Rate of Decay and Distribution Volume of MB Isoenzyme of Creatine Kinase, Intravenously Injected into the Baboon

Walid G. Yasmineh, Robert B. Pyle, and Demetre M. Nicoloff

We determined the decay constant and distribution space of the MB isoenzyme of creatine kinase after intravenous injection of 440 to 720 U, in a group of seven adult baboons. The rate of decay of the isoenzyme was monoeponential, with a mean decay constant of 0.00175 min⁻¹ (SD, 0.00019). The mean distribution volume obtained by extrapolation of the monoeponential curve to zero time was 5.22% (SD, 1.06) of body weight. We have determined these parameters with the purpose of using this isoenzyme in the baboon as an experimental model for measuring myocardial infarct size.

Currently, one of the most promising approaches to the quantitation of myocardial damage after infarction is the specific determination of the activity of some enzyme(s) that is released in the circulation from the injured cells. In 1971 Shell et al. (1) experimentally induced infarction in dogs by ligating a left anterior descending coronary artery; they proposed equations for estimating infarct size from the total creatine kinase (EC 2.7.3.2, CK)¹ activity in serum specimens obtained at various intervals following infarction. The equations included two important parameters; the decay constant (Kd) and distribution volume (DV), which were determined experimentally by bolus intravenous injection of purified CK. Although the DV then reported for serum CK in the dog (11.4%) was later revised (5.0%) by these authors (2), the equations remain basically sound because infarct size measurements thus obtained correlated well with those obtained by determining infarct size directly from the amount of CK depleted from the myocardium.

Here we report the Kd and DV of MB isoenzyme of creatine kinase (CK-MB) after bolus injection in the baboon (Papio cynocephalus).

¹ Nonstandard abbreviations used: CK, creatine kinase; Kd, decay constant; DV, distribution volume; CK-MB, MB isoenzymes of creatine kinase; Tris, tris(hydroxymethyl)aminomethane.

The use of CK-MB and the baboon as an experimental model offers two great advantages. First, CK-MB is a much more specific and sensitive indicator of myocardial damage than is total CK and comprises about 20% of the total CK activity of the myocardium in both baboon and man (3–9), in contrast to dog myocardium, in which CK-MB is only about 2–3% (3). Second, the pattern of myocardial infarction that is experimentally induced in the baboon better simulates that of man because of the genetic closeness of the two species. Both man and the primates have a relatively constant coronary circulation and a collateral circulation in the coronary arterial system that varies little from one baboon to another (10, 11).

Materials and Methods
Preparation of CK-MB for Injection

CK-MB was prepared as previously described (3, 4) by chromatography of baboon heart extracts on DEAE-Sephadex A-50. The isoenzyme was prepared on a large scale by loading about 300 ml of extract containing the equivalent of 10 g of tissue on a bed of the anion exchanger in a 10 × 10 cm Büchner funnel. After elution of the MM isoenzyme with 1500 ml of 0.1 mol/liter NaCl–50 mmol/liter Tris (pH 8.0), the MB and BB isoenzymes were eluted collectively with about 600 ml of 0.4 mol/liter NaCl–50 mmol/liter Tris (pH 7.0). We did not attempt to separate the BB isoenzyme, because it comprised only 4% of the combined CK activities. The solution of MB and BB isoenzymes was then dialyzed extensively against 0.1 mol/liter NaCl–50 mmol/liter Tris–10 mmol/liter mercaptoethanol (pH 8.0), and loaded again onto a 15 × 3 cm column of the anion exchanger, to concentrate the isoenzyme. The enzyme was eluted stepwise with 10-ml aliquots of 0.4 mol/liter NaCl–50 mmol/liter Tris (pH 7.0). Most of the activity (>80%) was usually recovered in about 40 ml of the buffer, which was then dialyzed against physiological saline containing 10 mmol of mercaptoethanol per liter. About half of the original CK-MB activity was usually
recovered. Before injection into baboons, the enzyme solution was sterilized by passage through a Swinnex-25 filter unit (Millipore Corp., Bedford, Mass. 01730). Usually about 20 ml of the solution was injected into each baboon, without further purification.

Baboon Studies and CK Isoenzyme Assay

Seven baboons, ranging in weight from 22.2 to 24.5 kg, were sedated with ketamine hydrochloride (4 mg/kg body weight). After sedation, an intravenous infusion was started and 20 ml of the enzyme preparation injected into each baboon. Five-milliliter aliquots of blood were drawn immediately before and within 5 to 10 min after injection, and thereafter at 3-h intervals for the first 24 h and 4-h intervals for the following 12 h. The serum was separated within 1 h after blood collection and kept at −20 °C until assayed. Serum CK isoenzymes were separated by discontinuous gradient elution from micro-columns of DEAE-Sephadex A-50 and assayed by the method of Rosalki, as previously described (3). Although only the MB isoenzyme of CK was injected, a considerable amount of MM isoenzyme activity appeared in the serum after the injection, a result of handling the animals (which are routinely squeezed in their cages to be sedated) and also possibly a result of some tissue damage caused by the cutaneous incision required before an intravenous catheter is inserted for enzyme injection and blood sampling.

Results

Pattern of CK-MB Decay

Figure 1 illustrates the decay pattern of CK-MB after the injection of 691 U of the isoenzyme into a 22-kg baboon (see also baboon No. 1 in Table 1). The rate of decay was monoexponential for the first 24 to 28 h, after which it decreased significantly. The slower rate, however, did not start until more than 90% of the injected CK-MB was cleared. This biphasic pattern was essentially the same in each of the seven baboons.

Estimation of Kd and DV

We used a computer program for simple regression analysis to derive the equations of the best-fit lines relating the natural log of the serum CK-MB activities (y-axis) to the decay pattern of CK-MB after bolus injection in a baboon

Fig. 1. Decay pattern of CK-MB after bolus injection in a baboon

<table>
<thead>
<tr>
<th>Baboon no.</th>
<th>Weight, kg</th>
<th>CK-MB Injected, U</th>
<th>DV %</th>
<th>Kd, min⁻¹</th>
<th>SD of Kd, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.2</td>
<td>691</td>
<td>4.6</td>
<td>0.00175</td>
<td>0.997</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>7</td>
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<td>443</td>
<td>6.7</td>
<td>0.00184</td>
<td>0.992</td>
</tr>
</tbody>
</table>

Mean: 5.22, 0.00175
SD: 1.06, 0.00019
CV, %: 20.4, 10.8%
Kd at Low CK-MB Activities

We also studied the decay pattern in two additional baboons after injection of relatively small amounts of CK-MB (96 and 116 U), to determine whether the biphasic decay pattern of CK-MB observed at 24 to 28 h after injection may not be affected by the initial concentration of enzyme in the serum. In both experiments, the results were similar to those obtained when four- to sevenfold more enzyme was injected (see Table 1). The initial monoexponential phase lasted about 28 h, at which time the serum CK-MB concentrations were approximately 15 U/liter. The Kd values were 0.00154 and 0.00155 min⁻¹. These results suggest that injected CK-MB is distributed into two compartments: a major compartment, which is represented by the plasma volume, and a minor one, which is extravascular.

Discussion

Present methods for assessment of myocardial damage after infarction and/or surgery include S-T segment mapping, scanning of the heart after isotope administration, and the use of organ-specific enzymes that are released into the circulation as a consequence of damage. Of these methods the first two more adequately describe the location of an infarct, while the third estimates its size. In this investigation, we have determined the magnitude of two parameters that must be evaluated before the enzymatic estimation of infarct size: the decay constant and distribution volume. We used the MB isoenzyme of CK for this purpose because it is found primarily in myocardial tissue, as was recently shown in man by Roberts et al. (7) and, in our laboratory (3, 4), in the baboon.

The mean DV of 5.2% of body weight obtained in this study for CK-MB in the baboon is very close to that reported for total CK (4.5 to 5.5%) in the dog by Roberts et al. (2). The mean Kd of 0.00175 min⁻¹ is to the best of our knowledge the first estimate for CK-MB obtained by bolus injection in any experimental animal. This value is also very close to the mean Kd (0.000172, n = 12) reported by Roberts et al. (2) for humans after uncomplicated myocardial infarction. We have estimated from their data, however, that the SD is 0.0039, or twice that found in the baboon by bolus injection (0.00019, Table 1). In studies currently in progress in this laboratory, the Kd of CK-MB was calculated from the activities of sera of 16 unselected patients after acute myocardial infarction (13). The mean Kd was 0.00123 (SD = 0.00020), which is significantly (P < 0.01) lower than that obtained by bolus injection, or after uncomplicated myocardial infarction. These observations raise the important question of what Kd value should be used in calculating infarct size for individual patients. Recently, Norris et al. (14) and Shell et al. (1) used patients’ individual Kd values to calculate infarct size. In view of our observations, however, it appears that this problem should be further investigated in experimental animals, where a correlation can be made between serum CK-MB infarct size and infarct size based on the amount of CK-MB depleted from the myocardium.

References