Elevated CK-MB Isoenzyme after Exercise Stress Test and Atrial Pacing in Patients with Ischemic Heart Disease

Alon T. Marmor, M.D., F.C.C.P.,*† Roberto Klein, M.D.;*
Michael Plach, M.D., F.C.C.P.,† David Groshar, M.D.;*
and Adam Schneeweiss, M.D.‡

Using a highly sensitive monoclonal antibody kit for CK-MB, significant release of small amounts of CK-MB isoenzyme after exercise stress test was detected 4 to 6 h after induction of ischemia. This occurred in ten out of 15 patients with ischemic heart disease (66 percent) and in only one of the 18 healthy subjects (5.6 percent) serving as a control group. In five patients with coronary artery disease in whom atrial pacing was performed with simultaneous blood sampling from coronary sinus, a drastic elevation in CK-MB isoenzyme (from 2.04±2.06 ng/L to 10.88±6.9 ng/L; p<0.001) was detected within 10 to 30 min after induction of acute ischemia. A small but significant increase in total CK also was detected (from 21±12 IU/L to 52±14 IU/L; p<0.01). These preliminary observations have to be further investigated in a larger group of patients before a definitive conclusion can be reached about the clinical significance of CK-MB release during exercise. (Chest 1988; 84:1216-20)

LAO = left anterior oblique

Elevation of CK-MB isoenzyme in the blood of patients with ischemic heart disease has been established as a very specific and sensitive diagnostic indicator of acute myocardial infarction.1,2 Usually the elevation of CK-MB isoenzyme in acute myocardial infarction is associated with an elevation of total CK far above the normal limits. Recently, however, several studies reported elevated CK-MB isoenzyme in the presence of normal total CK in patients with acute ischemic events resembling in their clinical manifestation acute coronary insufficiency rather than a fully developed picture of acute myocardial infarction.3,5 The significance of these findings is controversial. The majority of the investigators regard pathologically elevated CK-MB isoenzyme with normal total CK as micronecrotic events (small myocardial infarctions).4,6 Others view the phenomenon of small elevation of CK-MB isoenzyme as indicating reversible myocardial damage rather than myocardial necrosis.7,8 A strong support for the latter hypothesis was provided by the work of Heynicks et al10 demonstrating in a thorough histologic examination a lack of myocardial necrosis in the hearts of baboons in whom acute myocardial ischemia was induced and in whom, consequently, CK-MB isoenzyme elevation was found. Most investigators agree that patients with elevated CK-MB in the presence of normal total CK have certain clinical characteristics which have yet to be elucidated.

In order to clarify this topic the present study was performed. Using a highly sensitive kit for quantitative measurements of CK-MB isoenzyme levels, the changes in the absolute level of CK-MB isoenzyme in peripheral and coronary sinus blood during exercise- or atrial pacing-induced ischemia were measured. The ischemic changes on ECG were correlated with the transient perfusion defects observed on thallium 201 exercise scintigraphy.

METHODS

Patient Population

Forty-one subjects, 36 men and five women, participated in the study. Thirty-five subjects underwent exercise stress test (ergometry) with ECG recordings and thallium 201 scintigraphy. For the remaining six patients, atrial pacing was carried out. The study population then was divided into three groups.

Group 1 comprised 20 subjects (14 men and four women aged 22 to 50 years; average, 41.2±12 years), referred with atypical chest pain and without any history of ischemic heart disease. These subjects underwent an exercise stress test and thallium 201 scintigraphy for evaluation of their complaints. All subjects in this group had negative exercise stress test and had no evidence of persistent or transient perfusion defects on the thallium 201 scintigraphy. In two subjects, a thallium perfusion defect was detected, but due to divergence of opinion between the observers regarding its significance the subjects were excluded from the study.

Group 2 consisted of 15 male subjects (aged 34 to 64 years; average, 52.7±10 years) with angiographically documented triple vessel coronary artery disease with strong clinical evidence of ischemic heart disease. Thirteen had typical effort angina and two had silent ischemia documented in previous exercise tests and Holter monitoring. These subjects underwent exercise thallium 201 scintigraphy. In all the patients all the drugs were discontinued 24 h before the exercise stress test. All patients had typical electrocardiographic ischemic changes during or after exercise, with at least 1.5 mm downward sloping for at least 0.08 s of the ST segment in one or more leads and transient perfusion defects in thallium 201 scintigraphy with redistribution after 4 to 24 h.

*Division of Cardiology, Rebecca Sieff Hospital, Safed, Israel.
†Faculty of Medicine, Technion, Israel Institute of Technology; Haifa, Israel.
‡Geriatric Cardiology Research Foundation, Tel-Aviv, Israel.

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Reprint requests: Dr. Marmor, Department of Cardiology, Sieff Hospital, Safed, Israel 13100
Group 3 was composed of six patients (four men and two women aged 52 to 64 years; average, 60 ± 7.8 years) with a high probability for coronary artery disease. These subjects were incapable of performing a diagnostic exercise stress test and underwent atrial pacing. One of the patients developed atrial fibrillation during the initiation of the pacing and was excluded from the study. All patients developed chest pain and ischemic ECG changes according to the previously mentioned criteria. During the atrial pacing and after the occurrence of the acute myocardial ischemia, sequential blood samples were taken from the coronary sinus and the peripheral vein simultaneously.

Enzyme Measurements

Blood samples, for both total CK and CK-MB isoenzyme levels, were obtained during each stage of the study. In groups 1 and 2 the samples were taken before and at 1, 4, 6 and 25 h after the exercise test. In group 3, blood was drawn simultaneously from the peripheral vein and the coronary sinus before and during pacing and every 10 min after the acute ischemia for 60 min. The blood samples immediately were centrifuged and frozen at −20°C and the samples were evaluated within three days of their being taken. All the measurements were performed in duplicate by laboratory technicians unaware of the patients’ identity and diagnosis. Total CK was determined by Enzymine CK-Nac, a kinetic determination of CK activity after activation by N-acetylcysteine and expressed as international units per liter of serum. For measurement of CK-MB isoenzyme we employed the Tandem CK-MB kits (Hybritec Europe SA). By this method, serum containing CK-MB isoenzyme reacts with a plastic bed (solid phase) which is coated with a monoclonal antibody directed toward an antigenic site on the M subunit of the CK-MB molecule and a second monoclonal antibody oriented toward an antigenic site on the B subunit of the same isoenzyme molecule. After the formation of the solid phase, all nonenzyme elements are washed out. The CK-MB isoenzyme being bound to the antibody remains on the plastic bed and is incubated with an enzyme substrate. The concentration of CK-MB isoenzyme is calculated from the conventional spectrophotometric readings. The results are expressed in nanograms per milliliter according to the manufacturer’s instructions (Tandem CKMB Isoenzymetric Assay Instruction Manual, 1986). In our laboratory, the analysis of CK-MB concentrations in serum samples obtained from 22 apparently healthy nonhospitalized individuals showed that 99 percent of the samples had less than 3 ng CK-MB/ml with a mean concentration of 2.1 ± 1 ng/ml. The intraobserver and interobserver variability in 20 healthy subjects was <1 percent and 3 percent, respectively, the differences not exceeding 1 to 1.8 ng/ml.

Exercise Stress Test and Thallium 201 Scintigraphy

The subjects from groups 1 and 2 underwent multistage exercise stress testing on supine bicycle ergometry. A 12-lead ECG was obtained for every stage of the exercise and during recovery. Exercise was stopped when the patients complained of severe chest pain, shortness of breath, extreme weakness or when hypotension occurred. A test was considered positive when ST depression of at least 1.5 mm during at least 0.08 s (horizontal or downward in shape) appeared with or without chest pain. A test was considered negative when 90 percent or more of the predicted heart rate for age and sex was achieved without chest pain or any ischemic change on the ECG.

At peak exercise, 2 to 4 mCi of thallium 201 was injected intravenously, and the patients were asked to continue exercise for 60 s more. Myocardial images were obtained with an Apex 410 Elscint gamma camera equipped with a low-energy parallel hole medium resolution collimator. Imaging was begun 10 min after completion of exercise in four projections: anterior (supine and standing), 30° and 60° LAO (supine). Delayed images were obtained 4 and 24 h after the initial imaging.

Atrial Pacing

Right heart catheterization was performed in six patients. A Zucker 7FR bipolar pacing catheter was employed, and was advanced from the internal jugular vein into the coronary sinus. This catheter had a proximal electrode placed in the right atrium, and the distal tip was placed under fluoroscopy in the coronary sinus. Continuous recording of intracardiac pressures and oxygen saturations during catheterization confirmed the positioning in the coronary sinus. This catheter allows right atrial pacing and simultaneous blood sampling from the coronary sinus. A standard 12-lead ECG recording was obtained by a three-channel ECG recorder at rest and at each stage of the atrial pacing. The pacing was begun at 90 beats per minute, with the rate increased gradually by 10 beats per minute every three minutes. It was stopped at 150 beats per minute or when chest pain or ECG changes of myocardial ischemia appeared. The average pacing rate needed in all patients did not exceed 140 beats per minute. All patients had blood samples drawn according to the previously mentioned protocol.

Statistical Method

Student’s paired t test was employed to compare the changes in the enzyme levels in the same patient, and unpaired t tests were used to compare the enzyme levels between the various groups.

Results

In all patients, acute myocardial ischemia documented by ST depressions, horizontal or down-sloping of more than 2 mm (2.7 ± 0.5), and thallium reversible perfusion defects were induced. Eight patients had an anteroseptal defect, five patients had apical and septal defects, and two patients had an inferior wall defect. In the 15 patients studied, there was an average elevation in CK-MB isoenzyme levels from 1.5 ± 1.1 to 2.8 ± 1.6 ng/ml (p < 0.05) detected within 6 h after exercise-induced ischemia. In ten patients (66 percent) an elevation of more than 2 SD from the baseline values was measured. In contrast, in the 18 normal subjects there was a small and nonsignificant change in the CK-MB isoenzyme level from 1.5 ± 1.7 to 1.5 ± 2.5 ng/ml.

Figure 1. CK-MB levels before and after exercise stress test at peak level in healthy subjects and in patients. Open circle = control group; solid circle = patient group.
2.0 ± 1.7 ng/ml, p = NS (Fig 1). Only one healthy subject (5.6 percent) had an elevation in CK-MB isoenzyme exceeding 2 SD, compared with 66 percent of the patients, the difference being statistically significant at p<0.001. None of the ten patients with significantly elevated CK-MB isoenzyme developed clinical, electrocardiographic or enzymatic signs of acute myocardial infarction. The total CK was 47.17 ± 31 IU/L in normal subjects and 37.33 ± 12 IU/L in patients at rest, and it did not show any particular trend during exercise testing, reaching a maximum of 50.22 ± 30 IU/L in normal subjects and 40.87 ± 21.56 IU/L in patients (Fig 2). Although no significant elevation in total CK occurred during exercise in both groups, the baseline values and the peak exercise values of the normal subjects were significantly higher than those of the patients, p<0.05. The explanation for this may be that the normal subjects were younger than the patients and they achieved higher exercise levels (in normal subjects the exercise was stopped at submaximal level, maximum load of 150 w; in the patients the exercise was symptom-limited and it did not exceed 100 w). The heart rate blood pressure product achieved was 15,700±250 in patients vs 22,000±1,750 in the control group, p<0.01.

In the five patients in whom blood samples were taken from the coronary sinus during atrial pacing, there was a dramatic elevation of CK-MB isoenzyme level between 10 and 30 min after the induction of ischemia, ranging from 4 to 24 ng/ml (Fig 3). Mean CK-MB isoenzyme level rose from 2.04±2.06 to 10.88±6.9 ng/ml (p<0.01). At the same time, total CK rose from 21±12 to 52±14 IU/L (p<0.01) (Table 1). All patients had documented myocardial ischemia of more than 2 mm ST depression and an average heart rate of 140±12 beats per minute. None of the patients developed clinical, electrocardiographic or enzymatic signs of acute myocardial infarction. The peripheral blood CK-MB isoenzyme level rose mildly from 1.4±1.1 to 2.06±1.4 ng/ml, p = NS.

**DISCUSSION**

The CK-MB isoenzyme is present in healthy individuals in minute amounts, undetectable by the routinely employed electrophoretic methods. Pathologically elevated CK-MB isoenzyme levels occur in massive skeletal muscular necrosis or myocardial necrosis. The percentage of CK-MB isoenzyme in peripheral blood will be significantly higher when myocardial necrosis occurs than when massive skeletal muscular necrosis occurs due to the relatively high percentage of CK-MB isoenzyme in the heart. Therefore, it is justified, in the everyday clinical setting, to
express the amount of CK-MB isoenzyme in blood as a percentage of total CK, and it is generally accepted that more than 4 percent of CK-MB isoenzyme in the blood indicates myocardial necrosis.11 When estimated by the electrophoretic technique, however, this approach is not sensitive enough to detect small amounts of CK-MB isoenzyme release when the amount of necrotic or ischemic tissue is relatively small causing almost imperceptible changes in total CK. The significance, however, of small amounts of CK-MB isoenzyme in ischemic events may be far-reaching especially in its prognostic value. In the present study, the highly sensitive and specific method of monoclonal antibody against CK-MB molecule was used, allowing detection of minute isoenzyme levels below 3 ng/ml in the subjects studied.

CK-MB Isoenzyme Levels after Exercise

Elevation of total CK after exercise was reported by numerous investigators.7,8,11 In most of these studies, no elevation of CK-MB isoenzyme was observed in healthy subjects or in patients.2,12 However, there are some reports mentioning a slight elevation in CK-MB isoenzyme after strenuous physical exercise in marathon runners.13,14 The consensus about this phenomenon seems to be that elevated CK in blood following a certain degree of exercise represents a physiologic enzyme leakage from skeletal muscle which is paramount to the normal muscular activity and is proportional with the metabolic demands of the muscle. Some studies address the issue of the release of total CK and CK-MB isoenzymes in exercise-induced ischemia. Klein et al14 found that no elevation in plasma CK-MB isoenzyme level occurred in myocardial ischemia induced by treadmill exercise. Our previous studies6,9 and the studies of Davies et al17 reported a small but significant elevation of total CK and CK-MB isoenzymes in acute ischemic episodes induced by exercise. The present study confirms these findings, showing a small but significant elevation in the absolute amounts of CK-MB isoenzyme in the ischemic group and a nonsignificant change in the control group (Fig 1). Surprisingly, total CK levels did not increase subsequently and were actually higher in the control group than in the patients (Fig 2). The higher level of total CK in the control subjects may be explained by the fact that they were significantly younger with a larger muscle mass, and they achieved a significantly higher exercise level than the patients. The absence of concordant total CK and CK-MB isoenzyme elevation may have several reasons, such as lack of sensitivity of the total CK measurement compared with the CK-MB isoenzyme measurement, the lag in time between the rise in CK and in CK-MB levels, the randomness of the sampling, and so on. In the present study, thallium 201 scintigraphy was employed in order to prove that reversible myocardial ischemia is associated with elevated CK-MB isoenzyme levels. Indeed, in all ten patients in whom CK-MB isoenzyme level was definitely elevated at least more than 2 SD than the baseline value, reversible perfusion defects were documented by thallium scintigraphy. This strong clinical evidence of elevated CK-MB isoenzyme level associated with myocardial ischemia does not rule out the possibility of small micronecrotic damage concomitantly occurring in the ischemic area.

Atrial Pacing-Induced Ischemia with Elevated CK-MB Isoenzyme Level

The existence of elevated CK-MB isoenzyme level in the presence of normal total CK is questioned19 due to the fact that small amounts of CK-MB isoenzyme released from the heart during a myocardial ischemic or micronecrotic event are diluted in the peripheral blood, rendering the detection of such minute elevations highly improbable. Thus, it is reasonable to assume that if samples from the coronary sinus are taken, before the enzyme is released into the peripheral blood, immediately after the ischemic event, the changes in CK-MB isoenzyme levels can be accurately monitored. In order to clarify this topic, blood samples were obtained in this study simultaneously from coronary sinus and from peripheral vein in six patients in whom acute myocardial ischemia was induced by rapid atrial pacing. The CK-MB isoenzyme levels in the first hour after induction of ischemia in each patient (Fig 3) clearly indicate a definite elevation in CK-MB isoenzyme and total CK levels in the coronary sinus, from 2.04 ± 2.06 to 10.88 ± 6.9 ng/L (p<0.001) for CK-MB isoenzyme and from 21 ± 12 to 52 ± 14 IU/L (p<0.01) for total CK, with some CK-MB isoenzyme elevation in the peripheral blood (Table 1). Already in 1974 an elevation of total CK in coronary sinus after atrial pacing-induced myocardial ischemia was reported by Chong et al.18 Recently, Ninomiya et al17 found elevation in CK-MB isoenzyme in coronary sinus after very short episodes of acute coronary insufficiency induced experimentally. Considering all

Table 1—CK-MB Isoenzyme Levels in Coronary Sinus before and after Atrial Pacing

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2 ng/L</td>
<td>9 ng/L</td>
<td>5.2 ng/L</td>
<td>5.4 ng/L</td>
</tr>
<tr>
<td>2</td>
<td>0.4 ng/L</td>
<td>5.2 ng/L</td>
<td>24 ng/L</td>
<td>4.9 ng/L</td>
</tr>
<tr>
<td>3</td>
<td>0.1 ng/L</td>
<td>1 ng/L</td>
<td>8.5 ng/L</td>
<td>9.8 ng/L</td>
</tr>
<tr>
<td>4</td>
<td>0.6 ng/L</td>
<td>0.7 ng/L</td>
<td>5.5 ng/L</td>
<td>0.7 ng/L</td>
</tr>
<tr>
<td>5</td>
<td>4.9 ng/L</td>
<td>5.4 ng/L</td>
<td>11.2 ng/L</td>
<td>15.5 ng/L</td>
</tr>
<tr>
<td>Mean</td>
<td>2.04 ng/L</td>
<td>4.24 ng/L</td>
<td>10.88 ng/L</td>
<td>6.46 ng/L</td>
</tr>
<tr>
<td>SD</td>
<td>2.07 ng/L</td>
<td>3.1 ng/L</td>
<td>4.92 ng/L</td>
<td>3.83 ng/L</td>
</tr>
</tbody>
</table>
Elevated CK-MB Isoenzyme Level with Normal Total CK

In recent years, several studies described a subgroup of patients admitted to coronary care units with clinical episodes resembling myocardial infarction or prolonged ischemia with elevated CK-MB isoenzyme and normal total CK levels. Some authors advocated that these patients have a good prognosis while others argued that this is a high-risk group of nontransmural myocardial infarction with a high rate of reinfarction and bad prognosis. However, all authors agreed that these patients with elevated CK-MB isoenzyme and normal total CK levels represent a distinct subgroup of patients or an entity, differing from the patients with fully developed acute myocardial infarction or from patients with episodes of acute coronary insufficiency and normal enzymatic profile. The present study further substantiates the existence of elevated CK-MB isoenzyme with normal total CK levels in patients with acute ischemic events adding additional information and revealing new aspects of this problem. Elevated CK-MB isoenzyme level was found in most of the patients (66 percent) with ischemic heart disease after acute coronary insufficiency induced by ergometry or atrial pacing. When the blood samples were taken from the coronary sinus instead of the peripheral vein, 100 percent of the patients had elevated CK-MB isoenzyme levels. Regardless of the mechanism involved and regardless of the reversibility of the cellular injury, this finding has to be taken into account in the clinical management of these patients. A long-term follow-up study of this subgroup of patients is needed to elucidate the clinical significance of this observation.

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REFERENCES
3 Hong RA, Licht JD, Wei JY, Heller GV, Blaustein AS, Pasternak RC. Elevated CK-MB with normal total creatine kinase in suspected myocardial infarction: associated clinical findings and early prognosis. Am Heart J 1986; 111:1041-47