

Circulating CK-MB and CK-BB isoenzymes after gastrointestinal surgery

SWEI H TSUNG

From the Department of Pathology, Methodist Hospital of Gary, Inc, 600 Grant Street, Gary, Indiana 46402, USA

SUMMARY The effect of gastrointestinal surgery on serum creatine kinase activity was studied in 30 patients. The MB isoenzyme was demonstrated in sera of 30% of the patients and BB isoenzyme in 23%. MB content varied from 0.8 to 10.3% of the total creatine kinase activity, and the BB content from 0.6 to 18.4%. The CK-BB was probably of gastrointestinal origin, since gastrointestinal tract contains high CK activity with BB isoenzyme predominating. A cardiac origin for the observed serum CK-MB isoenzyme increase would seem the most likely, although no patients showed evidence of electrocardiographic changes. Increased CK-MB activity has been observed in myocardial ischaemia without infarction.

Recent reports by workers using a variety of sensitive methods have indicated that the presence of the BB isoenzyme of creatine kinase (EC 2.4.2.7.) in human serum is not as rare as was originally supposed. CK-BB activity has been reported in sera of patients with severe acute brain injury,^{1,2} chronic renal failure, haemodialysis, renal transplant,^{3,4} and prostate resection.⁵ Although the findings appear to have little diagnostic significance in their own right, they are important in relation to certain isoenzyme separation techniques that are now in use.

Theoretically, damage of organs containing CK activity should release soluble enzyme into the general circulation. Gastrointestinal (GI) tissue has been reported to contain total CK activity only less than skeletal and cardiac muscles, and comparable to CK activity of brain per gram wet tissue with almost exclusively BB isoenzyme.⁶ It has been well documented that acute brain damage will effect the release of CK-BB into the blood circulation. However, there are few reports on CK isoenzyme in patients with damage of the GI tract. The present study was initiated to detect the frequency of occurrence of CK-BB and CK-MB isoenzymes in the sera of patients after GI surgery.

Material and methods

PATIENTS

Thirty patients (31-78 yr, median 68) undergoing GI

resection were studied: six patients underwent subtotal gastrectomy for benign peptic ulcer and one patient for resection of carcinoma; there were two patients undergoing small bowel resection and 18 patients undergoing large bowel resection for malignancy. Two patients had large intestine removed because of gangrene secondary to thrombosis of the mesenteric artery; one patient had small intestine resection for granulomatous ileitis. Thirty patients with various types of orthopaedic surgery were used as controls. All patients had surgery under general anaesthesia.

Blood samples were obtained from each patient within 24 h preceding surgery. Because a rigid time schedule could not always be adhered to, the postoperative samples were collected two to four hours after surgery. Blood samples were also taken 20-24 h after operation from 22 patients with GI surgery and 30 patients with orthopaedic surgery.

Total CK activity and CK isoenzyme were determined simultaneously on the pre- and post-operative specimens for each patient, either the same day or the next day after storage at -20°C . We also analysed tissue homogenates from samples consisting of two stomach, three small intestine, and nine large intestine. Tissue homogenates were prepared as described in a previous report.⁷

CK activity was estimated at 37°C with an HMA analyzer (Hycel, Inc, Houston, TX 77036) by the kinetic method of Rosalki⁸ with reagents from Roche Diagnostics, (Division of Hoffman-La Roche Inc, Nutley, NJ 07110).

The serum and supernatant fluid of tissue homogenates were chromatographed by discontinuous-gradient elution from DEAE Sephadex A-50 (Pharmacia Lab Inc, Piscataway, NJ 08854) as described by Yasmineh *et al.*⁹ Aliquots (1 ml) of serum or tissue extracts containing less than 2U of CK activity were loaded on 0.6 cm × 6 cm microcolumns. The MB and BB fractions were confirmed by electrophoresis at pH 8.8 on agarose film by the CK isoenzyme procedure of Corning ACI (Palo Alto, CA 94303). The eluate was concentrated using the Model B-15 Minicon Concentrator.

Results

Column reproducibility was evaluated by repeated analysis of a serum pool fortified with MB and BB isoenzymes from an extract of heart and intestinal tissue. The results proved satisfactory and are summarised in Table 1. A range of normal values

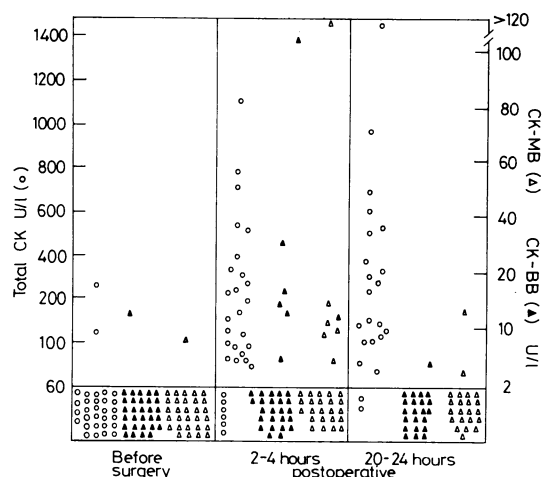
Table 1 Between-run precision of CK isoenzymes assay by column chromatography in a special MB and BB fortified serum control

	MM	MB	BB	Recovery %
Mean U/l	96	22	8	97.6
SD	3.45	1.32	0.8	2.5
CV%	3.6	6	10	2.6

n = 21 fortified serum samples.

for the total CK activity and CK isoenzymes was established on 40 hospital employees (25 men and 15 women, ranging in age from 18 to 51 yr). The range for total CK activity in this group was 24-60 U/l (42 ± 2 SD). MB activities ranged from 0 to 1.8 U/l. BB activities ranged from 0 to 0.9 U/l. Therefore, 2 U/l was used as the upper limit for the MB and BB isoenzyme. In the control group, total CK values before surgery ranged from 23 U/l to 58 U/l with a mean of 45 U/l. CK-MB or CK-BB was not found in any of the presurgical serum samples. Two to four hours after operation, total CK activity ranged from 26 U/l to 1092 U/l, with a mean of 175 U/l. Six of 30 patients (20%) demonstrated CK-MB isoenzyme ranging from 3 U/l to 7 U/l. Total CK activities on 20-24 h postoperative samples ranged from 35 U/l to 1340 U/l with a mean of 310 U/l. CK-MB isoenzyme was not measurable. No control patient had detectable CK-BB in 2-4 h or 20-24 h postoperative samples.

The Figure summarises the results of total CK and isoenzyme activities on 30 patients with GI surgery. Total CK values before surgery ranged from 8 U/l to 260 U/l with a mean of 51 U/l. One patient



Total creatine kinase and isoenzyme activities in serum of 30 patients before and after gastrointestinal surgery.

with carcinoma of colon had detectable CK-BB of 10 U/l (28% of total CK activity) although total CK activity was normal. The two patients with above-normal CK values had gangrene of the small and large intestine; CK-MB was 5 U/l or 2% of total activity in one patient.

Two to four hours after operation, total CK activities ranged from 26 U/l to 1054 U/l with a mean of 248 U/l. Seven of 30 patients (23%) demonstrated CK-MB isoenzyme activity ranging from 5 U/l (1.5%) to 109 U/l (10%), six of 30 patients (22%) demonstrated CK-BB isoenzyme activity ranging from 2.5 U/l (10%) to 105 U/l (10%). In two cases both CK-MB and CK-BB were demonstrable. Table 2 summarises the results of total serum CK activity and its isoenzyme distribution in those patients with detectable serum CK-MB and/or CK-BB.

Repeat CK and isoenzyme studies were done on 22 patients 20-24 h after the operation. Only two patients had normal total CK activity. The total CK activity was slightly greater in the samples taken 20-24 h (mean 316; range 52-1451 U/l) than in the samples taken two to four hours after surgery. Blood from two of these patients showed MB activity of 11 U/l and 3.5 U/l or 0.8 and 1.5% of total activity, while only one patient had BB activity of 3.5 U/l (0.6%). These three patients did not show CK-MB or CK-BB in the samples taken at two to four hours after surgery. CK-MB or CK-BB isoenzymes were more frequently demonstrated in samples taken two to four hours after surgery than in the samples taken 20-24 h after surgery. The

Table 2 CK concentration and isoenzyme distribution in 13 patients' sera and four patients' tissue homogenates

Patient		Serum CK activity U/l	MB%	BB%	Tissue CK activity U/g wet tissue	MM%	MB%	BB%
1	Ilectomy	576	1.1	0				
2	"	326	1.5	0				
3	Gastrectomy	258	3.5	0				
*4	"	216	1.6	0	175	0	3	97
5	Colectomy	417	2.8	1.1				
6	"	412	1.3	0				
7	"	82	6.5	0				
8	"	24	0	10.4	117	1	4	95
9	"	114	0	18.4				
*10	"	1451	0.8	0	93	0	1	99
11	"	1054	10.3	10				
*12	"	595	0	0.6				
13	"	158	0	7	167	3	0	97
*14	"	186	0	4				

*20-24 h samples.

presence of CK-MB or CK-BB, or both, in the blood was usually transient except for one patient whose blood still showed CK-BB activity of 4.5 U/l (11% total activity) in a 10 day-postoperative sample. This was the same patient who showed normal CK activity with 28% BB fraction before surgery. This patient had adenocarcinoma of colon with liver metastasis. The percent of BB activity declined after surgery.

Table 3 summarises the results of our CK enzyme

Table 3 CK concentration and isoenzyme patterns (in % total CK activity) of gastrointestinal tissue

Tissue	CK activity (U/g wet tissue)	MM%	MB%	BB%
Colon	117	1	4	95
	234	3	2	95
	280	0	1	99
	93	0	1	99
	167	3	0	97
	138	4	0	96
	205	0	0	100
	171	1	0	99
	111	2	4	94
Ileum	223	1	1	98
	118	0	0	100
	186	3	4	93
Stomach	175	0	3	97
	157	5	4	94

assays for the 14 GI specimens. The mean total CK activity was 170 U/g wet tissue (range, 93-280 U/g). Isoenzyme analysis showed CK-BB to be the major isoenzyme, ranging from 93 to 100% of total activity. The CK-MB and CK-MM isoenzyme ranged from 0 to 5%. In 10 patients, enzyme assays were simultaneously performed on both serum samples and surgical specimens. Four of seven patients had CK-MB or CK-BB detectable in the serum.

Discussion

Henry *et al*¹⁰ reported that surgery involving the GI tract does not increase serum MB values, nor is BB generally detected after such a procedure. In the present study, however, CK-MB was found in 33% and CK-BB detected in 23% of patients. The CK-BB found in nine of 30 patients was probably of gastric or intestinal origin, since this isoenzyme study showed that CK-BB is the major isoenzyme in GI tissue. It is unlikely that the effects of anaesthesia on the brain could have resulted in transient release of the CK-BB into the circulation since no CK-BB increase was seen in controls after orthopaedic operations. Two reasons speak against the theory that CK-MB observed in nine patients is of GI origin. Firstly, enzyme assays on tissue and serum were simultaneously performed on 10 patients; two patients (patients 4 and 10 in Table 2) had CK-MB of 3.6 U/l and 11 U/l respectively, with serum CK-BB undetectable. These two patients had tissue CK-BB of 99 and 97% respectively, while tissue CK-MB ranged from 1 to 3%. If the CK-MB was of GI origin due to surgical trauma, CK-BB should also be present with much higher activity unless the release mechanism of both CK-MB and CK-BB isoenzyme is different. Secondly, CK-MB was more frequently detected than CK-BB as shown in Table 2. This also contradicts the theory that CK-MB is of GI origin, because the GI tract contained considerably less CK-MB than CK-BB.

Itano¹¹ demonstrated raised BB activity in a patient with membranous colitis. Doran¹² reported the presence of CK-MB and CK-BB in a patient suffering from infarction of the colon. Although the source of CK-BB was attributed to intestinal necrosis, the source of CK-MB was not explained. In this study, two similar cases were encountered showing extensive necrosis of the small and large

intestine as a result of mesenteric artery thrombosis. Both patients had CK-MB and CK-BB in their postoperative samples.

Silverman *et al*¹³ found CK-BB to be associated with untreated or progressive malignant disease. This study consisted of 20 patients having carcinoma of the intestine. All had normal CK activities before surgery. However, one patient had CK-BB isoenzyme of 10 U/l or 28% of total activity. Ten days after operation, the total CK activity was 41 U/l with BB isoenzyme of 4.5 U/l (11%). This patient was found to have liver metastasis by liver scan and this was confirmed at surgery which was performed to relieve the intestinal obstruction. Using radioimmunoassay, Silverman *et al*¹³ claimed that CK-BB might be used as a tumour marker. They observed CK-BB in 19% patients with metastasis, and 2% without metastasis. It would be expected that CK-BB would be less frequently found using less sensitive methods.

This study demonstrated that CK-MB or CK-BB, or both, can be measurable in some patients after GI surgery, with infarcted intestine and with progressive malignant disease. The CK-BB was probably of GI origin, or was produced by malignant neoplastic cells. Transient release from brain due to anaesthetic effect should also be considered, but would seem unlikely because none of the patients in the control group had measurable CK-BB. A cardiac origin for the observed serum CK-MB isoenzyme increase would seem the most likely, although no patients studied showed evidence of electrocardiographic changes. Increased CK-MB activity has been observed in myocardial ischaemia without infarction. Incubation of CK-BB in human plasma has been shown to convert most of the enzyme into a form in which electrophoretic mobility corresponds to the MB isoenzyme, and it has been suggested that similar change can occur in the circulation.¹⁴ Therefore, this mechanism might account for the observed CK-MB in this study.

The presence of significant amounts of BB isoenzyme, whatever its tissue origin, will falsify MB assays which use methods unable to separate CK-MB from CK-BB.^{15 16} The laboratories using column chromatography to identify CK-MB should be aware that in the presence of CK-BB, the ionic strength of the MB eluting buffer is critical. Eckfeldt and Kershaw¹⁶ demonstrated that using the Harleco Ultrazyme kit with 0.25 mol/l sodium chloride buffers, very significant amounts of CK-BB were eluted in the CK-MB fraction. Our routine clinical

chemistry laboratory using kits from Roche Diagnostics Division experienced a similar phenomenon because their CK-MB buffer also contains 0.25 mol/l sodium chloride.

References

- 1 Kaste M, Somer H, Konttinen A. Brain-type creatine kinase isoenzyme: occurrence in serum in acute cerebral disorders. *Arch Neurol* 1977;**34**:142-4.
- 2 Somer H, Kaste M, Troupp H, *et al*. Brain creatine kinase in blood after acute brain injury. *J Neurol Neurosurg Psychiatry* 1975;**38**:572-6.
- 3 Galen RS. Creatine kinase isoenzyme BB in serum of renal diseased patients. *Clin Chem* 1976;**22**:120.
- 4 Weseley SA, Byrnes A, Alter S, *et al*. Presence of creatine phosphokinase brain band in the serum of chronic renal disease patients. *Clin Nephrol* 1977;**8**:345-8.
- 5 Kimler SC, Sandhu RS. Circulating CK-MB and CK-BB isoenzymes after prostate resection. *Clin Chem* 1980;**26**:55-9.
- 6 Tsung SH. Creatine kinase isoenzyme patterns in human tissue obtained at surgery. *Clin Chem* 1976;**22**:173-5.
- 7 Tsung SH, Huang TY, Han D, *et al*. Total creatine kinase and isoenzyme MB activity in serum and skeletal muscle of a patient with dermatomyositis. *Clin Chem* 1980;**26**:1912-3.
- 8 Rosalki SB. An improved procedure for serum creatine phosphokinase determination. *J Lab Clin Med* 1967;**69**:696-705.
- 9 Yasmineh WG, Hanson NG. Electrophoresis on cellulose acetate and chromatography on DGAE-Sephadex A-50 compared in the estimation of creatine kinase isoenzyme. *Clin Chem* 1975;**21**:381-6.
- 10 Henry PD, Roberts R, Sobel BE. Important to separate creatine kinase isoenzyme? *Clin Chem* 1975;**21**:1845.
- 11 Itano M. The detection of CPK₁ BB in serum. *Am J Clin Pathol* 1976;**65**:351-5.
- 12 Doran GR. Appearance of creatine kinase BB isoenzyme in the serum of a patient suffering from infarction of the colon. *Clin Chim Acta* 1979;**92**:415-9.
- 13 Silverman LM, Derner GB, Zweigh MH, *et al*. Creatine kinase BB: a tumor associated marker. *Clin Chem* 1979;**25**:1432-5.
- 14 Cho HW, Meltzer HY, Young JJ, Goode D. Effect of incubation in human plasma on electrophoretic mobility of brain-type creatine phosphokinase. *Clin Chim Acta* 1976;**73**:257-65.
- 15 Gerhardt W, Waldernstrom J. Creatine kinase B-subunit activity in serum after immunoinhibition of M-subunit activity. *Clin Chem* 1979;**25**:1274-80.
- 16 Henry PD, Roberts R, Sobel BE. Rapid separation of plasma creatine kinase isoenzyme by batch adsorption on glass beads. *Clin Chem* 1975;**21**:644-9.
- 17 Eckfeldt J, Kershaw M. Increased circulating creatine kinase isoenzyme BB in a patient with metastatic prostatic carcinoma gave spuriously high isoenzyme MB values with Harleco Ultrazyme kit. *Clin Chem* 1980;**26**:348-9.

Requests for reprints to: Dr SH Tsung, Department of Pathology, Methodist Hospital of Gary, Inc, 600 Grant Street, Gary, Indiana 46402, USA.