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Differentiating Muscle Damage from Myocardial Injury by Means of the Serum Creatine Kinase (CK) Isoenzyme MB Mass Measurement/Total CK Activity Ratio

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We immunoenzymometrically measured creatine kinase (CK) isoenzyme MB in extracts of myocardium and in homogenates of five different skeletal muscles. CK-MB concentrations in the former averaged 80.9 μg/g wet tissue; in the skeletal muscles it varied widely, being (e.g.) 25-fold greater in diaphragm than in psoas. CK-MB in skeletal muscles ranged from 0.9 to 44 ng/U of total CK; the mean for myocardium was 202 ng/U. In sera from 10 trauma and 36 burn patients without myocardial involvement, maximum ratios for CK-MB mass/total CK activity averaged 7 (SEM 1) ng/U and 18 (SEM 6) ng/U, respectively. Except for an infant (220 ng/U), the highest ratio we found for serum after muscular damage was 38 ng/U. In contrast, the mean maximum ratio determined in 23 cases of acute myocardial infarction exceeded 200 ng/U. Among seven determinations performed 8 to 32 h after onset of symptoms, each infarct patient demonstrated at least one ratio ≥110 ng/U. Ratios observed after infarct were unrelated to treatment received during the acute phase. We propose a CK-MB/total CK ratio of 80 ng/U as the cutoff value for differentiating myocardial necrosis from muscular injury.

Additional Keyphrases: *acute myocardial infarction · immunoenzymometric assay · enzyme mass vs catalytic activity*

The major clinical application of assay of the MB isoenzyme of creatine kinase (CK, EC 2.7.3.2)[7] in serum is to assess the possibility and extent of acute myocardial infarction (AMI) or, more broadly, to differentiate myocardial injury from skeletal muscle damage. The ratio of CK-MB to CK-MM isoenzyme is markedly higher in myocardium than in skeletal muscles, and CK-MB activities in serum are generally considered as indicative of AMI when they exceed 3 to 5% of total CK activity (*1, 2*).

In some studies, however, proportions of CK-MB have exceeded 6% in serum of subjects with multiple trauma or severe burns but without apparent myocardial injury. These unexpectedly high CK-MB contents in skeletal muscles have called into question the efficiency of assay of this isoenzyme for diagnosing AMI in patients with muscular damage (*3, 4*). Moreover, analytical methods measuring the catalytic activity of CK-MB have produced conflicting results as to the proportions of the CK isoenzymes in the human tissues (*5, 6*). Thus we have used a new immunoenzymometric assay, designed to determine the mass concentration of CK-MB with improved specificity (*7*), to reexamine the CK-MB content of skeletal and heart muscles. We have also applied this technique to serum specimens taken serially from patients with multiple trauma, burns, or AMI, to appraise the patterns of CK-MB release into the blood and to assess the magnitude of the changes in the serum concentration of this isoenzyme after muscle and myocardium damage. Finally, we wanted to investigate the value of CK-MB mass measurements for the differential diagnosis of AMI and skeletal muscle injury.

Materials and Methods

Patients

We studied three distinct categories of patients, from whom blood samples were taken serially during their hospital stay:

- 10 trauma patients (nine men and one woman, mean age 40, SD 18 years) admitted with multiple fractures after motor-vehicle accidents. Criteria of selection of subjects for the study were short delays before hospitalization (<75 min) and absence of any clinical evidence of myocardial necrosis or ischemia. Blood was sampled on admission and every 6 h during the following two days.
- 36 burn patients (20 males and 16 females—including two children, one- and 17-months old—mean age 24, SD 19 years). Myocardial involvement was also ruled out in all these patients, but the intervals before hospitalization were less uniform than was the case for the trauma patients (0.5–7.5 h, mean 3.5, SD 2.9 h). Blood was sampled upon admission and 24 and 48 h later. The Burn Unit Skin (BUS) classification was used to rate the severity of burns (*8*). The BUS score was <100% for 21 of these patients, between 100 and 150% for nine, and >150% for six. Seventeen patients survived without complication, 14 survived but contracted septicemia, and five died within six days.
- 23 AMI patients (17 men and six women, mean age 58, SD 9 years) who were admitted to the Coronary Care Unit (CCU) comprised the third category of patients. AMI was diagnosed on the basis of a typical clinical history, electrocardiographic evidence, and the characteristic increase and decrease of total CK activity in serum. To be included in the study, patients had to reach the CCU within 4 h after the onset of chest pain and initial CK values had to be less than 150 U/L. Eleven patients received fibrinolytic therapy (intracoronary perfusion of 0.8–1.0 × 10^6 units of streptokinase

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[7] Nonstandard abbreviations: AMI, acute myocardial infarction; CCU, coronary-care unit; CK, creatine kinase (EC 2.7.3.2); MB, CK isoenzyme; BUS, Burn Unit Skin.

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within 30 min) within 15 min of admission, whereas the other 12 patients were treated in a more conventional manner (administration of continuous intravenous heparin, 1000 units/h). For all these patients, blood was sampled upon admission, every 4 h during the first 36 h, and every 12 h thereafter until 72 h.

Tissue Homogenates

From specimens of muscle (diaphragm, psoas, pectoralis major, intercostal, femoralis) and myocardium tissue taken at autopsy within 6 h after death from three different subjects, homogenates were prepared as previously described (*9*). In brief: precisely weighed muscle and myocardium fragments (about 1 g) were homogenized in ice-cold pH 7.0 Tris acetate buffer in an Ultra-Turrax blender. Cell fragments were removed by centrifugation at 4000 × *g*. The supernates, kept on ice, were analyzed the same day for total CK activity and CK-MB mass.

Biochemical Constituents

Total CK. Total CK activities in serum (reference interval: 0–100 U/L) were determined at 37 °C with an optimized spectrophotometric method (CK UV test, no. 3388; Merck, Darmstadt, F.R.G.) according to Rosalki (*10*), by using a discrete analyzer (ABA 100; Abbott Labs., North Chicago, IL 60064). The linearity limit was 1500 U/L. Dilutions were made in heat-inactivated serum, in the ratio 1:10 or, exceptionally, 1:100. In contrast to isotonic saline, heat-inactivated serum has no "activity" effect on CK (*11*).

CK-MB

CK-MB. We measured CK-MB concentrations with a solid-phase, two-site immunoenzymometric assay (Tandem-E CKMB; Hybritech Europe S.A., Sart Tilman, Liège, Belgium), using the Hybritech "Photon" photometer (*7*). In this technique, samples containing CK-MB are reacted with a plastic-bead solid phase that is coated with a monoclonal antibody directed toward the M subunit of CK-MB, and with an enzyme-labeled monoclonal antibody directed toward the B subunit of the molecule. After the formation of the solid phase/CK-MB/labeled-antibody "sandwich", the bead is washed, then incubated with enzyme substrate (*p*-nitrophenyl phosphate). The amount of substrate turnover, determined colorimetrically, is directly proportional to the concentration of CK-MB in the test sample. All measurements were done in duplicate. The mass concentrations of CK-MB (μg/L) were numerically about double the activities (U/L) obtained with the immunoinhibition method at 37 °C (CK-MB UV test, Merck) (*12*).

Sample Stability

The serum samples collected in the three participating centers (Centre des Brûlés at Lyon and CCU and Department of Surgery, Liège) were stored at −22 °C and transferred within 15 days to the Laboratory of Clinical Chemistry of the University of Liège. Total CK activity and CK-MB mass were measured immediately after thawing. Total CK activity is stable during storage at −22 °C (*13*). We did not find any statistical differences in the CK-MB mass concentrations measured before and after storage at −22 °C (10 replicates) during 30 days for three serum pools with increasing CK-MB content (17, 54, and 152 μg/L). Moreover, seven successive freezings and thawings of these specimens at approximately 12-h intervals only lowered the CK-MB mass concentration by 5%.

Results

Patterns of Total CK and CK-MB Release after Injuries to Muscle and Myocardium

Patients with multiple trauma. In the 10 trauma patients, total CK activities in serum markedly increased during the first day of hospitalization and the peak value (mean 15 588, SEM 7648 U/L) was recorded after 30 h (Table 1).

The mean curve for CK-MB mass concentrations peaked after 18 h (20.6, SEM 7.7 μg/L). CK-MB decreased during the second day after trauma and, in contrast to total CK activities, had nearly become normal after 48 h (Figure 1*A*). The highest values for the nine CK-MB mass concentrations measured in each patient ranged from 5.5 to 67.1 μg/L (mean 21.4, SEM 7.6 μg/L). Regularly decreasing values were obtained for the CK-MB mass/total CK activity ratio during the period of investigation (Figure 1*B*). Maximum ratios averaged 7 (SEM 1) ng/U (range 2–13 ng/U).

Patients with burns. In the serum of the 36 burn patients, mean total CK activities regularly increased during the first two days of hospitalization (Table 1). In contrast, close mean values about 7 μg/L were obtained for CK-MB mass concentrations. The highest of the three individual values obtained for CK-MB ranged from 0.1 to 69.0 μg/L (mean 11.7, SEM 3.0 μg/L). Because of lower total CK activities, the CK-MB mass/total CK activity ratios were, however, greater than in the trauma patients at the corresponding measurement times. Maximum ratios averaged 18 (SEM 6) ng/U. In our series, only the one-month-old child demonstrated CK-MB mass/total CK ratios >40 ng/U: 220 ng/U upon admission (total CK 76 U/L, CK-MB 16.7 μg/L), 90 ng/U (total CK 185 U/L, CK-MB 16.6 μg/L) after 24 h, and 63 ng/U (total CK 125 U/L, CK-MB 7.9 μg/L) after 48 h. In the 35 remaining

Table 1. Total CK Activities and CK-MB Mass Concentrations in Patients with Multiple Trauma, Burns, and AMI (Conventional Treatment), at Admission and 24 and 48 h Later

	Multiple trauma			Burns			AMI		
	Adm	24 h	48 h	Adm	24 h	48 h	Adm	24 h	48 H
Total CK activity, U/L									
X±SEM	349±127	13274±6280	14028±6076	1284±372	1840±684	2711±1502	105±18	1283±272	511±99
Range	8–1090	1240–52500	1400–52000	42–8650	19–19000	15–49200	50–260	343–2990	116–1220
CK-MB concentration, μg/L									
X±SEM	1.7±1.2	17.4±6.3	3.3±1.7	7.7±2.4	6.9±2.1	6.8±2.4	2.6±0.9	164.5±30.3	23.9±6.3
Range	0–9.6	3.6–54.4	0.4–14.1	0–69.0	0–61.7	0–64.3	0–8.7	37.3–314.0	0.4–64.2
CK-MB mass/total CK activity, ng/U									
X±SEM	4±2	3±1	0.2±0.1	17±7	9±3	7±2	25±98	164±29	32±9
Range	0–13	1–4	0.1–0.3	0–220	0–90	0–63	0–72	65–371	11–72

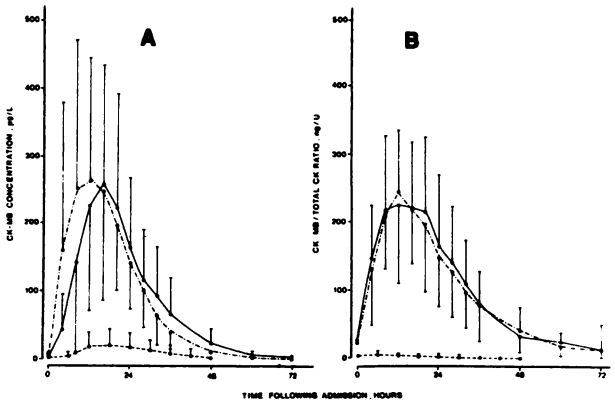

Fig. 1. Comparison of the mean (±SD) serum CK-MB concentrations (*left*) or of the mean (±SD) CK-MB/total CK ratios (*right*) in patients with multiple trauma (——, n = 10) and in patients with AMI, treated with heparin (——, n = 12) or by intracoronary perfusion with streptokinase (—·—, n = 11)

patients, the maximum ratios averaged 11 (SEM 2) ng/U (range 1–38 ng/U).

The patient population was subdivided into three different categories according to the BUS score (*8*) calculated for each patient (Table 2). While constantly decreasing in the 20 subjects with the lowest BUS values (<100%), the CK-MB mass concentrations regularly increased during the first 48 h of hospitalization in the patients with the most severe burns. The most important differences in CK-MB values among the three groups of burn patients were recorded at the end of day 2 ($F = 3.6$, $p < 0.05$). The CK-MB mass/total CK activity ratios were not related to burn severity.

AMI patients. Serum total CK activities and CK-MB mass concentrations peaked earlier ($p < 0.05$) in the streptokinase-treated subjects than in the patients who received intravenous heparin (Table 3). There was, however, no statistical difference between the maximum values recorded in the two groups. The highest of the seven values obtained for CK-MB mass concentrations between 4 and 28 h after hospital admission in the streptokinase- and heparin-treated group ranged from 85 to 693 μg/L and from 115 to 635 μg/L, respectively. The evolution of the CK-MB mass/total CK ratios in the course of AMI was also independent of treatment (Figure 1*B*). During the corresponding 24-h period, the individual maximum ratios averaged 230 ng/U (range 110–460 ng/U) in the patients who received a fibrinolytic therapy and 220 ng/U (range 120–400 ng/U) in the subjects treated with heparin.

Table 2. Serum CK-MB Mass Concentrations ($\bar{X}$ ± SEM) Recorded at Admission and 24 and 48 h Later for the Burn Patients, Classified According to Severity of the Burns

	BUS score, %		
	<100 (n = 20)[a]	100–150 (n = 9)	>150 (n = 6)
CK-MB concentration, μg/L			
Admission	6.0 ± 4.4	4.4 ± 2.9	1.2 ± 0.3
24 h	3.3 ± 1.9	7.0 ± 3.1	17.1 ± 8.6
48 h	2.0 ± 0.7	7.9 ± 4.5	21.4 ± 10.7
CK-MB mass/total CK activity, ng/U			
Admission	11 ± 3	3 ± 1	8 ± 4
24 h	6 ± 2	8 ± 4	3 ± 1
48 h	5 ± 2	5 ± 2	2 ± 1

[a] Results for the one-month-old infant excluded from this group.

Total CK Activity and CK-MB Mass Concentration in Tissue Homogenates

Total CK activity and CK-MB mass concentration were determined in homogenates prepared from myocardium and skeletal muscle (psoas, pectoralis major, diaphragm, femoralis, and intercostal) specimens obtained at autopsy.

In skeletal muscles, the CK-MB mass concentrations ranged from 0.8 (psoas) to 21.4 μg (diaphragm) per gram (wet weight) of tissue (Table 4), and the CK-MB mass/total CK activity ratio from 0.9 (psoas) to 44 ng/U (diaphragm). One gram of myocardium contained, on average, 80.9 μg of CK-MB, and the mean CK-MB/total CK ratio was 202 ng/U.

Discussion

Determination of the CK-MB content of skeletal muscles has led to conflicting results (*14*). Some authors reported CK-MB activities >20% of total CK (*15, 16*); others failed to demonstrate CK-MB in the muscular tissue (*17*). Various drawbacks of the analytical methods used to measure CK-MB activities are probably partly responsible for these discordances. Electrophoresis is known to overestimate CK-MB (*18*); mitochondrial CK, macro-CK, and adenylate kinase (EC 2.7.4.3) interfere with the chromatographic and immunoinhibition assays (*19–21*). Hence, after correcting for residual adenylate kinase the MB activities measured by immunoinhibition in skeletal muscle homogenates, Urdal et al. (*22*) found that the contribution of CK-MB to the total CK activity was only 1.1%.

Quantitative differences in the CK isoenzyme pattern from one muscle to another are also sources of divergence between results. In 1965, Rosalki (*23*) noted that the CK-MB content of the human skeletal muscles could vary in relation to the proportion of fibres of type I and II. Tsung (*24*) observed large differences in the CK-MB proportions of different skeletal muscles. Bentz et al. (*25*) found that the CK-MB activity represented 0.1% of the total CK in rectus abdominus and 4.2% in diaphragm. Recently, Sylvén et al. reported a positive correlation between CK-MB and the percentage of type I fibres in the muscle (*26*).

Our use of the "Tandem-E CKMB" assay allowed us to demonstrate that the differences in the CK-MB content of the human skeletal muscle were greater than usually

Table 3. Characteristic of Total CK and CK-MB (Mean ± SEM) Release in the Streptokinase Treated and Non-Treated AMI Patients

	Heparin treatment (n = 12)				Streptokinase fibrinolysis (n = 11)			
			Time, h				Time, h	
	Maximum values		Peak	Return to normal	Maximum value		Peak	Return to normal
Total CK activity	1486±281	U/L	20.4±2.2	—	1608±429	U/L	15.0±1.9	—
CK-MB concentration	270.4±45.5	μg/L	16.7±2.0	60.0±3.6	298.8±57.8	μg/L	13.0±1.8	55.5±1.9

Table 4. Activity of Total CK and Mass Concentration of CK-MB in Human Myocardium and Skeletal Muscles

	Myocardium	Psoas	Diaphragm	Femoralis	Pectoralis major	Intercostal
Total CK activity, U/g						
	406	602	881	938	1051	883
	350	925	433	851	870	689
	438	1064	370	773	944	784
$\bar{X}$	*398*	*864*	*561*	*854*	*955*	*819*
CK-MB concentration, μg/g						
	93.7	0.3	18.0	3.5	12.5	5.3
	63.0	0.6	34.0	4.9	7.3	0.9
	85.9	1.6	12.3	3.1	9.5	2.1
$\bar{X}$	*80.9*	*0.8*	*21.4*	*3.8*	*9.8*	*2.8*
CK-MB mass/total CK activity, ng/U						
	231	0.5	20	4	12	5
	180	0.6	79	6	8	1
	196	1.5	33	4	10	3
$\bar{X}$	*202*	*0.9*	*44*	*5*	*10*	*3*

reported: the concentration of CK-MB per gram of wet tissue was, for example, 24-fold greater in diaphragm than in psoas. We also showed that the CK-MB content of the myocardium was only four-fold that in diaphragm. However, due to the fact that total CK activities per gram of tissue were higher in skeletal muscles than in myocardium, the differences in the CK-MB mass/total CK activity ratios were more important: for four of the five skeletal muscles we investigated, the CK-MB mass/total CK activity ratio (≤10 ng/U) was more than 20-fold lower than that for myocardium (202 ng/U).

Published results also differ concerning serum CK-MB concentrations after muscle injury. In polytrauma patients without myocardial involvement, CK-MB activities reportedly ranged from 0 to 5.7% (*27*) or were about 1% of total CK (*28*). The CK-MB activities in serum of patients with electrical burns, thermal burns, and blunt trauma averaged 8.6, 4.6, and 5.7% of total CK, respectively, with an overall range of 0.5 to 22% (*4*).

In this work, we studied the dynamics of the CK-MB release in the blood after muscular injury. Immunoenzymometric measurements performed at 6-h intervals after admission in the trauma patients indicated an increase and decrease of CK-MB mass concentrations (Figure 1*A*), with maximum values 18 h after admission. In the patients with the most severe burns, CK-MB peaked later (48 h) but the maximum concentration (mean 21.4 μg/L) was very close to that calculated for the trauma patients (mean 20.6 μg/L). Similarly, there was excellent agreement between the ranges of maximum CK-MB concentrations in the trauma patients (5.5 to 67.1 μg/L) and in the burn patients (0.1 to 69.0 μg/L). There was also little difference between the maximum CK-MB mass/total CK activity ratios recorded for these two groups: 2 to 13 ng/U (mean 7) in the trauma patients and 1 to 38 ng/U (mean 11) in the burn patients, after exclusion of results for the one-month-old infant. The high ratios (63 to 220 ng/U) found in the latter are likely to be related to an increased CK-MB fraction during development of the human muscle (*29*).

The excellent agreement between the range of the CK-MB mass/total CK ratios in muscle tissue (0.9–44 ng/U) and the range of the maximum serum ratios measured after muscle injury (1–38 ng/U) in the overall population of trauma and burn patients must also be emphasized. The same holds true for the CK-MB mass/total CK activity ratios determined in myocardium specimens and in the sera of AMI patients.

Early intracoronary perfusions with streptokinase are now widely used in AMI patients who reach the CCU shortly after the attack, in order to recanalize the obstructed coronary artery and, consequently, to limit the infarcted area (*30*). Successful fibrinolytic therapy is known to modify the kinetics of enzyme release, because the increased washing-out from the ischemic area leads to an earlier appearance of the tissue markers in the patient's plasma (*31, 32*). Although serum total CK activity and CK-MB mass concentrations peaked earlier in the patients treated with streptokinase than in those who received heparin (Table 3), the treatment had no influence on the magnitude of the changes of CK-MB concentrations and on the evolution of the CK-MB mass/total CK activity ratios (Figure 1*B*) in the course of the disease. The maximum serum CK-MB concentrations recorded during the 4th and the 28th hours after admission—that is, given the delays in hospitalization, about 8 to 32 h after the onset of chest pain—ranged from 115 to 635 μg/L in the patients with conventional treatment and 85–693 μg/L in those who received fibrinolytic therapy. During this 24-h period, the patients of these two groups demonstrated at least one CK-MB mass/total CK activity ratio ≥110 ng/U.

The use of two monoclonal antibodies, directed towards the M and B subunits of the CK-MB molecule, makes the Tandem method highly specific. As previously demonstrated (*12*), large excess of CK-MM and of CK-BB did not interfere with the assay, confirming the results obtained by others with a two-site immunoradiometric assay (*33*). The fact that no modification of the CK-MB results were observed after addition of hemolysates up to a final hemoglobin concentration of 1.5 g/L (*12*) also indicated that adenylate kinase does not influence the Tandem assay. Thus, the immunoenzymometric assay does not require dilution of the sample when CK-MM is present in large quantities; moreover, it yields reliable results in the presence of adenylate kinase originating from muscles or erythrocytes. The excellent stability of the CK-MB molecule at −22 °C is an additional advantage of mass measurements over catalytic activity determination.

Conclusion

We previously determined the reference range for CK-MB mass concentrations in presumably healthy subjects to be 0–6 μg/L (*12*). These values were close to those (0–4 μg/L) established in normal individuals by Chan et al. (*7*), who

recommended, however, use of a broader reference interval (0–9 μg/L) for non-infarct patients. Our study demonstrates that in fact CK-MB mass concentrations as great as 69 μg/L may be present in serum of patients with skeletal muscle injury, and therefore that a cutoff value of 9 μg/L would lead, in trauma patients, to numerous false indications of AMI. We propose, therefore, that the assessment of myocardial necrosis be based on the serum CK-MB mass/total CK activity ratio rather than on the absolute concentration. None of the trauma or burn patients older than one year demonstrated a CK-MB mass/total CK activity ratio >40 ng/U at any time during the first 48 h following the accident. Thus we chose the value of 80 ng/U—twofold the maximum ratio recorded in the patients with muscular injury—as cutoff for differentiating skeletal muscle and myocardium necrosis. When several CK-MB mass measurements were performed between 8 and 32 h after the onset of the symptoms, all AMI patients demonstrated at least one value ≥110 ng/U for the CK-MB mass/total CK activity ratio whether or not they had received fibrinolytic therapy. Consequently, use of the cutoff value of 80 ng/U will completely differentiate AMI patients from those with trauma or burns, thus allowing detection of myocardial necrosis even in the presence of pre-existing muscular damage.

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