Immunological determination of serum m-AST activity in patients with acute myocardial infarction

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SUMMARY Serum m-AST (mitochondrial isoenzyme of AST) activity in patients with acute myocardial infarction was determined quantitatively by a new immunological technique which is sensitive and easily available. All 31 patients with acute myocardial infarction showed abnormally high levels of serum m-AST (more than 5 KU/ml); the mean serum m-AST activity attained its peak (42.0 ± 4.9 KU/ml) on the first day after the onset of infarction 5 hours later than that of serum t-AST (total AST) activity in 15 patients whose peak m- and t-AST activities were identified clearly. The individual peak m-AST activity correlated with the total CK released ($r = 0.83$, $n = 15$), indicating that the release of m-AST also reflects the infarct size. The ratio of serum m-AST/t-AST increased following myocardial infarction and showed the maximal value (average 25.7%) on the third to seventh day after onset. This ratio in the patients with acute myocardial infarction was also greater than that in patients with liver disease or with heart failure from causes other than acute myocardial infarction. In the patients who had the additional complication of heart failure and/or cardiogenic shock the ratio was also greater than that in the patients without these hazards. These results indicate that the ratio of serum m-AST/t-AST reflects the severity of the myocardial cellular damage in acute myocardial infarction.

Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, E.C.2.6.1.1.; AST) consists of two isoenzymes, one associated with the cytoplasmic fraction (s-AST) and the other with mitochondria (m-AST) (Fleisher *et al.*, 1960; Borst and Peeters, 1961; Boyd, 1961; Massarrat and Lang, 1965). Since AST is mainly contained in striated muscle, myocardium, and liver, its serum activity has been found to rise in diseases of these organs (Schmidt *et al.*, 1967; Farmer *et al.*, 1970; Idéo *et al.*, 1971). Though the ratio of serum m-AST activity to total serum AST (t-AST) activity has been reported to represent the severity of cellular damage in liver disease (Schmidt and Schmidt, 1961; Idéo *et al.*, 1972), the clinical significance in myocardial infarction of m-AST and the ratio m-AST/t-AST remains unsettled, probably because the previous methods for the determination of m-AST using electrophoresis or column chromatography were inadequate in sensitivity as well as quantitatively (Fleisher *et al.*, 1960; Boyd, 1961; Bodansky *et al.*, 1966; Idéo *et al.*, 1971).

Modern medical treatment of acute myocardial infarction, however, attempts to salvage the jeopardised myocardium surrounding the fully infarcted area. Thus, the evaluation of the degree of the cellular damage within the ischaemic myocardium and the assessment of the extent of infarcted area are of great importance.

Recently Wada *et al.* (1976) described a new immunological technique for the determination of serum m-AST activity which is more sensitive and more easily available than previous methods. Accordingly, in the present study we used this new method to investigate the serial changes of serum m-AST activities and also the ratio of the serum m-AST/t-AST in patients with acute myocardial infarction. The results were compared with those in patients with liver disease and with cardiac failure as well as with normal controls.

Subjects

NORMAL SUBJECTS

Eighty-two healthy subjects (42 men and 40 women, whose ages ranged from 20 to 71 years, average 42

years) were studied to determine the normal range of serum m-AST activity. In order to match the age range of a control group with that of the patients with acute myocardial infarction, 27 subjects of more than 50 years were also selected; the average age was 56.3 years.

ACUTE MYOCARDIAL INFARCTION

Serum m-AST, t-AST, and creatine kinase (CK) activities were determined in 31 patients with acute myocardial infarction whose onset of infarction was clearly identified. There were 26 men and 5 women, whose ages ranged from 35 to 76 years (average 61.5 years). None of these patients died within a week of onset of the illness. The diagnosis of myocardial infarction was established by a history of typical chest pain, characteristic electrocardiographic abnormalities, and an increase in serum enzyme activities (CK, lactic dehydrogenase, and α -hydroxybutyric dehydrogenase).

In all patients we measured (i) the individual peak levels of serum m-AST and t-AST, (ii) the interval from the onset of symptoms to the peak levels of serum m-AST and t-AST activities, and (iii) the serial changes in the ratio m-AST/t-AST. The total CK released, which represents the infarct size, was also obtained by the method of Sobel *et al.* (1972) modified by Norris *et al.* (1975) in 15 patients without cardiogenic shock or severe arrhythmia in the acute phase who survived for more than 7 days.

In order to investigate the relation between serum m-AST levels and the complication of pump failure, the 31 patients were divided into two groups according to the criteria of Killip and Kimball (1968): group 1 consisted of 19 patients (average age 57.6 years) without heart failure, and group 2 of 12 patients (average age 67.2 years) with heart failure and/or cardiogenic shock.

CARDIAC FAILURE

In order to investigate the contribution of the m-AST released from liver in the patients with heart failure but without muscular necrosis, serum m-AST and t-AST activities were determined in 16 patients (average age 44.5 years) with cardiac failure caused by valvular disease (11 patients) or congestive cardiomyopathy (5 patients).

LIVER DISEASE

The ratio of m-AST/t-AST in 23 patients with acute or chronic hepatitis, not including fulminant hepatitis (average age 40.3 years), and 12 patients with liver cirrhosis (average age 53.3 years) were compared with the ratios in patients with acute myocardial infarction.

Methods

A new method for the determination of serum m-AST activity is based on the difference in the immunological properties of the isoenzymes. Serum m-AST activity was assessed as the total residual AST activity after complete removal of s-AST (cytoplasmic fraction) with the immunoadsorbent, red blood cells sensitised with anti-s-AST antibody.

(i) PREPARATION OF IMMUNOADSORBENT

Anti-s-AST antibodies were prepared in rabbits by injecting the purified human s-AST with Freund's complete adjuvant. Purification of s-AST was performed by the method of Morino *et al.* (1963). The IgG fraction was purified by the modified method of Petersen *et al.* (1961) from the anti-sera; its anti-s-AST titre is higher than 1:10 000. IgG fraction was mixed with 2 volumes of phosphate buffer solution containing 50 per cent sheep red cells. One volume of 5 per cent glutaraldehyde solution was added to this mixture with stirring. Red cells precipitated centrifugation were thoroughly washed with phosphate buffer solution and suspended in phosphate buffer solution so as to give a concentration of 20 per cent. This suspension was used as the immuno-adsorbent of s-AST.

(ii) ASSAY OF m-AST ACTIVITY

To determine the serum m-AST activity, s-AST was removed from the test serum with the immuno-adsorbent described above. The immuno-adsorbent (1 ml) was suspended in 5 ml of 0.15 M phosphate buffer solution (pH 7.2). The mixture was centrifuged at 1500 *g* for 3 minutes and the precipitate was mixed thoroughly with 0.5 ml of the serum specimen. The mixture was allowed to stand at room temperature for about 5 minutes, with occasional stirring. The immuno-adsorbent was completely removed by centrifugation at 1500 *g* for 5 minutes and 0.2 mg/ml of the supernatant solution was used to assay the m-AST activity. M-AST and t-AST activity was measured by the spectrophotometric method coupled with malic dehydrogenase and expressed in Karmen units.

(iii) RELIABILITY OF METHOD

In order to evaluate the reliability of this method we prepared the standard AST sera containing known amounts of m- and s-AST with various ratios of these isoenzymes (see Table 1). The standard sera were prepared from crystallised m- and s-AST obtained from the pig heart by the method of Morino *et al.* (1977); the respective activities of these isoenzymes can be determined by the spectrophotometric technique as described above. Measure-

Table 1 Test of accuracy of m-AST determination by immunoadsorbent method. Standard sera were prepared from crystallised m-AST and s-AST of pig heart

AST standard serum	s-AST	200	200	200	200	200
	m-AST	10	20	40	80	160
Measured activity of m-AST		9	22	40	82	163

Activity is expressed in KU/ml.

ment of m-AST activity of these standard sera by our new method revealed the excellent reliability of the method and the electrophoresis of the serum from which the s-AST is removed with immunoadsorbent showed only a band which was completely identical with a band produced by crystallised m-AST, indicating that s-AST activity can be assessed as the difference between the activities of t- and m-AST for routine work (Table 1).

Results

(1) NORMAL VALUE OF SERUM m-AST ACTIVITY

The mean serum m-AST activity determined in 82 healthy subjects was 1.0 KU/ml, ranging from 0 to 6 KU/ml. The mean value of serum m-AST in 17 patients more than 50 years old was 1.5 KU/ml (0 to 5 KU/ml).

To evaluate the reproducibility of our method, we determined in triplicate the m-AST activity of each of 15 serum samples from patients with myocardial infarction. The maximum error in determination

was 8.4 per cent (mean error was 6.4%), indicating a good reproducibility of the method.

(2) SERUM m-AST ACTIVITY IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

(i) Serial changes of serum m-AST activities

Fig. 1 shows the serial changes of the mean serum m-AST activities in 31 patients with acute myocardial infarction. On the day of onset the mean m-AST activity was raised (20.2 ± 2.9 KU/ml) and increased to the maximum (42.0 ± 4.9 KU/ml) on the first day after infarction, decreasing gradually thereafter to the normal level (< 5 KU/ml) on the ninth day of infarction. When more than two determinations were performed in a day, a value obtained early in the morning was illustrated as a representative value of an individual for that day after onset. The mean t-AST activity also showed a peak value (280.5 ± 38.2 KU/ml) on the first day after onset of infarction (see Fig. 2) but returned to normal on the seventh day after onset. In 15 patients whose serum m-AST and t-AST activities were determined every 4 hours within the first 24 hours and thereafter every 6 hours until CK activity returned to normal, the mean peak m-AST activity (65.7 ± 9.8 KU/ml) was obtained on average at 34.6 ± 3.2 hours after the onset of infarction, returning to

Fig. 1 Daily serum m-AST activities in 31 patients with acute myocardial infarction.

Fig. 2 Daily serum t-AST activities in 31 patients with acute myocardial infarction.

Table 2 Ratio m-AST/t-AST after onset of infarction in groups 1 (without heart failure) and 2 (with heart failure and/or cardiogenic shock (mean $\pm$ SE (%))

	Day of onset	Day after onset								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Group 1	14.5 $\pm$ 2.1	17.5 $\pm$ 2.0	17.6 $\pm$ 2.1	21.3 $\pm$ 2.2 ↓ *	21.8 $\pm$ 2.1	23.6 $\pm$ 2.2	25.3 $\pm$ 2.0	26.4 $\pm$ 2.2	19.0 $\pm$ 4.1	20.5 $\pm$ 2.6
Group 2	20.3 $\pm$ 4.4	16.5 $\pm$ 3.0	20.3 $\pm$ 4.0	33.6 $\pm$ 5.1	26.3 $\pm$ 3.7	25.4 $\pm$ 4.0	23.1 $\pm$ 1.5	23.0 $\pm$ 4.5	24.4 $\pm$ 6.0	32.5 $\pm$ 10.5
Total	16.8 $\pm$ 2.1	17.1 $\pm$ 1.6	18.0 $\pm$ 1.7	25.7 $\pm$ 2.5	23.7 $\pm$ 2.0	23.8 $\pm$ 1.9	24.6 $\pm$ 1.4	24.3 $\pm$ 2.9	21.5 $\pm$ 3.3	23.1 $\pm$ 3.4

* $P < 0.01$.

normal on the ninth day after the onset, while the mean peak t-AST was 420.7 ± 50.5 KU/ml which was obtained on average 29.4 ± 2.9 hours after the onset (about 5 hours earlier than that of m-AST), returned to normal on the seventh day of infarction.

The mean ratio of the individual serum m-AST to t-AST activity (m-AST/t-AST) increased gradually after infarction reaching a maximum ($25.7 \pm 2.5\%$) on the third day after onset (Table 2). Though individual values of this ratio showed a wide range at each study interval, the mean of the ratios m-AST/t-AST in 12 patients in group 2 (with heart failure and/or cardiogenic shock) was higher than that in 19 patients in group 1 (without heart failure) at every study interval except the first, sixth, and seventh day after onset (see Table 2). On the third day after infarction the maximal mean m-AST/t-AST was obtained. This ratio in group 2 was 33.6 ± 5.1 per cent which was significantly greater than that in group 1 ($21.3 \pm 2.2\%$, $P < 0.01$).

(ii) Relation between peak value of serum m-AST activity and total CK released

In 15 out of 31 patients serum m-AST and CK activities were determined every 4 hours within the first 24 hours and thereafter every 6 hours until CK activity returned to normal, and the relation between the peak serum m-AST activity and the total CK released (which directly reflects infarct size) was investigated. The individual peak m-AST activities (Fig. 3) showed a close correlation with the total CK released ($r = 0.83$, $n = 15$), indicating that the peak m-AST activity also reflects infarct size.

(3) SERUM m-AST ACTIVITY IN PATIENTS WITH HEART FAILURE

The average serum m-AST and t-AST activities were 19.3 ± 6.0 (3-102) KU/ml, and 164.1 ± 51.0 (37-830) KU/ml, respectively, in 16 patients with heart failure caused by valvular heart disease or congestive cardiomyopathy but without infarction. The ratio m-AST/t-AST averaged 12.2 ± 1.0 per

Fig. 3 Relation between the peak m-AST activity and the total CK released.

cent ($6.0-21.7\%$) which was substantially less than that of the patients with acute myocardial infarction—statistically significant from the third to seventh day after onset ($P < 0.01$).

(4) SERUM m-AST ACTIVITY IN PATIENTS WITH LIVER DISEASE

The mean serum m-AST activity was 8.9 ± 2.0 (0-70) KU/ml in 23 patients with acute or chronic hepatitis, while the mean serum t-AST activity was 70.5 ± 11.3 (11-246) KU/ml. In 12 patients with liver cirrhosis the mean m-AST activity (17.3 ± 5.4 KU/ml) was slightly greater than that of the patients with hepatitis.

The mean values of the ratios m-AST/t-AST in the patients with hepatitis and liver cirrhosis were 12.7 ± 1.8 per cent and 11.3 ± 2.0 per cent, respectively, both being significantly less than the values obtained from third to seventh day after onset in acute myocardial infarction ($P < 0.01$).

Discussion

In 1961 Borst and Peeters (1961) reported that aspartate transaminase has two isoenzymes corres-

ponding to cytoplasmic and mitochondrial fractions (s-AST and m-AST). In patients with liver disease, the detection of serum m-AST activity has been considered to reflect severe cellular damage (Schmidt and Schmidt, 1963). The clinical significance of serum m-AST activity in acute myocardial infarction, however, was unknown. This is partly because the previous methods using electrophoresis or column chromatography allow only semiquantitative determination with inadequate sensitivity.

In the present study we assessed serum m-AST activities quantitatively in patients with acute myocardial infarction by an immunological method with high sensitivity which was recently reported by Wada and his associates (1976). All patients in our series showed abnormal high m-AST activity (18 to 132 KU/ml). Their peak values correlated with the total CK released, which has been shown to be related to infarct size (Sobel *et al.*, 1972; Inoue *et al.*, 1977). This finding strongly suggests that in patients with acute myocardial infarction m-AST activity is derived from the myocardium and hepatic m-AST contributes only minimally to serum m-AST activity. Myocardial and hepatic m-AST are immunochemically identical (Morino *et al.*, 1964).

Our results also showed that the peak values of m-AST were obtained on the average 5 hours after the peak of t-AST activity. These results agree with the report by Murros *et al.* (1973) that serum m-AST activity determined by electrophoresis increased to above normal in 23 of 24 patients with acute myocardial infarction and that its peak level was usually obtained later than that of t-AST, indicating the delayed release of m-AST into the circulation.

Although Calva *et al.* (1974) also described this delay in the peak time of m-AST activity in coronary occlusion in 5 of 10 dogs, they could not detect serum m-AST activity in two infarcted dogs. In contrast to this and other previous reports (Fleisher and Wakim, 1961; Bodansky *et al.*, 1966; Murros *et al.*, 1973), all the patients with acute myocardial infarction in our study had abnormally high m-AST activities. This difference suggests that our immunological method, using the sensitised erythrocytes of sheep to eliminate the antigen-antibody complex of s-AST, has higher sensitivity than the previous technique. Indeed we detected serum m-AST activity in 31 of 35 patients with liver disease, though Murros *et al.* (1973) could not detect m-AST activity in the serum of their patients with liver disease whose serum t-AST was less than 100 KU/ml, even with the immunological technique.

The improvement in sensitivity for determination of m-AST activity, however, could result in a higher

value for the ratio m-AST/t-AST. In our study the mean value of the individual maximal m-AST/t-AST was 30.2 ± 1.7 per cent (12 to 64) and this value is significantly greater than those in the patients with liver disease previously reported (Farmer *et al.*, 1970; Idéo *et al.*, 1971). In the present study, the mean ratio of m-AST/t-AST in 35 patients with liver disease was 12.2 ± 1.9 per cent and was similar to the previous reported results (Fleisher and Wakim, 1961). Accordingly our results strongly suggest that the ratio of m-AST/t-AST in acute myocardial infarction is larger than it is in liver disease and confirm the work of Farmer *et al.* (1970).

There are two possible reasons for this difference in the ratio m-AST/t-AST between acute myocardial infarction and liver disease. The first possibility is that the ratio m-AST/t-AST of the myocardial tissue is greater than that of liver, and this difference is reflected in the larger ratio of serum m-AST/t-AST in patients with acute myocardial infarction. Bodansky *et al.* (1966) reported that the ratios of m-AST/t-AST in myocardial tissue and in liver tissue were 68 and 49 per cent, respectively. Recently, however, one of the authors (H.W.) also determined these ratios in the myocardium and liver by our method, and showed that the liver contained a higher percentage of m-AST (71.0%) than the myocardium (64.0%, unpublished data). This finding made it difficult for us to accept the first possibility that the higher ratio m-AST/t-AST in myocardial infarction was the result of the higher content ratio of the myocardium.

The second possibility is that the larger ratio of serum m-AST/t-AST reflects severe cellular insult to the mitochondria. Calva *et al.* (1974) reported that higher levels of m-AST were observed when the necrosis was extensive and transmural, and in dogs with extensive infarction the ratio of serum m-AST/t-AST was more than 25 per cent. In our cases also the serum m-AST/t-AST was smaller in group 1 (without heart failure). This result was incompatible with the study of Calva *et al.* (1974) which showed the mean total CK released was significantly greater in group 2 than in group 1. These results strongly suggest that the serum m-AST/t-AST reflects the degree of the myocardial tissue damage in acute myocardial infarction. The reduction of the ratio of serum m-AST/t-AST (the mean maximal ratio; 30.2%) from that of myocardial m-AST/t-AST (64.0%) might be the result of the higher lability of m-AST than s-AST (cytoplasmic AST) as suggested by the previous studies of Fleisher and Wakim (1961).

However, the substantially large ratio of m-AST/t-AST was obtained on the third to seventh

day after onset, a few days after the peak of m-AST, which indicates that there is a gradual release of m-AST from the damaged myocardium several days after infarction since the disappearance rate of m-AST in serum was reported to be more rapid than that of s-AST (Fleisher and Wakim, 1961). Though our results indicate that the m-AST/t-AST could be an index of severity of infarction, further investigations are needed to elucidate the quantitative relation between the release of m-AST and the degree of myocardial damage.

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