STUDIES ON THE SIGNIFICANCE OF SERUM MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE ACTIVITY FOLLOWING ISCHEMIC CARDIAC ARREST

JUN AMANO, M.D., MAKOTO SUNAMORI, M.D., TAKAO OKUMURA, M.D., TAKAAKI KAMEDA, M.D., AND AKIO SUZUKI, M.D.

Aspartate aminotransferase (EC 2.6.1.1.: AST) is known to have two isoenzymes, one associated with the cytoplasm (c-AST) and the other with the mitochondria (m-AST). We studied the relationships of m-AST activity in the coronary sinus blood to left ventricular function, coronary blood flow, water content and high-energy phosphate stores of the left ventricle following hypothermic ischemic cardiac arrest.

Under cardiopulmonary bypass with hypothermia of 20°C of myocardial temperature, 120 min of aortic occlusion was employed in 15 mongrel dogs. Left ventricular function (peak left ventricular pressure, left ventricular end-diastolic pressure, max dp/dt, cardiac index, left ventricular stroke work index), coronary blood flow, myocardial oxygen consumption, myocardial enzyme activity (m-AST, CK-MB), myocardial water content and high-energy phosphate stores (adenosine triphosphate, creatine phosphate) of the subendocardium of the left ventricle were measured. Data was obtained in the control state, and after 0, 30 and 60 min of reperfusion.

Significant negative correlations were obtained between m-AST activity and peak left ventricular pressure ($r = -0.81, p < 0.001$), max dp/dt ($r = -0.83, p < 0.001$), cardiac product ($r = -0.73, p < 0.01$), coronary blood flow ($r = -0.59, p < 0.05$), adenosine triphosphate level ($r = -0.72, p < 0.01$) and creatine phosphate level ($r = -0.72, p < 0.02$) after 60 min of reperfusion. Significant positive correlations were obtained between m-AST activity and left ventricular end-diastolic pressure ($r = 0.75, p < 0.01$) and water content ($r = 0.78, p < 0.01$) after 60 min of reperfusion.

These results led to the assumption that serum m-AST activity in the coronary venous blood is a useful index to evaluate the degree of myocardial injury.

Key Words:
- Mitochondrial aspartate aminotransferase (m-AST)
- Mitochondria
- Myocardial ATP
- Ischemic cardiac arrest
- Reperfusion

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A number of enzymes have been proposed as a diagnostic measure for the evaluation of myocardial ischemia.

Since the first report that the serum aspartate aminotransferase (EC 2.6.1.1.: AST) activity elevated in myocardial infarction by LaDue and Karmen in 1954, AST has been widely accepted as a guide for the diagnosis of acute myocardial
### TABLE I  CHANGES OF HEMODYNAMIC PARAMETERS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>30 min of reperfusion</th>
<th>60 min of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Peak LVP (mmHg)</td>
<td>127.7 ± 3.6</td>
<td>96.7 ± 7.4</td>
<td>84.8 ± 5.7</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>5.8 ± 0.4</td>
<td>10.2 ± 1.1</td>
<td>10.6 ± 1.3</td>
</tr>
<tr>
<td>LV max dp/dt (mmHg/sec)</td>
<td>1,388 ± 63</td>
<td>863 ± 97</td>
<td>741 ± 80</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>124.7 ± 7.6</td>
<td>106.6 ± 12.3</td>
<td>96.4 ± 8.9</td>
</tr>
<tr>
<td>LVSWI (gm-cm/kg/beat)</td>
<td>134.1 ± 8.5</td>
<td>103.5 ± 12.8</td>
<td>90.2 ± 10.1</td>
</tr>
<tr>
<td>Cardiac product</td>
<td>18,988 ± 943</td>
<td>11,780 ± 1,230</td>
<td>9,791.2 ± 914.6</td>
</tr>
<tr>
<td>CBF (ml/min/100g)</td>
<td>82.7 ± 8.2</td>
<td>85.0 ± 13.0</td>
<td>60.3 ± 6.5</td>
</tr>
<tr>
<td>MVO₂ (ml/min/100g)</td>
<td>6.5 ± 0.9</td>
<td>4.0 ± 0.8</td>
<td>3.0 ± 0.7</td>
</tr>
</tbody>
</table>

Peak LVP = peak left ventricular pressure, LVEDP = left ventricular end-diastolic pressure, LV max dp/dt = left ventricular maximal rate of pressure rise, CI = cardiac index, LVSWI = left ventricular stroke work index, CBF = coronary blood flow (left coronary artery), MVO₂ = myocardial oxygen consumption

### TABLE II  CORRELATIONS BETWEEN m-AST AND VARIOUS PARAMETERS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>Significance</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodynamics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>peak LVP</td>
<td>-0.81</td>
<td>p &lt; 0.001</td>
<td>12</td>
</tr>
<tr>
<td>LVEDP</td>
<td>0.75</td>
<td>p &lt; 0.01</td>
<td>12</td>
</tr>
<tr>
<td>LV max dp/dt</td>
<td>-0.83</td>
<td>p &lt; 0.001</td>
<td>12</td>
</tr>
<tr>
<td>CI</td>
<td>-0.34</td>
<td>ns</td>
<td>11</td>
</tr>
<tr>
<td>LVSWI</td>
<td>-0.31</td>
<td>ns</td>
<td>11</td>
</tr>
<tr>
<td>Cardiac product</td>
<td>-0.73</td>
<td>p &lt; 0.01</td>
<td>12</td>
</tr>
<tr>
<td>CBF</td>
<td>-0.59</td>
<td>p &lt; 0.05</td>
<td>12</td>
</tr>
<tr>
<td>MVO₂</td>
<td>-0.23</td>
<td>ns</td>
<td>12</td>
</tr>
<tr>
<td>CK-MB</td>
<td>0.40</td>
<td>ns</td>
<td>12</td>
</tr>
<tr>
<td>Water content</td>
<td>0.78</td>
<td>p &lt; 0.01</td>
<td>12</td>
</tr>
<tr>
<td>High-energy stores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>-0.72</td>
<td>p &lt; 0.01</td>
<td>12</td>
</tr>
<tr>
<td>CP</td>
<td>-0.72</td>
<td>p &lt; 0.02</td>
<td>10</td>
</tr>
</tbody>
</table>

ATP = adenosine triphosphate, CP = creatine phosphate, r = correlation coefficient, ns = not significant. Other abbreviations are the same as in Table I.

ischemia.

Furthermore, Boyd and Katunuma et al. have reported that AST had two isoenzymes, one localized in the cytosolic fraction (C-AST) and the other in the mitochondria (m-AST) of the rat or pig liver. It has been shown that m-AST levels in the myocardium decreased in relation to the damage of the myocardium in experimental myocardial infarction. Correlation between the weights of infarcted heart muscle and the values of m-AST in the serum was also observed in an experimental study. In addition, serial measurements of serum m-AST activity in acute myocardial infarction revealed an elevation of this activity and it rose in relation to the degree of heart failure.

Here we studied the relationships between m-AST activity in the coronary sinus blood and left ventricular function, coronary blood flow, water content and high-energy phosphate stores in the left ventricle following 120 min of ischemic cardiac arrest.

### MATERIALS AND METHODS

Fifteen mongrel dogs, weighing from 10 to 25 kg, were used in this investigation. All dogs were anesthetized with 30 mg/kg of sodium pentobarbital intravenously and respiration was controlled using a ventilator with an oxygen supplement to keep partial oxygen pressure of arterial blood about 80 mmHg. Left thoracotomy through the fourth or fifth intercostal space was employed for exposure of the heart. Arterial and left ventricular pressures were monitored.
m-AST in Myocardial Ischemia

Fig. 1. Changes of m-AST and CK-MB activities during reperfusion period. M-AST elevated significantly immediately after ischemic arrest (p < 0.01) and 60 min of reperfusion (p < 0.001).

using pressure transducers (Century, CP-01) and recorded simultaneously with an electrocardiogram using a polygraph (Nihon Kohden, RM-85). Cardiac output was measured by the thermodilution method using Swan-Ganz catheter. Coronary blood flow was measured at the proximal portion of the left anterior descending coronary artery and the left circumflex coronary artery by electromagnetic flow meters (Nihon Kohden, MF-27). A coronary sinus catheter was inserted from the left external jugular vein for sampling coronary venous blood.

Cardiopulmonary bypass was instituted at flow rate of 80 ml/kg/min with left femoral arterial cannulation and right atrial venous cannulation. A pump oxygenator was primed with 1,000 ml canine blood, 200 ml of 20% mannitol and 60 ml of 7% sodium bicarbonate. Moderate hemodilution up to a hematocrit of about 25% was used.

Systemic hypothermia about 20°C was employed using a heat exchanger. The aorta was cross-clamped when myocardial temperature reached 20°C and this temperature was maintained throughout the period of the global ischemia of the heart.

After 120 min of ischemia, the aortic clamp was released and the heart was rewarmed about 15 min to reach 36°C of myocardial temperature. Defibrillation at 5 watt-sec was performed at a myocardial temperature of 34°C and cardiopulmonary bypass was weaned when esophageal temperature reached 36°C.

Hemodynamic parameters such as left ventricular pressure, left ventricular max dp/dt, cardiac output (CO) and coronary blood flow (CBF) were measured before cardio-pulmonary bypass, and 30 and 60 min after unclamping of

Fig. 2. Correlation between m-AST and left ventricular pressure. M-AST was correlated to peak LVP and LVEDP (r = -0.81, p < 0.001, n = 12; r = 0.75, p < 0.01, n = 12, respectively).
m-AST activity during the control period, immediately after unclamping and 60 min of reperfusion. Isoenzymes of AST were isolated using a DEAE-Sephadex column (Nippon Chemiphar) and m-AST activity was measured by Karmen method using spectrophotometer (Centriflicm 500) with the absorbance at 340 nm.

Serum CK activity was determined with the ultraviolet method (Boehringer Mannheim) and isoenzymes of CK were measured by immuno-electrophoresis (Helena Lab.). All data was analyzed by Student's t-test and a difference with p < 0.05 was accepted as significant.

RESULTS

All dogs showed ventricular fibrillation on ECG during aortic clamping. Three dogs could survive no longer than 30 min reperfusion. Following data was obtained from the surviving 12 dogs.

Hemodynamically, peak left ventricular pressure, left ventricular end-diastolic pressure, cardiac index and left ventricular stroke work index were significantly decreased after 60 min of reperfusion. Left coronary blood flow was unchanged after 30 min of reperfusion, but decreased after 60 min of reperfusion (Table I).

Myocardial ATP and CP levels were $3.8658 \pm 0.5146$ and $3.6315 \pm 0.8766 \mu$ mole/g, respectively, after 60 min of reperfusion. The water content of the left ventricular endocardium was $80.9 \pm 0.9\%$.

Isoenzyme activity such as CK-MB and m-AST
in the coronary sinus blood elevated after aortic unclamping. Serum CK-MB activity increased from control level (0.7 ± 0.2 IU) to 2.6 ± 0.5 (p < 0.05) immediately after unclamping and to 6.7 ± 2.0 (p < 0.05) after 60 min of reperfusion. Mitochondrial AST activity also significantly elevated from control level (6.5 ± 1.0 KU) to 13.6 ± 2.0 (p < 0.01) immediately after aortic unclamping and to 22.9 ± 3.2 (p < 0.001) after 60 min of reperfusion (Fig. 1).

From these data after 60 min of reperfusion, following correlation were obtained (Table II):

Hemodynamics: Peak left ventricular pressure, left ventricular max dp/dt and cardiac product showed significant negative correlation with correlation coefficients of \( r = -0.80 \) (p < 0.001, \( Y = 117.8 - 1.4X \), n = 12), \( r = -0.83 \) (p < 0.001, \( Y = 1,220.3 - 29.9X \), n = 12) and \( r = -0.73 \) (p < 0.01, \( Y = 14,536 - 20X \), n = 12), respectively (Figs. 2 and 3). There was a positive correlation between m-AST activity and LVEDP with a correlation coefficient of \( r = 0.75 \) (p < 0.01, \( Y = 3.68 + 0.3X \), n = 12) (Fig. 2).

However, there was poor correlations between m-AST and cardiac index \( (r = -0.34) \) and left ventricular stroke work index \( (r = -0.31) \).

Coronary Blood Flow and Myocardial Oxygen Consumption: Myocardial oxygen consumption (MVO\(_2\)) showed no correlation to m-AST, but coronary blood flow correlated to m-AST after 60 min of reperfusion with a correlation coefficient of \( r = -0.59 \) (p < 0.05, \( Y = 87.3 - 1.2X \), n = 12) (Fig. 4).

Myocardial Water Content: Water content was correlated to m-AST with a correlation coefficient of \( r = 0.78 \) (p < 0.01, \( Y = 75.2 + 0.2X \), n = 12) (Fig. 5).

Myocardial High-energy Stores: Mitochondrial AST was correlated to myocardial ATP level with a correlatve coefficient of \( r = -0.72 \) (p < 0.01, \( Y = 6.50 - 0.12X \), n = 12), and also myocardical CP level showed significant correlation to m-AST with a correlation coefficient of \( r = -0.72 \) (p < 0.02, \( Y = 7.53 - 0.18X \), n = 10).

CK-MB: Poor correlation was obtained between m-AST and CK-MB during the 60 min reperfusion period (\( r = 0.40, n = 12 \)).

**DISCUSSION**

Mitochondrial AST significantly elevated immediately after hypothermic ischemic cardiac arrest and after 60 min of reperfusion. Furthermore, our present study demonstrated that m-AST showed negative correlations to peak left ventricular pressure, cardiac product, max dp/dt, coronary blood flow, adenosine triphosphate and creatine phosphate, and also showed positive
correlation to LVEDP and myocardial water content after 60 min of reperfusion following 2 hours of ischemic cardiac arrest with hypothermia.

There are numerous enzymes in the myocardium and it is well known that these enzymes appear in the serum following myocardial damage. The majority of enzymes are present mainly in the cytosol and these enzymes tend to be released more easily and more rapidly subsequent to myocardial cellular damage than other enzymes existing in subcellular organelles.

Creatine kinase is one of the myocardial enzymes and 90% of it exists in cytosol, and its kinetic, chemical and physical properties have been extensively studied. Serum CK level and its isoenzyme activity are well established as a sensitive and relatively specific indicator of acute myocardial infarction. Moreover, it has been demonstrated that the quantitative estimation of myocardial damage is possible by the determination of CK-MB efflux.

Since Katunuma and his associates and Boyd have reported independently that liver AST fraction could be separated into two fractions, i.e., cytosolic and mitochondrial fractions, by electrophoresis in 1961, much information has been accumulated concerning its physical, biochemical and kinetic properties. Molecular weights of c-AST and m-AST are 110,000 and 70,000, respectively, and Km for L-aspartate are 8.0 \times 10^{-3} and 1.8 \times 10^{-3}, respectively, at a pH of 8. Mitochondrial enzymes such as malate dehydrogenase and glutamate dehydrogenase are rarely employed in routine diagnostic enzymology because of the technical difficulties in their measurement. Recently, using the different affinity to DEAE cellulose, it has become more convenient to separate AST from other isoenzymes and information on AST isoenzymes has contributed to clinical cardiology.

Izumi has reported that the c-AST and m-AST activity ratio in the myocardium was 4:1 and that m-AST activity began to decrease 5 days after coronary artery ligation in experimental study in contrast to the earlier decrease of c-AST about after 24 hours. Furthermore, in experimental myocardial infarction Takemoto showed that serum m-AST activity reached its peak level within 24 hours after infarction and then decreased gradually. On the other hand, Farmer and Murro have reported that serum m-AST activity usually began to elevate between 8 and 24 hours after infarction, reached its peak at about 48 hours and, thereafter, gradually returned to normal level.

In the present study, m-AST activity in the coronary sinus blood elevated even immediately after ischemic arrest and then gradually increased after 60 min of reperfusion. Because we did not measure serum m-AST activity later, it is impossible to determine the peak-m-AST activity in this experiment. It requires a longer period and more frequent sampling to elucidate the time of peak m-AST.

As we employed direct current cardioversion at 5 watt-sec only one in all dogs, it seems that elevation of m-AST activity during reperfusion is not due to defibrillation but rather to the damage of ischemic insult itself.

It has been supposed that m-AST are released from myocardium which are almost irreversibly damaged, whereas lesser degrees of myocardial injury are capable of provoking cytoplasmic enzyme release.

In our present study with a mortality rate of 20%, the severity of myocardial injury seems to be on the borderline between reversible and irreversible damage. By employing 120 min of myocardial ischemia at 20°C of myocardial temperature, Rosenfeldt et al. demonstrated that a survival rate was 60% in the group without chemical cardioplegia after 30 min of reperfusion, and that recoveries of cardiac output, left ventricular minute work and dp/dt in the cardioplegia group were 92, 62 and 91%, respectively, whereas in the hypothermia group they were 38, 17 and 43%, respectively. Biochemical assessments simultaneously performed on biopsy specimens also revealed that postischemic myocardial adenosine triphosphate (ATP) content fell significantly to 56% of the control level in the hypothermic group but was unchanged in the cardioplegia group. They have also reported that electron microscopic assessments showed moderate mitochondrial damage in one heart and moderate edema in 4 hearts in the hypothermia group. They concluded that 60% of hypothermia group showed below average recoveries according to both functional and metabolic assessments.

In the present experimental condition it is postulated that the delayed recovery of ATP synthesis due to mitochondrial dysfunction, or the further energy deprivation provokes a decrease of myocardial contractility, an inhibition of Na⁺/K⁺-ATPase activity and cell acidosis. With a reduction of the normal Na⁺ gradient, the myocardial cell tends to accumulate water resulting in swelling. This cellular swelling is linked to the no-reflow phenomenon, which at least in the

m-AST in Myocardial Ischemia

ischemic myocardium relates to the initiation of an irreversible damage. At the same time, unusual quantities of Ca\(^{2+}\) begin to accumulate in the mitochondria, inhibiting the mitochondrial function and destroying the structural integrity. In addition, a reduced intracellular pH is likely to labilize lysosome membranes directly as well as to enhance greatly the activities of acid hydrolases liberated from lysosomes. High levels of these lysosomal enzymes can increase membrane fragility and permeability, and may lead to enzyme release from both mitochondria and cytoplasm. Sakai et al. stated that enzyme release after temporary ischemia may not necessarily reflect serious myocardial cell damage, but rather alterations of the membrane permeability of myocardial cells. The difference between m-AST and CK-MB activities during the reperfusion period seemed to be partly due to the coexistence of normal and destroyed myocardial cells. Therefore, it can also be supposed that the difference in the release mechanism and the pattern between mitochondrial enzymes and cytosolic enzymes provides additional information which cannot be obtained from the measurement of cytoplasmic enzymes. Murros has shown that peak serum m-AST increases in relation to the severity of myocardial infarction. Takemoto has also suggested that there is a correlation between the amount of infarcted myocardium and the values of m-AST in the serum. Furthermore, one may speculate that the determination of serum m-AST activity reflects more precisely the mitochondrial function and the ultrastructure than that of CK-MB in this situation. In our study, m-AST activity in the coronary sinus blood well correlated to left ventricular function, coronary blood flow, water content and myocardial high energy phosphate stores. Thus, these results suggest that the measurement of m-AST activity is beneficial in evaluating ischemic myocardial damage.

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