CREATINE KINASE BB ISOZYME IN THE BLOOD DURING OPEN HEART SURGERY

—Comparison with Creatine Kinase MB and MM Isozymes—

AKIHIKO USUI, M.D., KANEFUSA KATO, M.D. Ph.D*, TOSHIO ABE, M.D.
MITUYA MURASE, M.D., MINORU TANAKA, M.D.
AND EIJI TAKEUCHI, M.D.

By using a recently developed highly sensitive enzyme immunoassay method, concentrations of the 3 forms of cytoplasmic creatine kinases (CK-BB, CK-MB and CK-MM) were determined in blood samples serially taken from 18 patients who received mitral valve replacement. Blood CK-BB levels, 0.64 ± 0.32 ng/ml at the beginning of anesthesia, rose sharply after reperfusion reaching the peak level (23.3 ± 7.56 ng/ml) 2 hours after reperfusion, and then fell rapidly. The response of CK-BB in blood was rapider and more sensitive than that of CK-MB or CK-MM. The CK-BB concentrations were significantly higher in coronary sinus samples than in arterial samples. These results suggest that the major portion of elevated blood CK-BB level in the early phase after reperfusion are derived from the heart muscle.

CYTOPLASMIC creatine kinase (CK) is a mixture of similar proteins composed of 2 immunologically distinct subunits, B and M, and they exist in BB, MB and MM forms^{1,2}. It has been reported that CK-MB and CK-MM are localized predominantly in the heart and skeletal muscles, respectively^{3,4}. On the other hand, CK-BB is localized mainly in the central nervous tissues^{3,4}. Recently, we developed a highly sensitive enzyme immunoassay system for the assay of human CK-BB, CK-MB and CK-MM^{5,6}. The minimum detection limit is 1 pg/assay respectively, indicating that the present method is about 100-fold more sensitive than previously reported radioimmunosassay methods. Since it is

sufficiently sensitive for measurement of CK-BB, CK-MB and CK-MM in the human blood, the change of blood concentrations of each CK isozyme can be determined precisely. This method is useful especially for measurement of blood CK-BB levels because the blood CK-BB concentrations in healthy adults are very low (0.32 ± 0.14 ng/ml, $n = 60$)⁷.

Using this sensitive enzyme immunoassay system, we measured blood CK-BB concentrations during open heart surgery, comparing them with those of CK-MB and CK-MM, and estimated which organ is the origin of elevated blood CK-BB levels.

Patients and methods

Eighteen patients who underwent mitral valve replacement only are included in this study. Since each patient has neither coronary artery

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Department of Thoracic Surgery, Nagoya University School of Medicine, *Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Japan

Mailing address: Akihiko Usui, M.D., Department of Thoracic Surgery, Nagoya University School of Medicine, 65 Tsurumai-cho, Shouwa-ku, Nagoya 466, Japan

Fig.1. Perioperative blood concentrations of CK-BB, CK-MB and CK-MM in sequential samples in open heart surgery cases. The three forms of CK were determined by means of the enzyme immunoassay system as described in the text.

disease nor aortic valvular disease, the effect of cardioplegia is considered to be uniform. The operation was performed through a median sternotomy on a hypothermic cardiopulmonary bypass with cold oxygenated crystalloid cardioplegia and topical cooling. A Bjork-Shiley valve was used for the valve replacement. The ischemic time, the cardiopulmonary bypass time and the minimum nasopharyngeal temperature were 66.9 ± 12.5 min, 131 ± 19 min and $25.6 \pm 0.8^\circ\text{C}$ respectively. Arterial blood samples were serially taken through the peripheral arterial line 17 times during perioperative periods: at the beginning of anesthesia; at the time of clamping and unclamping of the aorta; at the 15th, 30th, 45th and 60th min; at the 2nd, 3rd, 4th, 6th, 8th, 10th, 12th and 18th hour mark after reperfusion; and the beginning of the 2nd and the 7th post operative day. Another series of samples was obtained simultaneously 9 times from the coronary sinus line placed through the right atrium under operation. They were taken at the time of the unclamping of the aorta, at the 15th, 30th, 45th and 60th min mark, and at the 2nd, 4th, 8th and 12th hour mark after reperfusion. Blood concentrations of CK-BB, CK-MB and CK-MM in the arterial samples were compared with those in coronary sinus samples and their respective differences were calculated.

Urine samples were taken eleven times serially during the perioperative period. They were taken at the time of clamping and unclamping of the aorta, and at the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th, 12th and 18th hour mark after reperfusion. Urinary secretions of CK-BB, CK-MB and CK-MM were estimated from the concentrations and the volumes of urine.

Seven mediastinum tumor patients who were operated through a median sternotomy were included as the control cases. Blood samples were taken from peripheral arterial lines four times during the perioperative period.

Within 30 min of collection, plasma was separated from the blood samples by centrifugation at 3000 rpm for 10 min and kept frozen at -80°C until analysis. Urine samples were kept at 4°C and were analysed within 1 week of collection.

Concentrations of the 3 CK isozymes were determined by the sandwich-type enzyme immunoassay system^{5,6}. The CK-BB and CK-MM assay were specific to the respective subunits (CK-B and CK-M), showing an approximately 15% cross-reactivity with the hybrid CK-MB form, but the CK-MB assay was specific without any cross-reactivity with CK-BB and CK-MM. CK-BB and CK-MM values were calculated by subtracting the cross-reacted values of CK-MB⁷.

Fig.2. Changes in relative concentrations of CK-BB and CK-MB in the total CK-isozymes during open heart surgery. The relative concentrations of CK-BB ($BB/BB + MB + MM \times 100$) or CK-MB ($MB/BB + MB + MM \times 100$) were estimated from the data shown in Fig. 1.

TABLE I DIFFERENCES OF BLOOD CONCENTRATIONS OF CK-BB, CK-MB AND CK-MM BETWEEN CORONARY SINUS AND ARTERIAL SAMPLES

Point of sampling (after reperfusion)	Differences of blood concentrations (ng/ml)		
	CK-BB	CK-MB	CK-MM
0 min	$4.76 \pm 3.75^*$	$15.4 \pm 18.1^*$	42.5 ± 78.6
15 min	$4.38 \pm 1.61^*$	$17.9 \pm 15.3^*$	$52.7 \pm 50.6^*$
30 min	$4.50 \pm 2.82^*$	4.71 ± 15.8	$92.6 \pm 107^*$
45 min	$7.78 \pm 4.30^*$	13.0 ± 24.8	$88.5 \pm 69.7^*$
60 min	2.00 ± 0.56	-4.34 ± 12.5	$70.8 \pm 70.3^*$
2 h	-1.44 ± 1.69	-1.83 ± 67.5	$150 \pm 127^*$
4 h	-0.64 ± 6.18	5.50 ± 10.6	130 ± 244
8 h	-0.18 ± 5.33	-6.00 ± 19.8	-40 ± 174
12 h	2.35 ± 0.59	-3.45 ± 7.71	-35 ± 192

*: $p < 0.05$

Statistical differences between the data obtained were evaluated by Student's or paired t-test.

RESULTS

Changes of blood concentrations of CK-BB, CK-MB and CK-MM during the perioperative period in the open heart surgery

Figure 1 shows blood concentrations of 3 CK isozymes in the sequential samples during

perioperative periods of open heart surgery. Blood CK-BB levels, 0.64 ± 0.32 ng/ml at the beginning of anesthesia, rose gradually during cardiopulmonary bypass and more sharply after reperfusion, reaching the peak level (23.3 ± 7.56 ng/ml) 2 hours after reperfusion. Then the levels fell quickly to 5.64 ± 2.54 ng/ml 12 hours after reperfusion and recovered to 0.82 ± 0.96 ng/ml, which were similar to those at the beginning of anesthesia, on the 2nd post operative day. Blood

Fig.3. Urinary secretion of CK-BB, CK-MB and CK-MM during open heart surgery.

TABLE II CK-BB CONCENTRATIONS OF BLOOD SAMPLES IN OPEN HEART SURGERY AND IN CONTROL SURGERY

Point of sampling	Blood CK-BB concentrations	
	OHS (n = 18)	control (n = 7)
Preoperation	0.64 ± 0.32	0.21 ± 0.05
During operation	21.8 ± 11.7	$0.25 \pm 0.08^{**}$
Postoperation	9.61 ± 5.04	$0.32 \pm 0.12^{**}$
The 1st day	4.02 ± 2.55	$0.28 \pm 0.08^{**}$

OHS: open heart surgery

** : $p < 0.01$, OHS vs control

CK-MB levels, 1.42 ± 0.86 ng/ml at the beginning of anesthesia, rose slowly during cardiopulmonary bypass and reached the peak level (149 ± 32.6 ng/ml) 3 hours after reperfusion. Then they fell gradually to 88.0 ± 3.54 ng/ml 12 hours after reperfusion and remained at higher levels (11.8 ± 6.37 ng/ml) than those at the beginning of anesthesia on the 2nd post operative day. Blood CK-MM levels, 42.6 ± 14.8 ng/ml at the beginning of anesthesia, rose slowly after reperfusion and reached the peak level (652 ± 449 ng/ml) 8 hours after reperfusion and then they remained at high levels (380 ± 116 ng/ml) on the 2nd post operative day.

These results indicate that blood CK-BB levels rise most rapidly, reach the peak level earliest and

fall most quickly and that, conversely, blood CK-MM levels rise most slowly and remain at abnormally high levels for the longest period.

Figure 2 shows the changes of both CK-BB and CK-MB percentage of total CK amount. The percentage of CK-BB, $1.4 \pm 0.7\%$ at the beginning of anesthesia, increased gradually, reaching the peak ($5.6 \pm 2.7\%$) 45 min after reperfusion and decreased gradually showing less than 1% after the 1st post operative day. On the other hand, the percentage of CK-MB, $3.2 \pm 1.8\%$ at the beginning of anesthesia, increased gradually after reperfusion, kept more than 20% from 1 to 6 hours after reperfusion and decreased thereafter.

Differences of blood CK-BB, CK-MB and CK-MM levels between coronary sinus and arterial samples

As shown in Table I, blood CK-BB levels in coronary sinus samples were higher than those in arterial samples and showed significant differences between levels at the start of reperfusion to 45 min after reperfusion ($p < 0.05$). Blood CK-MB levels of coronary sinus samples were also significantly higher at the start and 15 min after reperfusion. Blood CK-MM levels also showed significant differences between coronary sinus and arterial samples at the 15th, 30th, 45th and 60th min, and the 2nd hour after reperfusion. But in the other points there were no statistically significant differences between arterial and coronary sinus samples. These results may support the argument that the increased blood

CK isozymes are derived from the damaged heart muscles.

Urinary secretions of CK-BB, CK-MB and CK-MM

Figure 3 shows urinary secretion of CK-BB, CK-MB and CK-MM during open heart surgery. The urinary secretion of CK-BB was only 4.6 ± 5.0 ng/hour before reperfusion and reached 78 ± 64 ng/hour 2 hours after reperfusion and had a rate of about 18-times higher than that before reperfusion. The urinary secretion of CK-MB was 27 ± 21 ng/hour before reperfusion and 304 ± 146 ng/hour 3 hours after reperfusion which was about 10-times of that before reperfusion. The urinary secretion of CK-MM was 46 ± 14 ng/hour before reperfusion and increased 5-fold to 299 ± 216 ng/hour 2 hours after reperfusion.

Comparison between blood concentrations of CK isozymes of open heart surgery and those of control surgery

Blood CK-BB concentrations in control surgery were kept at low levels ranging from 0.21 to 0.32 ng/ml without any significant increase during the observational period, while blood CK-BB concentrations increased significantly during open heart surgery. Blood CK-BB levels of control surgery showed significant differences ($p < 0.01$) from those of open heart surgery cases except during the preoperative period (Table II).

DISCUSSION

Serum CK-MB levels or total serum CK activities are usually measured to estimate the degree of heart damage in patients with acute myocardial infarction or in those who received a surgical operation of the heart, since the MB and MM isozymes are present in the heart muscle at high concentrations^{3,4}. On the other hand, the BB isozyme is localized mainly in the brain, smooth muscle^{3,4} and some types of carcinoma tissues⁷⁻¹¹. Therefore, the measurement of CK-BB levels in cerebrospinal fluid or serum samples have been proposed to be useful as a biomarker of patients with neurological diseases^{12,13} and some types of carcinoma⁷⁻¹¹. However, concentrations of CK-BB, CK-MB, and CK-MM in human heart tissue were reported to be approximately 2,000, 2,500 and 12,000 ng/mg protein, respectively, by Kato et al^{5,6}. Since the heart has considerable amounts of CK-BB, it can be expected that CK-BB leaks from damaged heart

muscles into the blood stream. Actually, blood CK-BB levels rose during open heart surgery, but not in the cases of control surgery. Blood CK-BB concentrations were significantly higher in samples obtained from coronary sinus than in those from the peripheral artery in the early period after reperfusion. The patients did not show any symptoms of brain damage after the operation in all the cases of present study. These results suggest that the major portion of elevated CK-BB levels in the blood in the present study were derived not from the central nervous tissue, but from the heart muscle.

The response of CK isozymes in the blood after the operation was seen first in the BB, secondly in the MB, and finally in the MM forms. Since the molecular size of each CK isozyme is almost the same, the reasons for the rapid response of CK-BB followed by CK-MB remain to be clarified. The liberation of CK-BB from damaged heart muscles might be easier than that of the other CK isozymes.

It was reported by Roberts and Sobel that CK isozymes in the blood stream is mainly cleared by the reticuloendothelial system.¹⁴ But some amounts of CK isozyme in the blood stream were cleared by the kidney and secreted into the urine. Since blood concentrations of CK-BB were lower than those of the other CK isozymes, CK-BB in the blood stream might be eliminated easier by both the reticuloendothelial system and kidney than the other CK isozymes.

Blood CK-BB levels rose due to its leakage from the damaged heart muscle in the early period after reperfusion, however, its maximum level was low (23.3 ± 7.6 ng/ml) and its relative levels in total CK was less than 5.6%. If blood CK-BB concentrations rise to much higher levels or remain at abnormally high levels for long time, it should be considered that something happens in the brain or the gastrointestinal tract, which are the main sources of CK-BB in human body.

Although blood CK-BB levels were lower than the other CK isozymes, the detection of their changes in the early period after reperfusion are useful for estimating the damage of the heart muscle, and blood CK-BB levels in the late period after reperfusion might also be useful to locate the damage in the brain.

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