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Clinical application of subforms of creatine kinase MM and macro creatine kinases

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SUMMARY

The subforms of MM isozyme of creatine kinase (ATP:creatine N-phosphotransferase, EC 2.7.3.2, CK) in sera obtained from healthy adults and patients were determined by agarose gel isoelectric focusing (IEF). The patients were classified into six groups according to serum CK-MM activities and IEF patterns. The IEF spectra offered useful information on cell hyperplasia, augmented cell membrane permeability, cell destruction and release time of CK-MM in the circulation from the cells for diagnosis, progress observation and prognosis, especially in the cases of chronic hepatic diseases, acute myocardial infarction and muscular dystrophy. Macro CKs were also determined by IEF. Macro CKs could be completely distinguished from each other, and CK isozymes consisting of macro CK type 1 could be presumed by isoelectric points.

INTRODUCTION

The MM isozyme of creatine kinase (ATP:creatine N-phosphotransferase, EC 2.7.3.2, CK) exists as a family of multiple forms and can be separated into several subforms by isoelectric focusing (IEF) [1–4] and by high-performance liquid chromatography [5]. Naming of the subforms is varied at the present time [6–9]; therefore, the main subforms are designated here as MM1, MM2

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and MM3 from the anodal side. It has been reported that MM1 and MM2 are human plasma forms and MM3 is a tissue form [10]. When MM3 is released into the circulation from organ tissue, it is converted into MM2 and MM1 by carboxypeptidase-catalysed hydrolysis of C-terminal lysine residues [10]. Many studies on CK-MM subforms related to acute myocardial infarction [1, 11–13], exercise [2, 14] and other conditions [15–17] have been reported. In these clinical conditions, serum CK-MM activities are substantially increased. However, there has been little study on the CK-MM subforms of patients with normal to slight increases of CK-MM activities and macro CKs. In the present work, the CK-MM subforms in human sera of healthy adults and patients under various clinical conditions were determined by agarose gel IEF, and the clinical evaluation was studied. Further, macro CKs were also determined.

EXPERIMENTAL

Chemicals

Chemicals were purchased from the following sources: Monotest CK NAC, Monotest CK-MB, marker proteins for IEF (Boehringer–Mannheim, Mannheim, F.R.G.), DEAE Sephacel, Isogel and ampholytes (Pharmacia–LKB Biotechnology, Bromma, Sweden), anti-human CK-MM rabbit immunoglobulin G (IgG) and anti-human CK-BB rabbit IgG (Calbiochem, La Jolla, CA, U.S.A.) and peroxidase-conjugated rabbit immunoglobulins to goat immunoglobulins (Daco Immunoglobulins, Glostrup, Denmark).

Specimens

Serum samples were obtained from 30 healthy adults and 500 patients listed in Table I. Normal human cardiac muscle, skeletal muscle, brain tissue and liver were obtained at autopsy within 6 h of death. Liver cirrhosis and hepatocellular carcinoma tissues were obtained from 5 and 15 patients, respectively, at autopsy within 6 h of death.

Partial purification of CK-MM, CK-MB and CK-BB

CK-MM, CK-MB and CK-BB were isolated from skeletal muscle, cardiac muscle and brain, respectively [18]. The tissue was homogenised in 50 m*M* Tris–HCl buffer, pH 7.4, and centrifuged at 10 000 *g* for 30 min. The supernatant was applied to a DEAE Sephacel ion-exchange column (30 cm × 2.5 cm) and eluted with a linear gradient of 0–300 m*M* sodium chloride in 50 m*M* Tris–HCl buffer, pH 7.4. Fractions containing each CK isozyme were collected and used in the experiments.

Determination of CK-MM activity

Total serum CK activity and non-CK-M subunit activity were determined with Monotest CK NAC [19] and Monotest CK-MB, respectively, at 30 °C on

a Hitachi 705 autoanalyzer. CK-MM activity was calculated by the expression of [(total CK activity) – (non-CK-M subunit activity) × 2] with the exception of 22 malignant cases. Non-CK-M activity of these 22 cases was proved to be either CK-BB or mitochondrial CK by electrophoresis. Thus, their CK-MM activities were calculated by the expression of[(total CK activity) – (non-CK-M subunit activity)].

Isoelectric focusing (IEF)

IEF was carried out on supporting matrices [20] using an IEF system (Joko, Tokyo, Japan) consisting of 1% Isogel agarose (124 × 258 × 0.5 mm) and 2% ampholytes (1% of pH 5.0–8.0, 0.5% each of pH 4.0–6.5 and 8–9.5). Serum samples (5 μl) were applied on an agarose plate. 0.5 *M* Ethanolamine and 0.2 *M* citric acid were used as catholyte and anolyte, respectively. Focusing was performed at 300 V for 30 min at 4 °C, 600 V for 60 min, 900 V for 45 min and finally at 1000 V for 45 min. The gel was stained with a mixture of one pack of Monotest CK NAC, 10 mg of nitroblue tetrazolium and 0.2 mg of phenazine methosulfate at 37 °C for 30–60 min. The minimum sensitivity of the stain was 10 I.U./l.

Enzyme-labelled antibody staining

CK-MM was histochemically detected by an enzyme-labelled antibody technique with anti-CK-M goat antibodies in Monotest CK-MB. The autopsy tissue obtained was embedded in paraffin, cut into 3-μm thick sections and deparaffinised. Endogenous peroxidase was removed by reaction in 98% methanol containing 3% hydrogen peroxide. After rinsing with distilled water and phosphate-buffered saline (PBS), the tissue was allowed to react with anti-CK-M goat antibodies at 4 °C overnight and with peroxidase-conjugated rabbit immunoglobulins to goat immunoglobulins at 37 °C for 30 min. After each reaction with the antibodies, the tissue was rinsed with PBS and then stained with a mixture of 20 mg of 3,3′-diaminobenzidine tetrahydrochloride and 1% hydrogen peroxide in 100 ml of PBS at 28 °C for 3–5 min. As positive and negative controls, normal human skeletal muscle and normal liver tissue were stained, respectively.

Formation of macro CK type 1

Two types of macro CK type 1 were formed in vitro with anti-CK-MM and anti-CK-BB rabbit antibodies: 0.5 U of CK-MM and CK-BB were incubated with the antibodies, respectively, at 37 °C for 1 h. Formation of the macro CK type 1 was confirmed by electrophoresis on a Cellogel membrane [21] and thin-layer gel filtration [22]. The antibodies did not inhibit CK activities; therefore, the CK activity was stained after IEF.

RESULTS

CK-MM activities and IEF patterns of the sera obtained from healthy adults and patients are listed in Table I, and typical patterns are exhibited in Fig. 1. In the healthy adults, CK-MM activities were distributed less than 113 I.U./l. The enzyme was focused into three main bands (MM1, MM2 and MM3) with p*I* of 6.2, 6.7 and 6.9, respectively. Relative enzyme activities of the three main bands were very similar or MM1-dominant (track 1).

TABLE I

CK-MM ACTIVITIES AND ISOELECTRIC FOCUSING PATTERNS IN THE SERA OF THE HEALTHY ADULTS AND THE PATIENTS

Clinical cases	Number of patients	CK-MM activity[a] (I.U./l)	Isoelectric focusing pattern (%)			
			MM3 > MM2 > MM1[b]	MM3 < MM2 > MM1[c]	MM3 < MM2 < MM1[d]	Not detectable[e]
Healthy adults	30	57 ± 28	0	50	50	0
Acute hepatitis	15	62 ± 110	14	0	53	33
Chronic hepatitis	30	79 ± 56	17	40	43	0
Liver cirrhosis	30	70 ± 43	53	30	17	0
Hepatocellular carcinoma	42	44 ± 28	46	21	14	19
Gastric carcinoma	38	95 ± 137	35	21	26	18
Colon carcinoma	31	37 ± 42	13	26	29	32
Pulmonary carcinoma	37	89 ± 263	22	24	19	35
Brain carcinoma	8	40 ± 43	0	13	74	13
Acute myocardial infarction[f]	66	2235 ± 3341	79	18	3	0
Angina pectoris	39	71 ± 51	18	27	55	0
Open-heart surgery	38	1205 ± 1485	92	8	0	0
PTCA[g]	31	113 ± 74	32	55	13	0
Muscular dystrophy	14	3568 ± 4158	29	21	50	0
Polymyositis	6	251 ± 191	17	33	33	17
Myasthenia gravis	7	36 ± 18	14	29	43	14
Cerebral haemorrhage	26	25 ± 36	8	12	19	61
Cerebral embolism	25	27 ± 25	16	8	28	48
Hypothyroidism	17	121 ± 201	24	12	46	18

[a]Mean ± 1 S.D.
[b]MM3-dominant pattern.
[c]MM2-dominant pattern including the cases with very similar activities in three bands
[d]MM1-dominant pattern.
[e]Less than 10 I.U./l CK-MM.
[f]Isoelectric focusing patterns at admission.
[g]Percutaneous transluminal coronary angioplasty.

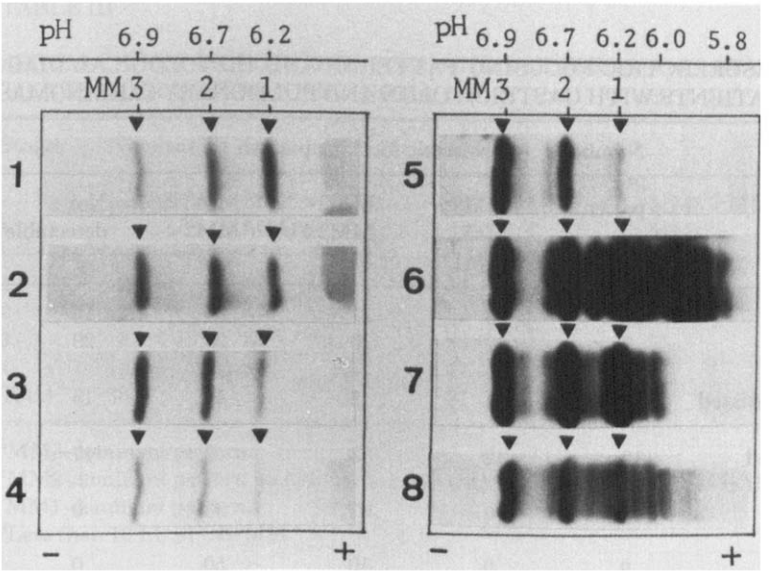

Fig. 1. Serum CK-MM IEF patterns of a healthy adult and patients. 1, Healthy adult; 2, chronic hepatitis; 3, liver cirrhosis; 4, hepatocellular carcinoma; 5, acute myocardial infarction, 2 h after onset of chest pain; 6, acute myocardial infarction, 24 h after onset of chest pain; 7, muscular dystrophy; 8, open heart surgery.

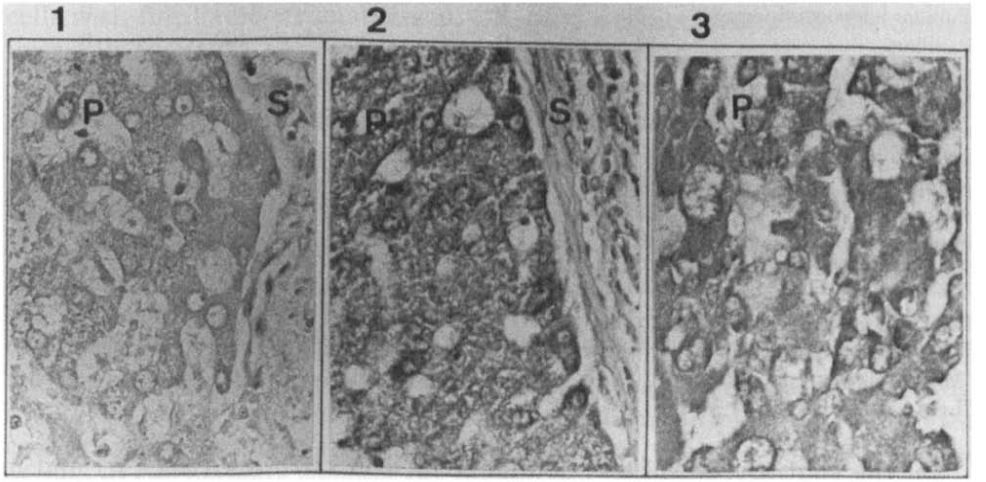

Fig. 2 CK-MM activities in liver tissue by enzyme-labelled antibody staining. 1, Normal; 2, liver cirrhosis; 3, hepatocellular carcinoma; P, parenchyma; S, stroma.

TABLE II

SERUM CK-MM ISOELECTRIC FOCUSING PATTERNS AND HISTOLOGICAL DIAGNOSES OF THE PATIENTS WITH GASTRIC, COLON AND PULMONARY CARCINOMAS

Primary diagnoses	Number of patients	Isoelectric focusing pattern (%)			
		MM3> MM2> MM1[a]	MM3< MM2> MM1[b]	MM3< MM2< MM1[c]	Not detectable[d]
Gastric	38	35	21	26	18
Well-differentiated adenocarcinoma	7	42	0	29	29
Moderately differentiated adenocarcinoma	13	31	8	46	15
Poorly differentiated adenocarcinoma	12	42	33	8	17
Papillary adenocarcinoma	3	0	67	33	0
Mucinous adenocarcinoma	2	0	50	50	0
Tubular adenocarcinoma	1	0	0	0	100
Colon	31	13	26	29	32
Well-differentiated adenocarcinoma	13	23	31	8	38
Moderately differentiated adenocarcinoma	10	0	30	40	30
Poorly differentiated adenocarcinoma	2	50	0	0	50
Tubular adenocarcinoma	3	0	33	67	0
Papillary adenocarcinoma	2	0	0	100	0
Mucinous adenocarcinoma	1	0	0	0	100
Pulmonary	37	22	32	11	35
Adenocarcinoma	22	32	32	4	32
Squamous cell carcinoma	8	0	38	24	38
Small cell carcinoma	7	14	29	14	43

[a]MM3-dominant pattern.
[b]MM2-dominant pattern including the cases with very similar activities in three bands
[c]MM1-dominant pattern.
[d]Less than 10 I.U./l CK-MM.

On the contrary, MM3 was dominant in liver cirrhosis (track 3) and in hepatocellular carcinoma (track 4), although serum CK-MM activities were within the normal range (Table I). There was a significant difference in the

TABLE III

SERUM CK-MM ISOELECTRIC FOCUSING PATTERNS AND STAGES OF THE PATIENTS WITH GASTRIC CARCINOMA

Stages	Number of patients	Isoelectric focusing pattern (%)			
		MM3 > MM2 > MM1[a]	MM3 < MM2 > MM1[b]	MM3 < MM2 < MM1[c]	Not detectable[d]
1	9	44	12	44	0
2	9	44	22	12	22
3	8	25	38	25	12
4	12	25	17	25	33
Total	38	35	21	26	18

[a]MM3-dominant pattern.
[b]MM2-dominant pattern including the cases with very similar activities in three bands.
[c]MM1-dominant pattern
[d]Less than 10 I.U./l CK-MM.

frequency of the MM3-dominant patterns between liver cirrhosis and chronic hepatitis (track 2, $p < 0.005$, χ^2 test) and between hepatocellular carcinoma and chronic hepatitis ($p < 0.005$, χ^2 test). To confirm the presence of CK-MM in the cirrhotic liver and hepatocellular carcinoma cells, CK-MM was histochemically detected by an enzyme-labelled antibody staining technique (Fig. 2). In liver cirrhosis (panel 2), CK-MM was clearly stained not only in the nodular parenchymal cells, but also in the diffuse fibrillated stromal tissue. In hepatocellular carcinoma (panel 3), CK-MM was stained in the carcinoma cells with fibrillated stromal tissue. CK-MM was not stained in the normal liver cells (panel 1).

In gastric carcinoma, MM3–dominant patterns were observed in 35% of the sera of the patients. Details of the IEF patterns and histological diagnoses of gastric, colon and pulmonary carcinomas are listed in Table II. Table III shows the relationship between the IEF patterns and stages of gastric carcinoma. There was no special finding in any of the cases.

In acute myocardial infarction, transition of the IEF patterns from onset of chest pain in the sera of patients is shown in Fig. 3A, and one example of the IEF patterns is shown in Fig. 4a. At the time of admission to the hospital, the CK-MM patterns were MM3-dominant in 79% of the sera, including that of one patient who was admitted to the hospital 26 h after onset of the pain (arrow). The frequency of the MM3-dominant patterns differed from that in the case of angina pectoris (Table I, $p < 0.005$, χ^2 test). Serum CK-MM activities of 79% of the patients covered the interval 42–12249 I.U./l and were higher than those of the residual 21% of the patients (55–301 I.U./l) whose IEF patterns were MM2- or MM1-dominant (Table IV, $p < 0.005$, Wilcoxon rank-sum

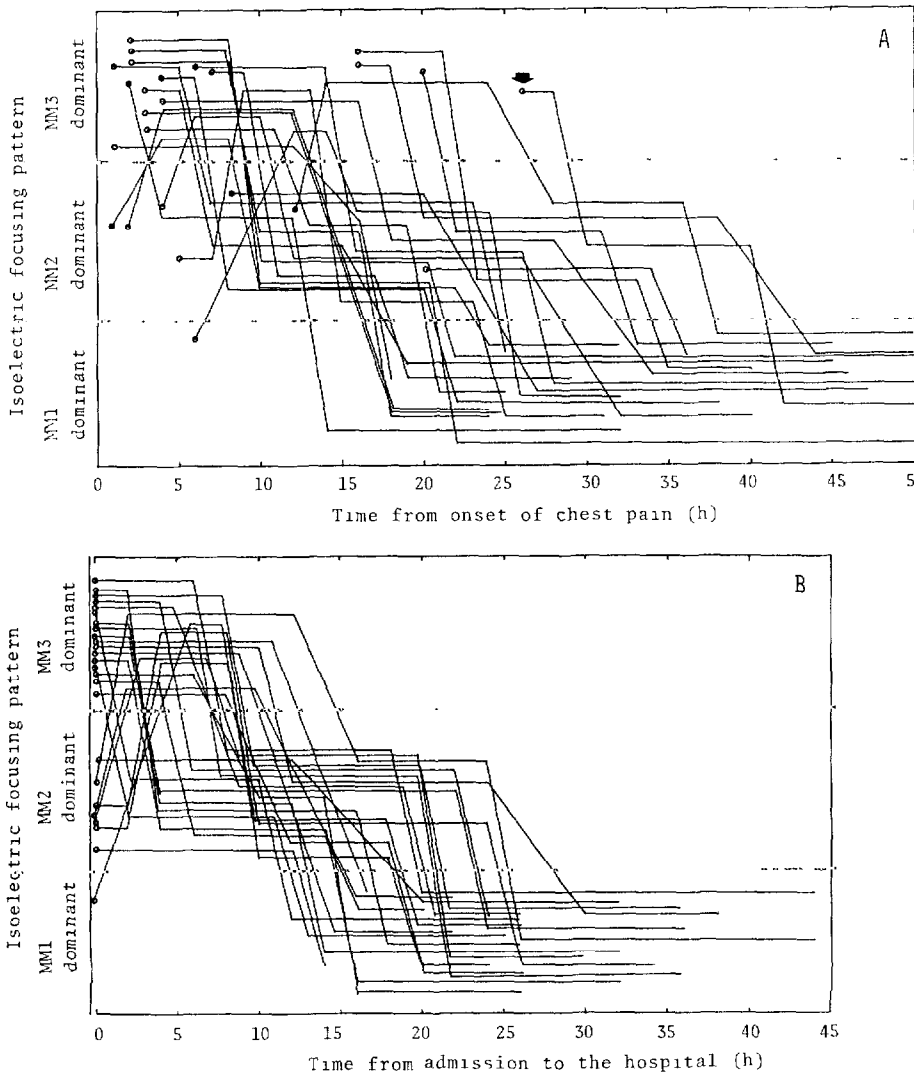

Fig. 3. Transition of CK-MM IEF patterns in sera of patients with acute myocardial infarction (A) Transition from onset of chest pain; (B) transition from admission to the hospital.

test). The MM3-dominant patterns converted into MM2-dominant patterns 7–30 h after onset of chest pain and finally to MM1-dominant patterns 14–44 h after pain onset. When CK-MM activity was the highest, the patterns were MM3- or MM2-dominant, but no case of an MM1-dominant pattern was found.

In Fig. 3B, the transition of MM3 to MM1 is expressed on a different time scale, i.e. taking as zero time the admission to the hospital rather than the onset of chest pain. The times at which the MM3-dominant pattern converted

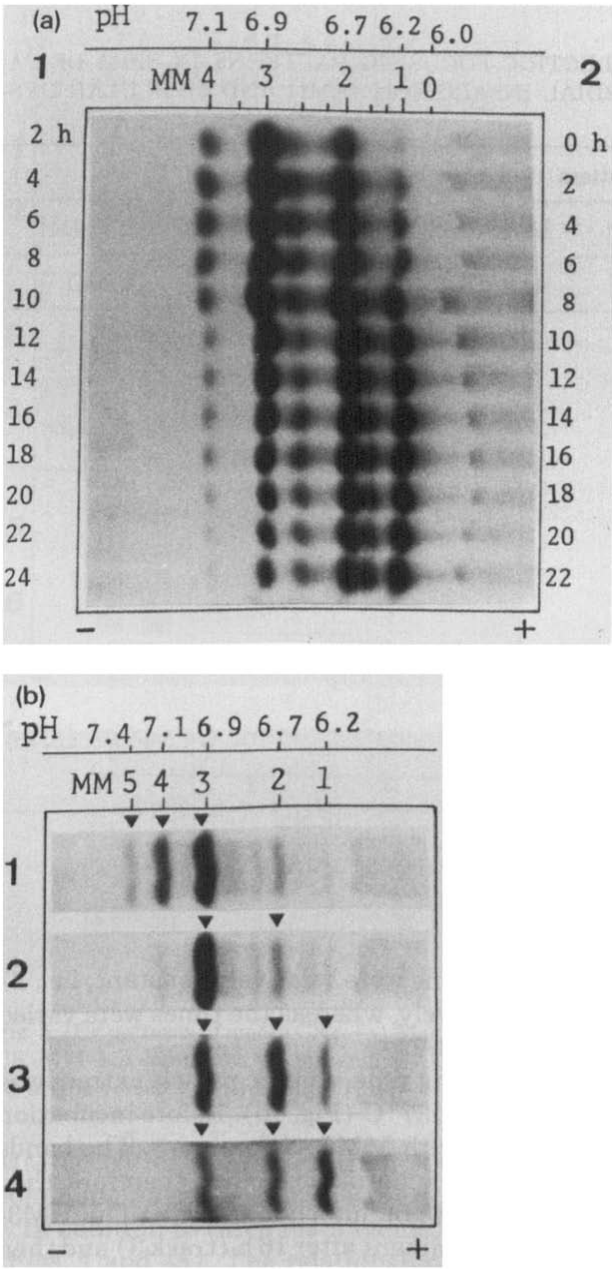

Fig. 4. (a) Transition of the CK-MM patterns in sera of a patient with acute myocardial infarction. 1, Time from onset of chest pain; 2, time from admission to the hospital (b) Conversion of CK-MM IEF patterns of cardiac muscle extract in serum of a healthy adult at 37°C in vitro. 1, Before incubation; 2, 1 h after; 3, 16 h after; 4, 24 h after.

TABLE IV

CK-MM ACTIVITIES AND ISOELECTRIC FOCUSING PATTERNS IN SERA OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION (AMI) AND MUSCULAR DYSTROPHY (MD)

CK-MM activity (I.U./l)	Isoelectric focusing pattern					
	MM3 > MM2 > MM1[a]		MM3 < MM2 > MM1[b]		MM3 < MM2 < MM1[c]	
	AMI	MD	AMI	MD	AMI	MD
0–99	2		4			
100–199	4		3		1	
200–299	6		3			
300–399	1		2		1	
400–499	1					1
500–599	2					2
600–699	3					
700–799	2					1
800–899	2					
900–999	2					
1000–1999	15			2		3
2000–2999	8			1		
3000–3999	1					
4000–4999	1	1				
>5000	2	3				
Total	52	4	12	3	2	7

[a]MM3-dominant pattern.
[b]MM2-dominant pattern including the cases with very similar activities in three bands.
[c]MM1-dominant pattern.

into the MM2- and MM1-dominant patterns were relatively constant, i.e., 2–16 h and 10–30 h after admission, respectively, whereas the times were varied from onset of chest pain, as shown in Fig. 3A.

In order to confirm the pattern-converting time, cardiac muscle extract was incubated in the serum of a healthy adult at 37 °C (Fig. 4b). Before incubation (track 1), several bands were confirmed with MM3 as dominant. The bands were completely inactivated by anti-CK-M subunit antibodies; therefore, they were recognized as subforms of CK-MM, but not mitochondrial CK. The MM3-dominant pattern converted into MM2-dominant after 16 h (track 3) and then to MM1-dominant after 24 h (track 4). The time required for the pattern conversion coincided with that in vivo. Similar results were obtained from CK-MM of the skeletal muscle extract.

In muscular dystrophy, the patterns were MM3-dominant when serum CK-

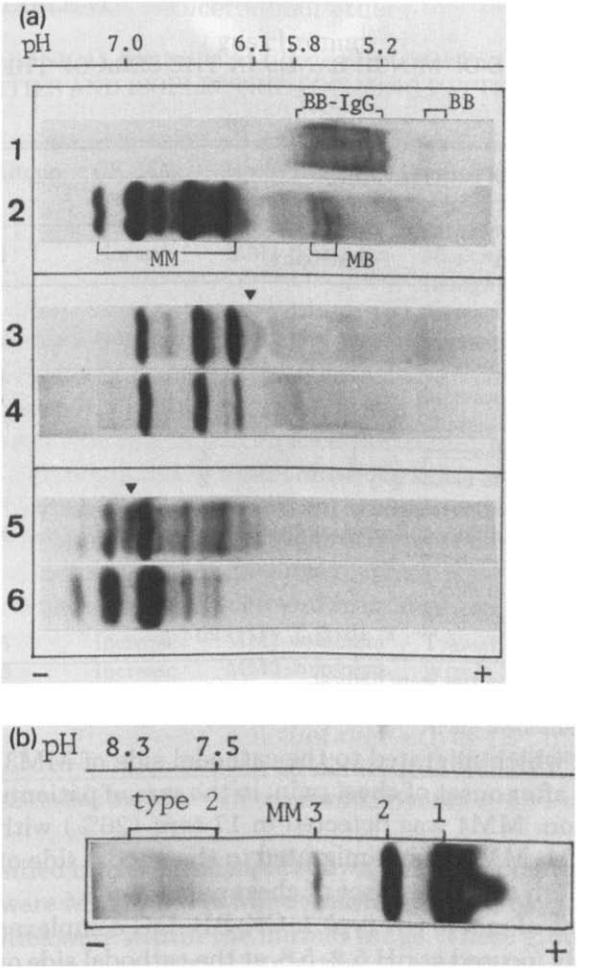

Fig. 5. IEF patterns of macro CKs. (a) Macro CK type 1; 1, CK-BB-IgG complexes; 2, marker; 3, CK-MM-IgA complexes; 4, marker; 5, CK-MM-IgG complexes, CK-MM used was skeletal muscle origin; 6, marker. (b) Macro CK type 2.

MM activities were more than 4000 I.U./l, and they were MM2- or MM1-dominant when the activities were less than 4000 I.U./l (Table IV).

In addition to three main bands, some minor bands appeared at pH 5.8–7.1 (Figs. 1 and 4a). The relationship between serum CK-MM activity and the appearance of the minor bands is shown in Table V. The minor bands began to appear in the sera when the serum CK-MM activity increased more than 100 I.U./l and were detected in all sera when the activities were more than 400 I.U./l. Appearance of the minor bands was not associated with the underlying

TABLE V

CK-MM ACTIVITIES AND APPEARANCE OF MINOR BANDS IN THE SERA OF THE PATIENTS

CK-MM activity (%)	Positive/total (%)	Number of patients		
		Acute myocardial infarction[a]	Muscular dystrophy	Others
0–99	0/353 (0)			
100–199	4/56 (7)			4
200–299	4/11 (36)			4
300–399	5/9 (56)	3		2
400–499	3/3 (100)		1	2
500–599	7/7 (100)	4	1	2
600–699	3/3 (100)	1		2
700–799	7/7 (100)	5		2
800–899	2/2 (100)	2		
900–999	2/2 (100)	2		
> 1000	47/47 (100)	37	8	2
Total	84/500 (17)	54	10	20

[a]Results at the time when serum CK-MM activities were highest.

diseases. Appearance of MM4, which migrated to the cathodal side of MM3, was the fastest, i.e., within 28 h after onset of chest pain, in the sera of patients with acute myocardial infarction. MM4 was detected in 17 sera (26%) with more than 900 I.U./l of CK-MM. MM0 which migrated to the anodal side of MM1 was detected more than 72 h after the onset of chest pain.

Fig. 5a shows the IEF patterns of macro CK type 1. CK-BB–IgG complexes of macro CK type 1 were broadly focused at pH 5.2–5.8, at the cathodal side of CK-BB. CK-MM–IgA in one patient's serum and CK-MM–IgG were detected as extra bands at pH 6.1 and 7.0, respectively.

Macro CK type 2, an oligomeric form of mitochondrial CK which is electrophoresed at the cathodal side of CK-MM, was detected as multiple bands with weak enzyme activity at pH 7.5–8.3 (Fig. 5b). The bands were not inactivated by anti-CK-M subunit antibodies and were not stained by a mixture eliminating creatine phosphate. Thus, they were mitochrondrial CK, but not CK-MM or non-specific reactions. Macro CK type 2 was distinguishable from the subforms of CK-MM and macro CK type 1.

DISCUSSION

Serum enzyme activities and IEF patterns of CK-MM under various clinical conditions were studied. From the results obtained, the patients could be clas-

TABLE VI

CLINICAL SIGNIFICANCE OF SIX GROUPS CLASSIFIED BY SERUM CK-MM ACTIVITIES AND ISOELECTRIC FOCUSING PATTERNS

Group	CK-MM activity	Isoelectric focusing pattern	Clinical conditions
1	Normal	MM1-dominant	Healthy adults Benign and malignant tumors without CK-MM increase
2	Normal	MM2-dominant[a]	Healthy adults Benign and malignant tumors without CK-MM increase
3	Normal	MM3-dominant	Diseases with cell hyperplasia and exaggeration of cell permeability Liver cirrhosis Hepatocellular carcinoma
4	Increase	MM1-dominant	More than 10–30 h after transient release of CK-MM Moderate amount of continuous release of CK-MM Acute myocardial infarction Muscular dystrophy
5	Increase	MM2-dominant[a]	Transition stage from group 6 to group 4
6	Increase	MM3-dominant	Within 2–16 h after transient release of CK-MM Large amount of continuous release of CK-MM Acute myocadial infarction Muscular dystrophy

[a]Including the cases with very similar activities in three bands.

sified into six groups, as shown in Table VI. In groups 1 and 2, the IEF patterns were MM1- and MM2-dominant, respectively, whereas serum CK-MM activities were within the normal range. These groups comprised of healthy adults and patients with various benign and malignant diseases.

Group 3 had normal serum CK-MM activity and MM3-dominant patterns which might indicate cell hyperplasia and augmented cell membrane permeability. The patterns were observed in liver cirrhosis and hepatocellular carcinoma. This was not an expected result because presence of CK has not been known in liver cells [23]. The findings obtained by an enzyme-labelled antibody technique indicated that as chronic hepatitis advances, CK-MM increased in the nodular parenchymal, carcinoma and stromal cells with active cell hyperplasia. As there was no relationship between the enzyme activity in the cells and in the sera, this clinical information on the liver diseases was only obtained by the serum CK-MM IEF patterns, but not by activities or zymograms of serum CK-MM. There was no relationship between histological diagnosis, stages or metastases of the tumors and the CK-MM IEF patterns, (Tables II and III). Thus, normal serum CK-MM activities and MM3-domi-

nant patterns will indicate cell hyperplasia and augmented cell membrane permeability, but not whether the cells are benign or malignant, histological cell types or tumor extensions.

On the contrary, groups 4–6 suggested CK-MM release from tissue cells to the circulation by an increase of serum CK-MM activity. In acute myocardial infarction, MM3-dominant patterns were obtained in most of the sera collected on admission to the hospital. The patterns continued at least 26 h after onset of chest pain unless the coronary artery reopened. Thus, when an MM3-dominant pattern is obtained on admission, it can be presumed that the onset of chest pain was within 26 h.

Once the coronary artery is reopened by percutaneous transluminal coronary angioplasty (PTCA; all the patients were treated by PTCA), any enzyme remaining at an infarcted area is hastily washed out into the circulation and may be catalysed by carboxypeptidase. Serum CK-MM activities and IEF patterns depend upon times and rates of enzyme release, distribution and catabolism, so no simple explanation of the results can be made; however, the IEF patterns of CK-MM were affected both by infarction and reopening of the coronary artery. A period of 2–16 h was required for the conversion from MM3-dominant to MM2-dominant patterns. The effects of PTCA itself on the patterns were not considerable because serum CK-MM activity was not significantly increased in the sera of patients treated by PTCA (Table I). Thus, the MM3-dominant pattern suggests that the patients were still within the 2–16 h period after the coronary artery reopening.

In muscular dystrophy with continuous CK-MM being released in large amounts into the circulation, the MM3-dominant pattern was observed, unless serum CK-MM activity increased more than 4000 I.U./l. This result was in contrast to that of acute myocardial infarction, where MM3-dominant patterns were observed even when serum CK-MM activities were within the normal range (Table IV). The finding suggests that serum carboxypeptidase activity increases with ordinal serum CK-MM activity.

Based on the above findings and the results obtained from sera of patients with muscular dystrophy, in group 6 showing MM3-dominant patterns, the sera were tested within 2–16 h of transient CK-MM release or when the patients were experiencing a continuous release of CK-MM in large amounts. Similarly, in group 4 showing MM1-dominant patterns, the sera were analysed 10–30 h after transient CK-MM release or the patients were experiencing a continuous release of CK-MM in moderate amounts. Group 5 sera indicate a transition stage from group 6 to group 4.

In serum with increasing CK-MM activity, several minor bands appeared in addition to main bands. The most intensive minor band converted from the cathodal side into the anodal side in a manner similar to the conversion of the main bands (Fig. 4a). From the IEF patterns of cardiac and skeletal muscle, it was found that a considerable amount of MM4 and some minor bands were

present at pH 6.2–7.4 in the tissue (Fig. 4b, track 1). Therefore, it can be presumed that appearance of the minor bands in serum is an indicator of CK-MM release in large amounts (Table V), and the cathodic minor bands are an early indicator of tissue damage. Some minor bands were reported to be associated with acute myocardial infarction [24]; however, none of the minor bands were recognized as being associated with certain disease groups in the present study.

Recently, Williams et al. [24] reported that in vitro incubation of serum with 0.015 *M* 2-mercaptoethanol induced conversion of MM1, -2, and -3 to b and c, d and e, and f and g, respectively (they designated main bands as MM1, -2 and -3 from the cathodal side and minor bands as b to g). We also confirmed the similar conversion of the main bands to the cathodic minor bands after incubation of serum with 2-mercaptoethanol in vitro, and the conversion seemed like a reverse phenomenon of the conversion shown in Fig. 4a and b. This finding suggests that a redox reaction of CK-MM molecules participates in the synthesis and disassimilation of the minor bands. These reactions shifted toward oxidation in the circulation (Fig. 4a and b), and reduction occurred with 2-mercaptoethanol added in vitro [24]. The reduction was designated as a second conversion and interpreted to be a reflection of a chemical imbalance requiring compensation for good prognosis in acute myocardial infarction [24]. However, the second conversion is not confirmed in the circulation. Releasing of lysine residues by carboxypeptidase and oxidation of CK-MM molecules should be for quick disassimilation and elimination of large amounts of CK-MM from the circulation.

One of the purposes of this study was to determine whether individual macro CKs could be distinguished by IEF. CK-BB–IgG complexes of macro CK type 1 were broadly focused at pH 5.2–5.8, CK-MM–IgA complexes at pH 6.1 and CK-MM–IgG at pH 7.0. On the other hand, macro CK type 2, an oligomeric mitochondrial CK, was focused at pH 7.5–8.3. From these results we can say that when additional CK bands are detected by electrophoresis, those bands can be characterised by IEF as macro CK either of type 1 or of type 2. Further, if they are type 1, it can be presumed that the isozyme consisting of the macro CK is CK-BB or CK-MM. MM4 has often been mistaken for macro CK type 2 because the subform was focused at the cathodal side of MM3 and appeared only in sera with an increased CK-MM as described above. However, MM4 was a minor band of CK-MM because the p*I* was pH 7.1, which differed from that of macro CK type 2. Further, MM4 was confirmed by inhibition with anti-CK-M subunit antibodies after an IEF run.

CK-MM and macro CKs in conjunction with possible clinical evaluation were studied by IEF. This technique has considerable diagnostic value, especially in the cases of chronic hepatic diseases, myocardial infarction and muscular dystrophy even without increase of serum CK-MM activity.

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