Radioimmunoassay of serum creatine kinase BB as index of brain damage after head injury

J P PHILLIPS, HILARY M JONES, ROWENA HITCHCOCK, N ADAMS, R J THOMPSON

Summary and conclusions

Brain-type creatine kinase isoenzyme (CK-BB) was measured by radioimmunoassay in the serum of 54 patients with head injuries. CK-BB was not detectable in 476 out of 1006 controls, the remaining 530 normal samples containing a mean of 1.5 ± SD0.75 μg/l. The mean CK-BB concentrations in patients with mild, moderate, and fatal head injuries were all significantly higher than the control value (p < 0.01 in each instance). Patients with serious head injury had serum concentrations many times the normal value, in two cases within 30 minutes after impact. Fatally injured patients continued to have high serum concentrations several days after injury. In less serious cases values approached normal within two or three days. Every patient with evidence of cerebral laceration, bruising, or swelling had a serum CK-BB concentration above normal. Raised concentrations were found in 14 out of 22 patients with concussion only.

The serum CK-BB concentration appears to be a sensitive index of brain damage and may prove useful in the management and follow-up of head-injured patients.

Introduction

A biochemical index of the extent of brain damage might be of great value in assessing and managing patients with head injury. Several proteins have been measured in serum and cerebrospinal fluid after trauma, including lactate dehydrogenase isoenzymes, a-creatine kinase isoenzymes, and myelin basic protein. Though such studies usually show a correlation between the serum concentration of the marker protein and the extent of the cerebral lesion, the methods may not be sensitive enough to monitor minor degrees of brain damage, and no one marker protein has become established in patient care. Brain-type creatine kinase isoenzyme (CK-BB) has been measured in head injuries by fluorescence and by spectrophotometry. Bell et al. measured CK-BB by radioimmunoassay in serum and cerebrospinal fluid from patients with neurological disorders and found significantly increased mean values in those with acute cerebrovascular accidents, patients with prolonged alterations of consciousness, and in a single patient with head injury. We developed a similar radioimmunoassay for CK-BB and found significantly raised mean serum concentrations in patients with dementia and also isolated raised values in patients with epilepsy, cerebral myelopathy, and cerebellar degeneration. Apart from neurological disorders radioimmunoassay has shown raised serum CK-BB concentrations in malignant diseases.

Radioimmunoassays for creatine kinase isoenzyme estimation are reportedly about 1000 times more sensitive than conventional spectrophotometry and furthermore recognise enzymically inactive but immunologically reactive protein. We have therefore used radioimmunoassay to measure CK-BB in the serum of 54 patients with head injury to see whether the concentration is a sensitive index of brain damage.

Patients and methods

The 54 patients were divided into three groups according to the early outcome (death or discharge).

Group 1 comprised 25 patients with mild head injury resulting in loss of consciousness for under 10 minutes. Three patients had transient neurological signs detectable after they regained consciousness, but the remaining 22 were concussed only. All patients fully recovered and showed no residual disability on discharge.

Group 2 comprised 19 patients with serious head injury resulting in some residual deficit on discharge.

Group 3 comprised 10 patients with isolated severe head injury resulting in death.
Most of the head injuries were sustained in road-traffic accidents. Patients with multiple injuries or suspected abdominal injuries were excluded. No patient had evidence of malignancy or of pre-existing neurological disorder. Control samples were obtained from 1006 blood donors and normal volunteers.

The CK-BB radioimmunoassay was performed exactly as described. Intra-assay and interassay variations were 4.5% and 10.5%, respectively. Sera from patients with head injury were either separated and assayed immediately or stored at −70°C for up to two weeks before assay, immunoreactivity being unaffected by storage. The immunoreactivity in the sera of patients with head injuries diluted out in parallel with the standard curve of the radioimmunoassay. The detection limit of the assay corresponded to a serum concentration of 0.5 μg/l.

Significance of differences in means between different groups was assessed with Student's t test.

Results

Of the 1006 control sera, 476 (47.3%) contained no detectable CK-BB. These comprised 258 (57.7%) of the 447 samples from women and 218 (39.0%) of the 559 samples from men. The mean serum concentration in the 189 women with measurable immunoreactivity was 1.44 ± SD 0.71 μg/l and in the 341 men with detectable immunoreactivity 1.36 ± 0.88 μg/l. The controls were aged 18-70 years, and in neither sex did the detectable mean CK-BB concentration differ significantly with age group (<30, −50, −70). Four control sera (0.003%) contained concentrations of over 3.0 μg/l; three were from men aged 40, 42, and 43 years, whose values were 5.3, 5.0, and 5.8 μg/l respectively, and one was from a 21-year-old woman, whose concentration was 8.2 μg/l. A value of 3.0 μg/l was taken as the upper limit of normal.

Figure 1 shows the serum CK-BB concentrations in the three groups of head-injured patients. Patients with mild head injury (group 1) had a mean concentration of 4.57 ± SD 2.55 μg/l, those with serious head injury resulting in some residual deficit (group 2) a mean concentration of 11.21 ± 1.27 μg/l, and those with fatal head injury (group 3) a mean concentration of 20.9 ± 11.9 μg/l. The mean concentration in group 3 was significantly higher than the means in groups 1 and 2, and all three means were significantly higher than the control value (p < 0.01 in each instance).

Fatal head injury—The 10 patients in group 3 were aged 18-76 years, and all suffered severe generalised or localised cerebral laceration or bruising and died within one half hours to nine days after admission. In each case the head injury was the only injury present. All but one of the patients were unconscious on admission. The earliest available measurement was 33 μg/l in a patient 30 minutes after injury, two other patients having values of 33 and 40 μg/l within one hour of injury. A fourth patient died on the ninth day from continuing cerebral oedema. His initial serum CK-BB concentration of 10 μg/l decreased over five days to 3 μg/l but rose again to 10 μg/l on day 6 and was 8 μg/l on the day of death.

Serious head injury with some residual deficit—The 19 patients in group 2 were aged 7-75 years, and initial CK-BB concentrations were obtained 30 minutes to 24 hours after injury (14 measurements within 12 hours). Fifteen patients were unconscious on admission, responding only to painful stimuli. Most of the 19 patients showed moderate generalised or localised bruising on CT scanning with corresponding high serum CK-BB values. A patient struck by a wing mirror had the highest CK-BB concentration recorded (60 μg/l), which was estimated 30 minutes after injury. In one patient the clinical deterioration on day 8 and the subsequent temporal lobe resection were reflected by an increase in CK-BB from a steady 5 μg/l to 8 μg/l on day 8 and to 23 μg/l after operation (fig 2). Subsequent values were between 6 and 10 μg/l and coincided with a continuing poor clinical state (eye opening/motor response/verbal response score 7, Glasgow coma scale).

Discussion

Other attempts to measure CK-BB in serum after head injury have relied on spectrophotometric procedures, which lack immunological specificity and are not usually sensitive enough to detect the protein in normal serum or in patients with concussion. Our radioimmunoassay detects high degrees of immunoreactivity in the serum after head injury. While large intestine and prostate contain appreciable amounts of CK-BB, high concentrations in patients with injuries confined to the head and the correlation with the degree of cerebral damage point to the brain as the source of the circulating CK-BB. Immunoperoxidase techniques localise CK-BB to astrocytes. Serum CK-BB concentrations of up to 6 μg/l may be found in dementia and cerebellar degeneration, but none of the present
patients had any evidence of neurological disorders before head injury.

Serum CK-BB concentrations apparently increase rapidly after serious head injury and may reach 30 to 40 times the mean control value (fig 1). Of patients for whom serial measurements were available at least three in the fatally injured group continued to have concentrations five to 10 times normal three to six days after injury. In less serious cases CK-BB concentrations approached normal within two or three days. Hence such preliminary serial CK-BB measurements appear to correlate with clinical improvement, and a high initial reading suggests severe cerebral injury. Possibly concentrations of diagnostic and prognostic value would be obtained if patients with serious head injury were assayed for serum CK-BB within six hours of injury and again after four to six days. The use of CK-BB concentrations as an indicator of continuing or increasing cerebral oedema would require daily samples, but as little as 200 μl of serum (or plasma derived from daily blood gas measurement) would suffice.

Serum CK-BB detected by radioimmunoassay is apparently a sensitive biochemical indicator of brain injury. Not one of our patients with evidence of brain laceration, bruising, or swelling failed to show a concentration above normal. CK-BB is a soluble cytoplasmic protein which presumably can diffuse readily into the blood stream and is consequently detectable even in patients with concussion or minor head injury. Further measurements of this protein as a potentially clinically useful adjunct in the management and follow-up of head injury appear to be justified, and such a study is in progress.

We thank Mr J R W Gleave and Mr A E Holmes for allowing us to study their patients. We also thank Miss H Wombwell for expert technical help. This work was supported by a grant from the Wellcome Trust to Professor C N Hales. JP is in receipt of a grant from the Beech Fund, University of Cambridge.

Requests for reprints should be addressed to: R J Thompson.

References


(Accepted 4 August 1980)