Reperfusion and the Plasma Isoforms of Creatine Kinase Isoenzymes: A Clinical Perspective*

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The three CK isoenzymes. The introduction and application of creatine kinase (CK) isoenzymes significantly improved the sensitivity and specificity of detection of myocardial infarction (1). Elevated plasma creatine kinase, MB fraction (MB CK) is now regarded as the most sensitive, specific and cost-effective means of diagnosing acute myocardial infarction (2,3). The recent purification, characterization and detection of plasma CK isoenzyme subforms offers promise for further improvement and new applications. Creatine kinase is composed of two subunits, each with a molecular weight of about 41,000. The subunit M, so designated because of its abundance in muscle, forms MM CK, the predominant isoenzyme in cardiac and skeletal muscle. The B subunit, most abundant in the brain, forms BB CK, which in minimal amounts is also present in organs such as the gastrointestinal tract. The hybrid form MB CK is, except for trace amounts, found primarily in the heart, where it comprises about 15% of total myocardial CK activity, the remainder being MM CK. Hence, the specificity of MB CK is relative for myocardial injury. The two subunits M and B combine to form three isoenzymes. During electrophoresis under conventional conditions, MM remains essentially neutral at the origin, whereas negatively charged MB and BB exhibit anodal migration. In the 1960s and 1970s occasional reports appeared of additional isoenzymes but these were usually discarded as artifact. In 1977, Wevers et al. (4) showed that MM CK, on release into the blood, exhibited three bands on electrophoresis. They presented convincing evidence that blood somehow induced the formation of two MM CK molecules with more anodal migration than the form released from the tissue.

Technique of chromatofocusing to identify three iso-enzymes. In an attempt to clarify the mechanism responsible for conversion of tissue MM CK into three isoforms and to determine their physiologic and diagnostic significance, we developed a technique using chromatofocusing to isolate and purify the plasma isoforms. Results of these and subsequent studies (5–7) showed a single form of MM CK to be present in the tissue, whether myocardial or skeletal muscle, which, on release into the plasma, is sequentially converted into MM-2, a more negatively charged molecule, and then into MM-1, which is the most negatively charged. This conversion is due to carboxypeptidase N-induced hydrolysis of the C-terminal lysine of MM CK. On the basis of hybridization experiments and peptide mapping, we showed that MM-2 results from removal of lysine, a positively charged amino acid, from one subunit and MM-1 from removal of lysine from both subunits. Each isoform of MM CK has a distinct isoelectric point, but the molecular weight and catalytic activity are the same. The increased anodal migration of MM-2 and MM-1 on electrophoresis separates them from the parent tissue MM CK. In referring to the isoforms, a plea is made to conform to the international numerical classification of designating the most anodal form MM-1 followed by MM-2 and the tissue form MM-3. Amino acid analysis shows lysine to be the C-terminus of MM CK in the electric eel, dog, rabbit and human (8). Recently, we (9) cloned and sequenced the cDNA for human MM CK and confirmed lysine to be the C-terminus. The M subunit of several other species has now been sequenced with recombinant DNA techniques, and the C-terminus in each case is lysine (10–12). Carboxypeptidase N (13), which specifically hydrolyzes C-terminal arginine or lysine, is a ubiquitous enzyme found in the plasma of all mammals and vertebrates; thus, it would appear to be universal that a single tissue form of MM CK, on release into the plasma, is converted into more negatively charged forms of MM CK. The C-terminus of the B subunit is also lysine, but is somehow protected from carboxypeptidase and does not appear to develop plasma isoforms. However, the M

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subunit of MB CK undergoes hydrolysis; thus, MB CK exhibits two isoforms after release into the circulation.

**Kinetics of three isoforms on myocardial infarction.** Myocardial necrosis is heralded by a marked increase of plasma MM-3 and MB-2 (tissue forms) over that of the hydrolyzed forms. Results of studies in patients with acute myocardial infarction, as well as after cardiac surgery, show the expected kinetics of the three isoforms for MM CK (14–16). Unfortunately, there are currently few clinical data on the MB isoforms. Release of the tissue form MM-3 occurs very early after the onset of infarction and continues for 24 to 36 hours. The ratio of MM-3 to MM-1 reaches a peak within the first few hours. Shortly after release of MM-3, there appears an increase in MM-2 and MM-1, and as the MM-3 decreases, there is a corresponding reciprocal increase in MM-2 and MM-1. Early reperfusion, which occurs with cardiac surgery or after successful thrombolytic therapy, is associated with a more rapid release and disappearance of MM-3. Implicit in the isoform kinetics is the potential for improved assessment of acute myocardial infarction, particularly in reference to the following: 1) dating the onset of myocardial infarction; 2) earlier detection of infarction; 3) detection of early reperfusion; 4) detection of coronary reocclusion and early reinfarction; and 5) estimation of infarct size during reperfusion.

**Isoenzymes in myocardial infarction before and after reperfusion.** It is highly desirable to diagnose infarction before thrombolytic therapy and to noninvasively determine if reperfusion was induced. Elevated plasma MB CK activity after acute myocardial infarction without reperfusion is first detected within 4 to 8 hours by conventional electrophoresis, or within 4 to 6 hours using the more sensitive quantitative techniques (17). Plasma total and MB CK activities usually remain within the normal range for the initial 3 to 4 hours after the onset of symptoms. Baseline plasma CK activity exhibits a predominance of MM-1 isofrom of MM CK, that abruptly changes with release of CK from injured tissue. The circulation is flooded with MM-3 (tissue form) resulting in a severalfold increase of the ratio of MM-1 to MM-3. This may occur initially while total plasma CK activity is within the normal range. The ratio of MM-3 to MM-1 reaches a peak within the first few hours after onset of infarction and declines gradually as release declines and MM-3 converts to MM-2 and MM-1. Elevated plasma levels of MM-3 in experimental infarction can be detected within 1 hour of coronary occlusion, and in humans, within 2 to 3 hours of the onset of symptoms, whereas total CK is within normal limits (18). If these results are confirmed and rapid, convenient and sensitive assays can be developed, it would also be possible on admission to determine whether patients with chest pain should be admitted to a routine or coronary care unit bed, facilitating treatment that would be more prompt, appropriate and cost-effective. Currently, only 20 to 35% of patients admitted to the coronary care unit are subse-

quently confirmed to have acute myocardial infarction. After successful reperfusion, the plasma MM-3 activity is significantly elevated within 15 to 30 minutes, permitting a very rapid diagnosis. Analysis of the MM-3 temporal profile and extrapolation to the ordinate provide a reliable estimate of time of onset of infarction.

**Identifying successful reperfusion.** Clinical noninvasive assessment of intravenous thrombolytic therapy is now being explored intensively. Several clinical markers have been assessed, including abatement of chest pain and changes in the ST segment, but none have been found reliable (16). On successful reperfusion, the plasma CK temporal profile is one of rapid appearance and disappearance, which is quite distinct from that exhibited by patients who do not undergo reperfusion. Initial results using the time to peak MB CK were encouraging but later were shown to lack specificity. In a study (19) involving 133 patients with acute myocardial infarction, the mean rate of decline in the proportion of MM-3 in patients undergoing successful thrombolysis with recombinant tissue plasminogen activator (rt-PA) or streptokinase, as documented by coronary angiography, was approximately twofold greater than in patients with unsuccessful lysis or in patients who did not receive thrombolytic therapy. The overall diagnostic accuracy for detecting successful reperfusion was 82% with a sensitivity of 79% and a specificity of 75%.

**The rate of decline in MM-3 proportion as a noninvasive marker** is now being assessed prospectively as an ancillary study in the Thrombolysis in Myocardial Infarction (TIMI) trial. A secondary elevation in plasma MB CK has been shown to be a very sensitive and specific diagnostic marker for early extension of infarction (early reinfarction), but this refers to extension occurring 48 hours or more after the onset of the preceding infarction. Detection of extension within 24 hours of acute infarction, such as may occur with reocclusion after thrombolytic therapy, is not reliable with MB CK. Plasma MB CK activity peaks on an average of 23 hours (range 13 to 28); thus, detection and interpretation of a secondary elevation against a high background is likely to be relatively insensitive as well as nonspecific for secondary elevation during this interval. Because MM-3 declines rapidly, particularly with early reperfusion, secondary elevation should be reliably detected even after 12 to 18 hours from onset. The potential of MM-3 to detect early reinfarction and reocclusion is yet to be explored.

**Estimation of infarct size.** The need for accurate estimation of infarct size in patients with myocardial infarction during early reperfusion is great (20). The use of infarct size as an end point considerably reduces the number of patients required to document efficacy compared with use of the conventional end point—mortality. For example, to detect a 20% reduction in mortality with a power function of 0.8 and a probability value of less than 0.05 in a population having a 15% mortality rate, about 2,000 patients
are required; in contrast, a similar reduction in infarct size could be detected with a sample size of only about 350 patients in each group. Infarct size estimated with CK is a valid end point for clinical trials and has been shown to correlate closely with clinical variables, including mortality, and most recently with histologic estimates determined at postmortem (21). However, estimates of infarct size in the presence of reperfusion requires a new release ratio. This release ratio has been determined (22), but estimates using the new constant have yet to be validated. Quantitative estimates of the release of MM-3 should provide more accurate estimates and should accelerate progress in this area. In addition, serial analysis of MM-3 will provide an accurate means of knowing when tissue release of CK has ceased and make it possible for the first time to obtain accurate estimates of the individual CK disappearance rate and avoid the present necessity of having to use a mean value. This would provide more precise estimates of infarct size and be applicable in patients with or without reperfusion.

Clinical limitations and implications. The quantitative chromatofocusing technique (7), used initially for detection and purification of plasma isoforms, though very sensitive as subsequently shown by others (18), is too cumbersome for routine clinical or isoelectric focusing (4). The immuno blot technique (18) appears sensitive and quantitative but is extremely tedious and requires several hours to perform. The use of electrophoresis on high resolution agarose is simple, convenient, rapid and has the distinctive feature of being available in all clinical laboratories (23). However, it is only semiquantitative and, although adequate for most clinical uses, requires improved sensitivity for early diagnosis of myocardial infarction. The potential diagnostic and therapeutic value of the CK isoenzyme isoforms is quite significant; however, full exploitation of their potential, particularly for early diagnosis, is limited at present by the lack of a simple, rapid quantitative assay. This problem is, however, surmountable, and it is reasonable to expect a satisfactory resolution within the very near future.

References