Serum β -enolase in acute myocardial infarction

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SUMMARY The enzyme β -enolase ($\alpha\beta$ and $\beta\beta$ forms) is present in skeletal and heart muscle and catalyses the glycolysis of 2-phosphoglycerate to phosphoenolpyruvate. The enzyme was measured in serum samples from patients with acute myocardial infarction, angina pectoris, congestive heart failure, and idiopathic cardiomyopathy. Serum concentrations of β -enolase were significantly increased in acute myocardial infarction but not in the other cardiovascular diseases. Activity peaked approximately 12 to 14 hours after an acute attack of chest pain, and then gradually decreased as the patient recovered. The rise and fall in β -enolase concentration were faster and steeper than those of creatine kinase activity, particularly in patients in whom activities of both these enzymes were less high. The assay of β -enolase, which is highly specific and sensitive, has considerable advantages for the early diagnosis of myocardial infarction and the diagnosis of a second episode of myocardial infarction because β -enolase concentration increases very early and rapidly and clears quickly.

These data imply that serum β -enolase may be a more effective marker for early myocardial infarction, particularly in milder cases, than measurement of creatine kinase activity.

Mammalian enolase (2-phospho-D-glycerate hydro-lase, EC 4.2.1.11) occurs as dimers composed of three immunologically distinct subunits— α , β , and γ .^{1,2} γ -enolase ($\alpha\gamma$ and $\gamma\gamma$ forms) is found in neuronal tissues and neuroendocrine cells and β -enolase ($\alpha\beta$ and $\beta\beta$ forms) is found mainly in heart and skeletal muscle.^{3,4} The characteristic distribution of these enolases suggests that they may be useful as disease markers.^{3,4}

Using a sensitive enzyme immunoassay method Kato *et al* have recently confirmed that β -enolase in serum is a useful marker for human muscle diseases.⁵ We have measured immunoreactive β -enolase in serum samples from patients with various cardiovascular diseases to determine whether this enzyme could be used as a marker for acute myocardial infarction.

Patients and methods

We studied 26 consecutive patients (aged 38–84, 21 men and 5 women) with a first acute attack of myocardial infarction and without neuromuscular diseases. They were admitted to the coronary care unit of our hospital. We also studied 10 patients (all men, aged 36–61) with angina pectoris, eight with congestive heart failure not caused by acute myocardial infarction, and three patients with idiopathic cardiomyopathy. Cardiac disease was diagnosed by the attending cardiologist according to standard criteria, which included clinical history, electrocardiographic and echocardiographic changes, and biochemical assessment. We excluded patients with cardiogenic shock and those on cardiotonic medication, except for dopamine, which did not affect this enzyme. We also studied 20 normal subjects (15 men and 5 women, aged 20–70) and 10 patients with renal failure or hepatic disease. Blood samples were collected every two hours for 34 hours after admission from the cubital vein via an indwelling tube contain-

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ing anticoagulant. Within 30 minutes of collection serum was separated from the blood samples by centrifugation at 3000 rpm for 10 minutes. Plasma was obtained from citrated blood by centrifugation.

We used the β -enolase immunoassay described by Kato *et al.*⁴ The assay system uses polystyrene spheres coated with immobilised purified antibodies to β subunit and β subunit antibodies labelled with β D-galactosidase from *Escherichia coli*. The β -enolase assay is specific and there is no cross-reaction with other forms of enolase.⁵ Intramuscular injections and cardioversion did not markedly alter concentrations of serum β -enolase.

Serum creatine kinase (EC 2.7.3.2) activity was assayed spectrophotometrically with Testomar-C CK mono from Calbiochem-Behring, or a Centrifi-Chem CK reagent set from Baker Instruments Co, by coupling the reaction with hexokinase and glucose-6-phosphate dehydrogenase.⁶ The normal value of total creatine kinase activity is < 35 mU/ml.

Technetium-99m pyrophosphate scintigraphy of the myocardium was carried out according to the method of Bonte *et al.*^{7,8} in order to assess the size of the infarct. There was a good correlation between infarct size and creatine kinase activity; $y = 12.2x - 12.8$, $r = 0.81$, $p < 0.001$.

Patients with acute myocardial infarction were divided into two groups. Those in whom the concentration of β -enolase was normal on admission and peak creatine kinase activity (≤ 300 mU/ml) and peak β -enolase concentration (≤ 110 ng/ml) tended to be low were identified as mild cases. Those with a higher than normal concentration of β -enolase on admission, higher peak of creatine kinase activity (> 300 mU/ml), and peak β -enolase (> 110 ng/ml) concentration were defined as severe cases. There were 13 patients in each group.

Results

The mean (SD) serum concentration of β -enolase in the 20 normal subjects was 5.6 (3.1) ng/ml (range 1.6–16.7 ng/ml). Concentrations of β -enolase were normal in haemolysed serum samples. In the 26 patients with acute myocardial infarction the serum concentration of β -enolase correlated well with serum creatine kinase activity ($y = 0.41x + 34.67$, $r = 0.813$).

The peak concentration of β -enolase in serial serum samples from patients with acute myocardial infarction was significantly raised (4–22 times higher than the normal value) (figs 1 and 2). β -enolase concentration peaked 12–14 hours after an acute attack of chest pain lasting more than an hour and then gradually fell during recovery. Changes in serum β -enolase concentration resembled changes in serum

creatine kinase activity in acute myocardial infarction (figs 1 and 2). In 13 mild cases the increase in β -enolase concentration occurred before the increase in creatine kinase activity and the rise and fall in β -enolase concentration were steeper than those of creatine kinase activity (fig 1a and b). Comparison of the t^* values of the gamma variate function for the changes in β -enolase concentration and creatine kinase activity confirmed these data. In 13 severe cases the difference in the increase in β -enolase concentration and creatine kinase activity showed a

Fig 1 Mean (SE) serum β -enolase concentration and creatine kinase activity in 13 patients with mild acute myocardial infarction. Increases in creatine kinase activities or β -enolase concentration were significantly higher than normal from 6 to 32 hours and from 4 to 26 hours respectively after the onset of acute myocardial infarction. (Student's t test for paired values.) Normal values are shown by the shaded area.

Fig 2 Mean (SE) serum β -enolase concentrations and creatine kinase activity in 13 patients with severe acute myocardial infarction. Increases in creatine kinase activity and β -enolase concentrations were significantly higher than normal from 4 to 34 hours and from 2 to 34 hours respectively after the onset of acute myocardial infarction. Normal values are shown by the shaded area.

similar pattern to that in the mild cases. In the patients with normal enzyme activity on admission serum β -enolase concentration was higher than normal for a shorter time (3.1 (1.5)h) than creatine kinase activity (6.1 (2.0)h) (fig 3) and the mean interval between onset of the symptoms and peak β -enolase was significantly shorter (12.3 (0.7)h in mild cases and 14.6 (1.0)h in severe cases) than the interval to peak creatine kinase activity (16.6 (0.7)h in mild cases and 18.6 (1.3)h in severe cases). Serum concentrations of β -enolase did not rise in patients with angina pectoris or other heart diseases, in those with acute hepatitis or renal failure, or in healthy subjects after exercising on a treadmill (table).

Fig 3 Comparison (by Student's *t* test for non-paired values) of periods during which serum β -enolase concentration or creatine kinase activity were higher than normal in patients with acute myocardial infarction. Means (SD) are shown.

Although the mean value in patients with acute hepatitis was twice that in normal subjects, most values came within the limits of normal variation (mean $\pm$ 2SD). We found a significant ($p < 0.05$) cor-

Table Mean (SE) β -enolase concentration in normal subjects before and after treadmill exercise and in patients with cardiovascular, hepatic, or renal disease

	β -enolase concentration (ng*/ml)
Normal subjects (20)	5.6 (1.4)
Treadmill exercise test (7):	
Before	4.2 (1.0)
After	4.6 (0.9)
Angina pectoris (10)	5.2 (1.0)
Congestive heart failure (8)	4.0 (0.8)
Idiopathic cardiomyopathy (3)	2.1 (0.5)
Acute hepatitis and liver cirrhosis (10)	10.8 (2.6)
Renal failure (10)	3.3 (0.5)

* $\beta\beta$ enolase equivalent.

Fig 4 Correlation between peak serum concentrations of β -enolase and ejection fraction in patients with acute myocardial infarction (mild and severe cases).

relation between the peak concentration of β -enolase and ejection fraction (fig 4) and infarct size measured by scintigraphy both in mild and severe cases.

Discussion

Many investigators have reported an increase in the activity of several enzymes in the serum of patients with acute myocardial infarction and have confirmed a relation between peak activity of the serum enzyme (particularly creatine kinase) and the size of the myocardial infarct.⁹⁻¹³ The activity of glycolytic enzymes, such as pyruvate kinase¹⁴⁻¹⁶ and enolase^{16,17} was also reported to be increased in acute myocardial infarction. But serum β -enolase had not been evaluated as a marker in acute myocardial infarction. We used a highly sensitive assay for β -enolase. We demonstrated that the concentration of serum β -enolase rose in an early phase of acute myocardial infarction. The increase correlated well with changes in creatine kinase activity and the increase in β -enolase was specific for acute myocardial infarction; it did not occur in patients with other kinds of heart disease who did not have concurrent neuromuscular disorders in which serum concentrations of β -enolase are raised.¹⁸

Serum concentrations of β -enolase were normal in patients with acute hepatitis and renal failure, which suggests that the increase that we found in patients with myocardial infarction was not caused by a decrease in the degradation rate of this enzyme. We

believe that release from necrotic heart muscle rather than from blood cells accounts for the increase in β -enolase because β -enolase is not found in blood cells.

The peak concentration of β -enolase paralleled that of creatine kinase MB^{19,20} and myoglobin.^{20,21} The β -enolase assay system may be more simple and sensitive than assays for creatine kinase and myoglobin. A very early and rapid increase in concentration after the onset of infarction and a quick clearance may make β -enolase a better marker than creatine kinase for the early diagnosis of infarction and the diagnosis of a second episode of necrosis occurring more than a day after the first episode. In cases of acute myocardial infarction in which creatine kinase activity is not very high it may be difficult to detect myocardial infarction, and in such patients the assessment of serial changes in serum β -enolase concentration may be more useful for the early detection of acute myocardial infarction.

The time required to assay β -enolase concentrations can be reduced to less than an hour if the reaction temperature is raised and the size of the polystyrene balls is increased.⁴ This enzyme immunoassay is cheaper than a radioimmunoassay and does not require radioactive reagents.

Serum β -enolase concentration has been used successfully to estimate the size of myocardial infarcts produced in dogs by experimentally induced myocardial infarction (unpublished observations).

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