injury are not clearly apparent at autopsy as in Group I in the present study. Our findings coincide completely with those of Baxter et al., [18] who performed similar graded experimental myocardial contusion in isolated rats' hearts and observed a marked significant decrease in CP and ATP levels in the cardiac tissue proportional to the severity of blow. These authors found that coronary artery flow was decreased immediately after 2 minutes from impact. Previous work with langendorff-perfused rat hearts has shown that greater than 30 minutes must elapse before cell death and enzyme release occur from ischemia alone [31]; but in myocardial contusion the early enzyme release reflects direct cellular injury [18]. Another work with langendroff-perfused rat hearts has shown that ischemia produced the heart arrest with a marked decrease in the tissue levels of creatine phosphate (CP) and adenosine triphosphate (ATP) [32]. The relationship between diminished coronary flow and decreased function is not straightforward because the oxygen demand of the impaired heart may be satisfied despite reduced coronary flow [18]. These authors said that, the high-energy phosphates adenosine triphosphate (ATP) and creatine phosphate (CP) have been studied extensively during ischemia and reperfusion to resolve this issue. CP falls precipitously with ischemia and buffers a change in ATP, which declines gradually [33]. The relationship between CP and ATP defines the metabolic status of the cell [32]. This ratio was very highly significantly increased above control values 25 minutes after injury in our study and this finding was also demonstrated by Baxter et al., [18], who mentioned that this is a relationship indicative of well-perfused viable myocardium recovering from transient ischemia. These authors also, reported that, these additional data substantiate a causal relationship between the early alterations in coronary flow and decreased ventricular function.

Changes in coronary artery flow have been implicated as a cause of postinjury

dysfunction and infarction [18]. Histopathological investigation in the present study disclosed lacerations in segments of coronary vessels in group II. Another study, during autopsies of patients with fatal blunt chest trauma, found that, direct laceration or thrombosis of coronary vessels occurs but is rare [34]. The present study microscopically showed some collections of blood bounded by endothelium and could be considered as giant capillary sinusoids to form arteriovenous (A-V) communicatins. Our findings are in harmony with those of other investigators, who have studied postmortem angiograms of experimental myocardial trauma in dogs and in humans following trauma, as well as when dogs were killed immediately after experimental contusion and examined angiographically and morphologically [8, 34, 35, 36, 37, 38]. These investigators found by angiographic studies large collections of contrast material accumulated in the terminal branches of small vessels in the area of damaged muscle, and by morphological examination showed these collections to actually be dilated vascular channels or' giant capillary sinusoids'a form of arteriovenous communication. It was concluded that it was these channels, with their associated local A-V shunting, and not coronary artery spasm and thrombosis that accounted for the fall in distal coronary vascular resistance and the resulting deficit in distal coronary artery perfusion that leads to regional myocardial ischemia [7].

Autopsy may play an important role in assessing diagnostic criteria of myocardial contusion [1]. Potkin et al., [15] demonstrated that in groups of patients at high risk of sustaining cardiac injury, clinical and pathological findings used to diagnose cardiac injury may not correlate completely with findings at autopsy. In two of five patients in that seattle study group, autopsy was positive for cardiac injury while clinical criteria were negative; in ten patients who were found to be negative for cardiac injury at autopsy, all had positive electrocardiogram (ECG) findings [15], and these patients could have had a concussion injury [1]. In our study gross and microscopic appearance on autopsy showed a little pathologic entity in Group I, contrarily to Group II in which a distinct pathologic entity could be identified grossly and microscopically. In spite of these minimal morphological alterations in Group I, the increasing serum enzyme release of total CPK and CPK-MB fraction, as well as the decrease in the high-energy phosphate CP and ATP levels in the cardiac tissue were very marked, since their changes were very highly significant in comparison with control.

In summary, this study clearly showed very highly significant increases in the serum total CPK and CPK-MB fraction isoenzymes, 20 minutes after blunt cardiac trauma and this increase was proportional to the severity of blow. In addition, the cardiac tissue revealed a very highly significant reduction in the high-energy phosphate

levels CP and ATP, 25 minutes after cardiac injury, proportional to the severity of impact. These results suggest that the serum total CPK and CPK-MB fraction isonzymes are most reliable indicators of myocardial contusion following blunt chest trauma; as well as, cardiac tissue CP and ATP levels can be used as diagnostic criteria for a suspected fatal cardiac injury and not clearly apparent on autopsy.

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