S-100a₀ Protein in Serum during Acute Myocardial Infarction

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Concentrations in serum of S100a₀ protein (αα form of S-100 protein, which is present at high concentrations in heart muscle) were successively measured by enzyme immunoassay in 21 patients with acute myocardial infarction (AMI) and six with angina pectoris (ANP). Results were compared with measurements of creatine kinase isoenzyme MB (CK-MB) concentrations in the same specimens. Mean S100a₀ concentrations in sera from 100 healthy adults were 0.12 (SD 0.08) µg/L. In patients with AMI, S100a₀ concentrations were 4.74 ± 5.27 µg/L at admission, peaked 8 h after admission (23.5 ± 27.7 µg/L), then decreased gradually. Among nine AMI patients who were admitted within an hour after their attack, eight showed abnormally high concentrations of S100a₀ in serum (>0.5 µg/L), whereas only four showed abnormally high CK-MB concentrations (>5 µg/L) in sera at the time of admission. Serum S100a₀ concentrations remained within the normal range in all six patients with ANP; however, serum CK-MB concentrations were increased in two of them. Therefore, serum S100a₀ is useful not only for detection of AMI but also for differentiating AMI from ANP.

Additional Keyphrases: creatine kinase · angina pectoris · enzyme immunoassay

Protein S100, an acidic and calcium-binding protein described first by Moore (1), belongs to the family of EF-hand proteins, which includes calmodulin, troponin C, parvalbumin, and the light chain of myosin (2–5). S100 is not a single component, but rather a mixture of similar proteins composed of two immunologically distinct subunits, α and β chains, which combine as αα (S100a₀), αβ (S100a₁), and ββ (S100b) forms (6, 7). S100 was initially considered to be specific to glial cells, but heart and skeletal muscle have recently been found to be rich in S100a₀ (8, 9). Nevertheless, most S100b is found in the brain (9).

The characteristic distribution of the isoforms of S100 suggests that they may be useful as disease markers (10). Given the high content of S100a₀ in heart muscle, one might expect S100a₀ from damaged heart muscle to be released into the bloodstream of patients with acute myocardial infarction (AMI).⁵ We report here our measurements of S100a₀ in serum samples from patients with AMI, determined with a recently developed and highly sensitive enzyme immunoassay. We compared the concentrations of S100a₀ with those of creatine kinase (EC 2.7.3.2) MB isoenzyme (CK-MB), to evaluate whether S100a₀ is useful as a marker for patients with AMI.

Materials and Methods

We studied 21 consecutive patients with a first acute attack of myocardial infarction, who were admitted to the coronary-care unit of Oogaki Municipal Hospital. They had no neuromuscular diseases. We also studied six patients with angina pectoris (ANP), who were also admitted consecutively to the same coronary-care unit with suspected AMI. Clinical data for the patients are shown in Table 1. The diagnoses were confirmed by the attending cardiologist according to standard criteria, e.g., clinical history, clinical findings, electrocardiographic changes, echocardiographic changes, and biochemical assessment. For controls, we used serum samples from 100 normal subjects (provided by the Aichi Red Cross Blood Bank).

Blood samples were collected serially from the cubital vein at the following times: (a) at admission to the emergency room; (b) 2, 4, 6, 8, 12, and 18 h after admission; and (c) on the 1st, 2nd, 3rd, 4th, and 7th day of hospitalization. Serum was separated from the blood by centrifugation (1000 × g, 10 min) within half an hour after sampling and was stored at −80 °C until analysis.

Antibodies to human S100-a were raised in New Zealand White rabbits by injecting S100a₀ purified from human pectoral muscles (9) mixed with Freund's complete adjuvant (11). The antibodies raised were purified by immunoaffinity chromatography with use of S100a₀-coupled Sepharose 4B (11), then passed through a column of bovine S100b-coupled Sepharose 4B.

Concentrations of S100a₀ were determined by the sandwich-type enzyme immunoassay system described by Kato et al. (10). The assay system consists of polystyrene balls (3-mm diameter) coated with immobilized purified antibodies to the α subunit of S100 protein, and the same antibodies labeled with β-d-galactosidase (EC 3.2.1.23) from Escherichia coli. This assay is specific and does not cross-react with other forms of S100 protein. CK-MB concentrations were also determined by a sandwich-type enzyme immunoassay system (12).

We measured each sample in duplicate. Continuous variables were summarized as mean and standard deviation in the text and tables and as mean and standard error in the figures.

Results

Concentrations of S100a₀ protein and CK-MB in serum from 100 healthy adults were 0.12 (SD 0.08, range 0.02–0.38) µg/L and 1.24 (SD 0.98, range 0.36–5.08) µg/L, respectively. We considered values exceeding the mean +4 SD as abnormally high: 0.5 µg/L for S100a₀ and 5.0 µg/L for CK-MB.

Figure 1 shows the results for serial determinations of concentrations of S100a₀ and CK-MB in serum from patients with AMI. Values for S100a₀ concentrations were 4.74 (SD 5.27) µg/L at admission and increased gradually, reaching the peak (23.5 ± 27.7 µg/L) 8 h after admission.

CLINICAL CHEMISTRY, Vol. 36, No. 4, 1990 639
Afterwards, they decreased gradually, becoming almost normal (0.51 ± 0.38 μg/L) by the 7th day after admission. CK-MB concentrations in serum were 7.04 (SD 8.86) μg/L at admission and increased rapidly, reaching a plateau between 6 and 18 h with the maximum (119 ± 60 μg/L) at 12 h after admission, afterwards decreasing quickly, and returning to normal by the 4th day after admission.

On the other hand, serum S100α concentrations were low and remained <0.5 μg/L without any significant increase during the observation period in patients with ANP. However, serum CK-MB concentrations were 0.9 (SD 0.2) μg/L at admission and increased slightly, the highest value, 3.46 (SD 2.78) μg/L, being attained 4 h after admission.

Figure 2 shows the distribution of values for S100α and CK-MB concentrations in serum of patients with AMI or ANP. Every ANP case remained within the normal range of serum S100α concentrations even at the peak, whereas serum CK-MB concentrations were abnormally enhanced in two of six ANP patients. We had nine AMI patients who were admitted within an hour after their attack. These cases showed only ST-T changes and no Q waves on the electrocardiogram at the time of admission. Among them, serum S100α concentrations were already abnormally high in eight at admission, while serum CK-MB concentrations were abnormally high then in only four. Serum S100α concentrations increased to abnormal values by 2 h after admission in 95% of AMI patients, and all AMI patients showed abnormal S100α concentrations 4 h after admission. On the other hand, serum CK-MB concentrations still remained within the normal range 2 h after admission in four of 21 AMI patients. All AMI patients finally showed abnormally high CK-MB concentrations by 6 h after admission.

CK-MB concentrations in serum decreased quickly, and all AMI cases showed normal values by the 4th day, although serum S100α concentrations still remained abnormally high in three quarters of them at that time.

### Discussion

S100 protein, previously believed to be a neuron-specific protein, is also localized at a high concentration as the α form (S100α) in heart and skeletal muscle (8, 9, 13); moreover, serum S100α concentrations are enhanced in patients with acute myocardial infarction or muscular dystrophy (12). To evaluate serum S100α as a marker for AMI, we determined the concentrations of S100α and CK-MB in serum samples taken successively from patients with AMI or ANP by the use of highly sensitive enzyme immunoassay systems. The results indicate (a) serum S100α concentrations become abnormally high in the early phase of AMI, at a higher rate than serum CK-MB; (b) serum S100α remains high in the late phase when values for serum CK-MB are within normal limits; and (c) serum S100α concentrations remain within the normal range in patients with ANP, while CK-MB concentrations in serum sometimes are still high. The rapid response of serum S100α in the early phase of AMI is due in part to its relatively small molecular mass (21 000 Da) as compared...
with that of CK-MB (about 80 000 Da) (6, 7). CK-MB is a cytoplasmic enzyme (14, 15), whereas S100A4 is known to be associated with various structural elements in the muscle cells. Immunohistochemically, S100A4 stains intensely in the intercalated disc (15). The characteristic localization of S100A4 in the heart muscle may result in its prompt release into the bloodstream during the irreversible changes of the heart cells due to hypoxia.

CK-MB is cleared from the bloodstream by the reticuloendothelial system (16), whereas circulating S100A4 is mostly excreted in the urine, and the biological half-life of S100A4 in serum is shorter than that of CK-MB in serum (17). However, S100A4 values for patients with AMI remained positive even when the CK-MB values had become normal. This apparent discrepancy might result from the continuous release of S100A4, which is loosely bound onto heart muscle, into the bloodstream after the injury.

S100A4 did not increase in serum of patients with ANP, but serum CK-MB increased slightly in several patients with ANP. Although small amounts of S100A4 in the heart might be liberated into the bloodstream as well as CK-MB in patients with ANP, the quick clearance of S100A4, as mentioned above, may keep its concentrations in serum within the normal reference interval.

Although the biological function of S100A4 in the heart remains to be clarified, our results indicate that serum S100A4 is a useful marker, not only for the diagnosis of AMI but also for the differential diagnosis of AMI vs ANP.

References