**Serum Adenylate Kinase Activity and Isoenzyme in Acute Cerebral Vascular Disease**

Hidetoshi ENDO,* Yoshio IWATA, and Fumio KUZUYA

Department of Geriatrics, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan

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**Summary** Serum adenylate kinase (AK) activity was measured in cerebral vascular diseases and the AK isoenzyme pattern was studied in the patients suffering from cerebral infarction, cerebral hemorrhage, myocardial infarction, and polymyositis. There was a significant difference in AK activity between the control subjects and patients with acute cerebral infarction. Serum AK activity showed a peak on the 2nd day to the 4th day after the stroke attack, maintained at high level for about one week, and decreased gradually thereafter. There were three isoenzymes (AK-1, AK-2, AK-3) of AK, and we found that the AK isoenzyme patterns were different in different disease. The AK isoenzyme patterns in patients with cerebral infarction and cerebral hemorrhage were different from those of control subjects. The AK-3 isoenzyme was dominant in the control subjects. The proportion of AK-1 isoenzyme was increased in cerebral infarction and cerebral hemorrhage compared with that for the control subjects, and the proportion of AK-2 isoenzyme was elevated in acute myocardial infarction and polymyositis. These results suggest that serum AK is a sensitive indicator of cerebral infarction and a useful marker to follow the course of patient recovery. Moreover, the analysis of AK isoenzymes is useful in the diagnosis of cerebral vascular diseases.

**Key Words:** adenylate kinase, isoenzyme, cerebral vascular disease

There are many reports concerning elevated creatine phosphokinase (CPK) activity and CPK isoenzymes in acute cerebral vascular diseases (CVD) [1-3]. Previously, we studied CPK activity and CPK isoenzymes in the early stage of cerebral infarction, and found that the CPK levels remained within the normal limit in many cases and the band of CPK-BB was not elevated. On the other hand,

*To whom correspondence should be addressed.*
we detected an atypical band on the cathodic side of the CPK-MM isoenzyme band. The size of this band area seemed to be a more sensitive indicator than CPK isoenzyme band of the early stage of cerebral infarction, and we found this band to be adenylate kinase (AK). AK is an enzyme discovered in 1943 by Colowick and Kalckar [4], who named it myokinase. The character of this enzyme has been widely studied by many researchers [5-7], and it is well known that contaminating AK can interfere with the measurement of CPK activity [8]. As reported by Kanazawa [9], AK has been found not only in muscular tissues, but also in cerebral tissue to an extent of about 10 to 20% of CPK. Accordingly, some of the elevated serum CPK in CVD may be attributed to increased activity of serum AK.

In the present study, we measured serum AK activity in patients suffering from acute cerebral infarction, cerebral hemorrhage, myocardial infarction, and polymyositis. Further we studied AK isoenzyme patterns in patients suffering from the above diseases to detect any difference in pattern among these diseases.

MATERIALS AND METHODS

Fifteen normal healthy subjects, 20 patients suffering from acute cerebral infarction (within 1 week), 5 patients with cerebral hemorrhage, 4 patients with myocardial infarction, and one patient with polymyositis were used in this study. The diagnosis was made through the findings of brain CT and symptoms. The blood samples were taken within 24 h after the onset of cerebral infarction and cerebral hemorrhage, and from the 5th to 7th day. Serum samples were either analyzed immediately or frozen at −20°C. All samples with visible hemolysis were excluded.

NADH, pyruvate kinase, lactic dehydrogenase, (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT), and phenazine methosulfate (PMS) were purchased from Sigma Chemical Co., St Louis, MO; ATP, ADP, AMP, NAD, and hexokinase/glucose-6-phosphate dehydrogenase, and tricyclohexylammonium salt, from Boehringer Mannheim Yamanouchi, Tokyo; triethanolamine HCl buffer, from Katayama Chem. Ind. Co., Osaka; MOPS and MES buffers, from Corning.

AK activity was measured in accordance with the method of Laudahn and Heyck [10]. The reaction was started by the addition of AMP and the change in

1. Adenylate kinase reaction
   $\text{ADP} \rightarrow \text{ATP} + \text{AMP}$

2. Hexokinase reaction
   $\text{ATP} + \text{glucose} \rightarrow \text{ADP} + \text{glucose-6-phosphate}$

3. Glucose-6-phosphate dehydrogenase reaction
   $\text{Glucose-6-phosphate} + \text{NAD} \rightarrow \text{6-phosphogluconate} + \text{NADH} + \text{H}^+$

4. NADH + PMS $\rightarrow$ NAD + reduced PMS

5. Reduced PMS + MTT $\rightarrow$ PMS + reduced MTT

Scheme 1. Sequence of reactions for detection of AK activity following cellulose acetate electrophoresis.

SERUM ADENYLATE KINASE ACTIVITY AND ISOENZYME

absorbance at 366 nm due to the decrease of NADH reflecting the production of ADP was measured with Hitachi Spectrophotometer Model 100-10.

AK isoenzymes were separated electrophoretically with MOPS buffer. Scheme 1 shows the sequence of enzyme reactions used to stain for AK activity following cellulose acetate electrophoresis. Electrophoretic patterns were scanned with a Densitron-5020 (Herena) and separated into 3 patterns (AK-1, AK-2, and AK-3). The relative proportion of each AK isoenzyme was calculated by its band area. Data were presented as mean±SD. Student’s t-test was used to assess the significance.

RESULTS AND DISCUSSION

Figure 1 shows AK activity in the serum of healthy controls and patients suffering from acute cerebral infarction. AK levels in control subjects were under 5 mU/ml, whereas those in the patients were on the average, 18.79 mU/ml. Thus, there was a highly significant difference between control subjects and patients with cerebral infarction.

Figure 2 shows the time of appearance of AK activity in the serum in 5 patients with acute cerebral infarction. The activity showed a peak on the 2nd day to the 4th day after the stroke attack, and it maintained a high level for about one week and decreased gradually thereafter.

Figure 3 shows AK activity and electrophoretic patterns of sera. AK activity

![Graph showing serum adenylate kinase levels](image)

**Fig. 1.** Serum adenylate kinase levels. The bars show mean levels.
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was increased in patients with cerebral infarction and cerebral hemorrhage. Their isoenzyme patterns were different from those of control subjects.

Fig. 2. Serum adenylate kinase levels in cerebral infarction. The time courses of serum AK activity in 5 patients with cerebral infarction are shown.

![Graph showing serum adenylate kinase levels over time.]

Fig. 3. Serum adenylate kinase activity and its isoenzymes of representative normal subject and patients. Electrophoretic patterns of AK isoenzymes were scanned with a Den-sitron, and the activity was separated into 3 peaks (AK-1, AK-2, AK-3).

<table>
<thead>
<tr>
<th>Subject</th>
<th>AK activity (mU/ml)</th>
<th>AK isoenzyme pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5</td>
<td>![Graph showing control isoenzyme pattern]</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>5.7</td>
<td>![Graph showing polymyositis isoenzyme pattern]</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>12.9</td>
<td>![Graph showing cerebral infarction isoenzyme pattern]</td>
</tr>
<tr>
<td>Cerebral hemorrhage</td>
<td>7.2</td>
<td>![Graph showing cerebral hemorrhage isoenzyme pattern]</td>
</tr>
</tbody>
</table>

was increased in patients with cerebral infarction and cerebral hemorrhage. Their isoenzyme patterns were different from those of control subjects.

The distribution of serum AK isoenzyme patterns of healthy controls and

patients is shown in Fig. 4. The differences in these patterns were significant; AK-3 isoenzyme was the dominant one in the control subjects. The proportion of AK-1 isoenzyme was increased in acute cerebral infarction and in cerebral hemorrhage compared with that in control subjects, and the proportion of AK-2 isoenzyme was higher in the early stage of myocardial infarction and in polymyositis.

The change in AK activity reflected the time course and prognosis of cerebral infarction. An increase in AK-1 isoenzyme activity was found in the early stage of cerebral infarction and cerebral hemorrhage, and an increase in AK-2 isoenzyme indicates the muscle diseases.

Terent et al. [11] reported that AK activity appeared in cerebrospinal fluid (CSF) of patients with cerebral infarction. Further, Ronquist et al. [12], and Ronquist and Frithz [13] reported that AK was consistently found in the CSF of patients with malignant brain tumors and cerebral infarction. However, Getaz et al. [14] claimed that there was no clear correlation between the presence of disease in central nervous system (CNS) and enzyme activity. In the present study, AK activity in patients with cerebral infarction was also elevated, but the correlation between AK levels and the size of cerebral infarction was not examined.

Although increased AK activity in the CSF of patients with neurological disease was reported [13–15], further detailed study is necessary.

There are several reports of the analysis of AK isoenzymes. For example, Khoo and Russell [15] reported the study of AK isoenzymes in tissues of humans and rabbits and suggested that the analysis of AK isoenzyme pattern was useful in the diagnosis of CVD. However, there are few reports recognizing the significance of its distribution pattern in the diagnosis of CVD.

In this study we noted an elevation of the activity of serum AK in the early
stage of CVD, and obtained results indicating that serum AK levels increased in the early stage of cerebral infarction and returned to the normal level later. These results suggest that serum AK is a sensitive indicator of cerebral infarction and a useful enzyme to follow the clinical course of acute CVD.

REFERENCES