CLINICAL AND EXPERIMENTAL STUDIES OF
THE DETERMINATION OF SERUM GUANASE ACTIVITY IN
ACUTE MYOCARDIAL INFARCTION

Susumu Ito, M.D., Takeshi Takaoka, M.D., Seiichiro Kishi, M.D.
Yutaka Nakaya, M.D., Yoshikazu Hiasa, M.D.
and Hiroyoshi Mori, M.D.

Serum guanase activity was measured by a new method using direct colorimetric determination of ammonia in 25 patients with acute myocardial infarction, 21 dogs with experimental myocardial infarction and 6 CCl₄-treated dogs, and compared with serum GOT and GPT activity.

We found normal serum guanase activity in patients with acute myocardial infarction and in dogs with experimental myocardial infarction without liver damage, even when the serum GOT and GPT activities increased. On the other hand, serum guanase and transaminase activities were elevated significantly in the patients with acute myocardial infarction who had prominent symptoms of cardiac failure and congestion of the liver and CCl₄-treated dogs.

These findings suggested that the serum guanase activity was more specific than serum GOT and GPT activity as an indicator of liver damage and determination of serum guanase activity in the patients with acute myocardial infarction might be useful in assessing the presence of liver impairment.

Since serum glutamic-oxalacetic transaminase (GOT) activity is known to increase during the acute phase of myocardial infarction, measurement of this enzyme has been widely used as a routine clinical test. In typical cases, the serum GOT activity rises sharply in 6–12 hours, reaches a peak within 24–48 hours, and then gradually decreases to the normal level in 4–7 days after the attack of acute myocardial infarction. Elevation of the glutamic-pyruvic transaminase (GPT) activity is usually less marked in this condition. Serial determinations of the transaminase activities are generally believed to be helpful for differentiation of liver and heart diseases. The myocardium, however, contains a considerable amount of GPT and thus serum GPT often increases considerably after an acute episode of myocardial infarction. Determination of the serum GOT and GPT levels alone, therefore, is not sufficient for differentiating between hepatic and cardiac disorders. This report demonstrates the clinical value of determination of serum guanase activity for the differentiation of liver and heart diseases.

MATERIALS AND METHODS

1. Clinical examinations

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The Second Department of Internal Medicine, School of Medicine, Tokushima University,
Kuramoto-cho, Tokushima City, Tokushima 770, Japan

Sera were obtained from 25 cases of acute myocardial infarction, diagnosed by the history of their illness, physical examinations and laboratory data, including the ECG and the serial determinations of serum enzymes such as GOT, GPT, CK and guanase.

2. Experimental studies

a. Dogs with experimental myocardial infarction

Myocardial infarction was induced in 21 mongrel dogs by anesthetizing the animals by intravenous injection of sodium pentobarbital, and then opening the thorax and ligating the left coronary artery. Electrocardiograms were recorded using McFee's system before and 7 days after the operation. GOT, GPT and guanase activity were measured in 17 animals when the electrocardiogram showed the findings of acute myocardial infarction.

b. Dogs with CCl₄-induced liver damage

Liver damage was induced in 6 dogs by subcutaneous injection of 0.2 ml of CCl₄ per kg body weight. Blood samples were taken from the animals before and after this treatment.

c. Histological examinations

On the first day after the operation or injection of CCl₄, four dogs with experimental acute myocardial infarction and two dogs with CCl₄-induced liver damage were laparotomized and specimens of liver tissue were obtained under intravenous anesthesia with sodium pentobarbital. Samples of blood were taken immediately before anesthetizing the animals.

3. Analysis of blood specimen

Samples of blood were drawn from patients with acute myocardial infarction 1, 2, 3, 4 and 7 days after the attack. Blood specimens were also taken from dogs before and after the induction of myocardial infarction or treatment with CCl₄, and were treated similarly. The sera were separated for the assay of various enzyme activities. The activities of GOT, GPT and CK in the serum were assayed with commercially available kits and that of guanase was measured by our new method in which the amount of ammonia liberated by hydrolysis of the substrate 8-aza-guanine was estimated by direct colorimetry.

4. Statistical methods

The unpaired t test was used for statistical analysis.
RESULTS

1. Clinical examinations

a. Serum GOT, GPT, CK and guanase levels 1 day after the onset of acute myocardial infarction without cardiac failure

Serum GOT, GPT, CK and guanase levels 1 day after the onset of acute myocardial infarction without cardiac failure was shown in Fig. 1. Serum guanase activity was remained within normal range, but serum GOT, GPT and CK activities were elevated significantly.

b. Serum GOT, GPT, CK and guanase levels 1 day after the onset of acute myocardial infarction with cardiac failure

Serum GOT, GPT, CK and guanase levels 1 day after the onset of acute myocardial infarction in which manifestations of cardiac failure were prominent together with marked enlargement of the liver, was shown in Fig. 2. Not only GOT, GPT, and CK but also guanase activities were elevated significantly in patients of cardiac failure and congestion of the liver.

c. Serial changes in serum GOT, GPT and guanase levels after the attack of myocardial infarction without cardiac failure

In 23 cases the serum GOT and GPT activities reached peaks in 1–2 days, and then gradually returned to the normal levels in 5–7 days, whereas the serum guanase activity showed no appreciable increase. Fig. 3 shows the sequential changes in the activities of GOT, GPT and guanase in Case 1 during first week after an attack of acute myocardial infarction. The activities of serum enzymes reached peaks at 24 hours after the onset of the acute episode (GOT 316 KU; GPT 74 KU). In contrast, the serum guanase activity remained within normal limits throughout the 7 days period of examination.

d. Serial changes in serum GOT, GPT and guanase levels after the attack of myocardial infarction with cardiac failure

Fig. 4 shows the sequential changes in the activities of GOT, GPT and guanase in a case during the first two weeks after an attack of acute myocardial infarction. This patient, who had pronounced symptoms of heart failure and marked enlargement of the liver due to congestion, showed increased activities of serum GOT, (500 KU), GPT (600 KU) and also guanase (1.4 mmol/L/h) one day after the episode. The serum GPT level increased further to 650 KU on day 3, and then decreased with the serum GOT,
Fig. 6. Changes of serum GOT, GPT and guanase activities in CCl₄ treated dogs (N = 3).

- - - : GOT  o-----o: GPT  •-----•: guanase
Statistically significant difference from the level before treatment (* p < 0.02, ** p < 0.01).

GPT and guanase activities, reaching the normal level on day 14 after the onset of the acute episode.

Fig. 7. Serum GOT, GPT and guanase activities 1 day after the operation in dogs with experimental myocardial infarction in which liver biopsy or autopsy was performed (N = 4).

Statistically significant difference from the level before operation (** p < 0.01).

2. Experimental examinations

a. Serum transaminases and guanase activities in dogs with experimental acute myocardial infarction

The average serum GOT, GPT and guanase activities, with their standard deviations, found before and 1, 2, 3, 4 and 7 days after the induction of acute myocardial infarction in 17 dogs are shown in Fig. 5. In almost all animals, the serum

Fig. 8. Histological appearance of an autopsied liver specimen from a dog with experimental myocardial infarction (No.23). The appearance is essentially normal.
Guanase Activity in Acute Myocardial Infarction

Fig. 6 shows the average serum GOT, GPT and guanase values with their standard deviations in 3 dogs determined before and 1, 2, 3, and 7 days after the treatment with CCl_4. In all animals, the levels of all three serum enzymes increased significantly 1–3 days after the injection of CCl_4 and then gradually decreased.

3. Histological examinations

a. Microscopic findings in the liver of dogs with acute myocardial infarction

Specimens of liver tissue were obtained from 4 dogs on the day after the induction of acute myocardial infarction. Histological findings were compared with the serum GOT, GPT and guanase levels in the animals. The mean values of GOT, GPT and guanase in the serum of 4 animals before and 1 day after the operation are shown in Fig. 7. As found in the previous 17 dogs, the levels of serum transaminases were elevated in all 4 days on the first day after the operation, whereas the level of serum guanase activity was not increased. Light microscopic examination of the liver did not reveal any significant indications of hepatocytic injury. Fig. 8 is a photomicrograph of the liver of a dog on day 1 after the induction of myocardial infarction, when the serum guanase level was normal but the GOT and GPT values were raised to 440 and 210 KU, respectively; no abnormalities are apparent.

b. Microscopic findings in the liver of dogs with CCl_4-induced liver damage

GOT and GPT levels reached peaks 1–2 days after the operation and then gradually decreased to the normal levels by day 7 after the operation, while the serum guanase activities remained within normal limits.

b. Serum transaminases and guanase activities in dogs with CCl_4-induced liver damage

Fig. 9. Serum GOT, GPT and guanase activities 1 day after the treatment in CCl_4 treated dogs in which the liver was examined histologically (N = 2).

Statistically significant difference from the level before treatment (* p < 0.02, **p < 0.01).

Fig. 10. Histological appearance of an autopsied liver specimen from a CCl_4 treated dogs (No.25).
Necrosis, vacuolar and hyaline degeneration are seen.

Tissue specimens were taken from the liver of two dogs 1 day after the injection of CCl₄, and microscopic findings were compared with the serum GOT, GPT and guanase levels in the animals. As in the CCl₄-treated animals described above, both dogs had increased levels of serum GOT, GPT and guanase 1 day after the injection of CCl₄ (Fig. 9). Microscopic examination revealed marked vacuolar degeneration, hyaloid degeneration (with occasional cytoplasmic acidophilic hyperchromasia) and necrosis (with occasional karyoysis) of the centrilobular to midlobular regions throughout the liver in both dogs (Fig. 10).

**DISCUSSION**

Measurements of serum GOT and GPT activities are widely used as liver function tests. Increases in these activities, however, are not specific indications of hepatic disorders, because the myocardium, skeletal muscle, pancreas and other tissues also have high concentrations of these enzymes and consequently high levels of serum GOT and GPT are found in pancreatitis, various type of myopathy and injury of the muscles as well as after acute myocardial infarction.

In the present series of 23 cases of acute myocardial infarction without hepatic congestion, the mean activities of serum GOT and GPT were 134 KU and 53 KU, respectively. The mean activity of GOT was higher than that of GPT. And the serum level of GPT was more than 50 KU in 11 of the 23 cases, but it is uncertain whether this increase was due to myocardial infarction only, or to infarction in combination with hepatic injury.

Passanenti first reported the liberation of guanase into the circulating plasma in patients with damage of liver cells, Subsequently McLeod, Mandel et al. and Konttinen et al. pointed out the clinical significance of measurement of serum guanase activity as a liver function test.

However, as the measurement of serum guanase activity has not yet been widely used for this purpose, mainly because of the problems involved in the assay. We have devised a new simple colorimetric method for the assay of serum guanase, in which the amount of ammonia liberated by enzyme hydrolysis of the substrate (8-azaguanine) is measured, and have reported the clinical significance of this test.

The guanase concentration is high in the liver, brain and kidneys, but low in the myocardium, skeletal muscle and pancreas, where the GOT and GPT concentrations are relatively high. This difference between the distributions of the transaminase and guanase suggests the possible clinical value of measuring serum guanase activity. It has been found that the serum guanase activity was greatly increased in acute hepatitis, but only slightly increased in chronic hepatitis, liver cirrhosis and carcinoma of the liver and that it is not elevated significantly in various other pathologic conditions. Moreover, in spite of the fact that the brain and kidneys have high guanase contents, this enzyme does not increase in the serum of patients with cerebral hemorrhage or nephritis. These findings suggest that serum guanase activity is more specific than serum transaminase activities as an indicator of liver damage. Thus the determination of serum guanase activity should be helpful in distinguishing disorders of the liver from acute myocardial infarction with high serum GOT and GPT levels, which poses the clinical problem of whether there is any liver damage or not.

In the present study, all 23 patients with acute myocardial infarction without significant liver congestion had normal levels of serum guanase activity, even when their serum GOT and GPT levels were elevated.

On the other hand, 2 cases, who had pronounced symptoms of cardiac failure and congestion of the liver, had increased levels of serum guanase activity as well as high serum transaminase activities. Guanase was all recovered in the supernatant fraction on disruption of liver cells. Thus it is conceivable that this enzyme, like transaminases, may be released into the blood stream of patients with hepatic congestion.

In 1930, Jolliffe first demonstrated that liver function was deranged in patients with congestive heart failure. White et al. and Sherlock attempted to correlate liver function tests with histological findings on hepatic cells by means of serial biopsies or postmortem examinations of patients with congestive heart failure. We also performed liver function tests including the determination of serum guanase activity in seven cases of chronic congestive heart failure. In 4 of these cases, in which the activity of serum guanase was high, the activities of GOT and GPT were also raised, whereas in the other 3 cases, in which the activity of serum guanase was within normal limits, the serum GOT and GPT levels were normal. These findings suggest that
the activity of serum guanase is within normal limits in cases of chronic congestive heart failure in which the serum transaminase levels are not increased, but that it is elevated in cases in which the serum transaminase levels are increased.

The activities of serum transaminases were increased in all our cases of acute myocardial infarction, but the activity of serum guanase was increased in only a few cases, which were severe cases with signs of shock and an enlarged liver. The high serum guanase activity in such cases might have been due to marked congestion of the liver.

No liver biopsy specimens could be taken in this study, because all the patients were in the acute phase of myocardial infarction. Therefore, for histological studies we induced in acute myocardial infarction in dogs. All 17 dogs with experimental acute myocardial infarction showed elevated serum GOT levels and 15 dogs (88.2%) showed elevated serum GPT levels, but only one dog (5.9%) showed a slight increase in serum guanase activity (No. 7). No histological abnormalities were found in the livers of dogs that showed elevations of GOT and GPT activity, but not of guanase activity.

However, in dogs with CCl₄-induced liver damage, the serum guanase activity as well as the serum transaminase activities were elevated at the time when light microscopic examination showed marked liver damage characterized by vacuolar degeneration, hyaloid degeneration and necrosis of hepatic tissues. Both serum guanase activity and serum transaminase activities increased in dogs with pronounced injuries of liver cells induced by CCl₄, but only serum transaminase activities increased in dogs with experimental acute myocardial infarction without the involvement of any significant hepatocytic injury. These results indicate that the elevations of serum GOT and GPT activities seen in dogs with experimental acute myocardial infarction were ascribable to injuries of the cardiac muscles and to thoracotomy for induction of infarction. Accordingly, it is considered that the activity of serum guanase does not increase in acute myocardial infarction and various other muscle disorders unless there is also some kind of liver disorder. These findings show that the measurement of serum guanase activity is a useful clinical test for the presence of liver damage in acute myocardial infarction and other muscle disorders.

REFERENCES