Clinical Measurement of Myocardial Infarct Size

Modification of a Method for the Estimation of Total Creatine Phosphokinase Release after Myocardial Infarction

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SUMMARY
A modified method for the measurement of total creatine phosphokinase release from venous blood samples taken four-hourly after myocardial infarction has been used in 43 patients admitted to a Coronary Care Unit. The fractional decay rate \( K_D \) of enzyme activity has been measured by a standardized method in each patient, and accuracy of the calculation of total enzyme release has been improved by allowance for individual variations in decay rate, and discarding of data from which decay rates cannot be measured within confidence limits of less than ± 15%. Total enzyme release was greater in cases of transmural infarction than in patients with subendocardial infarction, and showed a good positive correlation with clinical indices of the extent of myocardial damage. As noted by previous workers, this method allows for the measurement of the rate as well as the extent of enzyme release, and so should prove useful in the clinical evaluation of therapeutic agents which might accelerate or retard the rate of myocardial necrosis in patients with acute myocardial infarction.

Additional Indexing Words:
Decay of enzymes in the circulation
Prognosis in myocardial infarction
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Now that coronary care units have reduced mortality from ventricular fibrillation, most hospital deaths from myocardial infarction occur because of cardiogenic shock and heart failure consequent upon extensive myocardial damage. For this reason, recent demonstrations that the size of experimental myocardial infarcts can be altered by therapeutic interventions\(^{4-8}\) offer real hope of benefit for these patients. Any therapeutic trial, however, must be assessed in terms of the effect of the agent on infarct size, which should be measurable by a noninvasive method.

The present report describes a modification of the method of Sobel and associates,\(^{4-8}\) for the measurement of total creatine phosphokinase (CPK) release into the circulation after myocardial infarction. A standardized method for calculation of the disappearance rate of CPK from the circulation in individual patients, which should improve the accuracy of the method, is described. Further modifications include the substitution of four-hourly for two-hourly sampling of venous blood which was found not to affect the accuracy of the method, and expression of the results as units of CPK release per ml of serum instead of as "infarct size" in absolute terms.

Methods

Collection of Specimens and Measurement of CPK Activity
Patients admitted to the coronary care unit had sampling of peripheral venous blood from an intravenous line inserted on admission to the unit. A polyethylene catheter 12" in length (Intracath, Bardic) was inserted percutaneously into an antecubital vein, and attached via a plastic three-way tap to a slow-running infusion of isotonic heparinized dextrose-saline. This infusion was used for medications such as lidocaine, which was given as necessary for the control of ventricular arrhythmias. Catheters were changed after 24–48 hr in order to minimize the risk of thrombophlebitis. Sampling was carried out by trained nurses, using appropriate sterile techniques. A small quantity (2 ml) of blood was withdrawn into a plastic syringe to clear the dead space of the catheter, and a further 2–4 ml was withdrawn into a second syringe for measurement of CPK activity, which was carried out on the specimens in batches every 24 hr. Sampling was usually carried out four-hourly, but in approximately one half of the cases blood was taken every one or two hours for the first 24 hours, and then four-hourly; the purpose of this was to check on variation of the integral of CPK release when calculated from one, two, or four-hourly enzyme levels. Sampling was continued for 2–4 days, until...
CPK activity had returned to near-normal levels (approximately 100 mIU/ml); it was discontinued if cardioversion was necessary, and usually had to be abandoned because of the technical difficulty of withdrawing blood in cases of cardiogenic shock. As intramuscular injections can themselves cause a release of CPK activity, all injections were given either intravenously or subcutaneously.

The accuracy of the method was assessed by measuring the activity of paired sera in duplicate, and the 95% confidence limits of a single estimation were found to be ± 5%. As it was sometimes necessary to store the serum in contact with the clot, preliminary experiments were done to determine the effect on CPK activity when serum was stored in contact with the clot for up to three days. No difference was found when these were compared with identical specimens which were separated immediately and refrigerated.

CPK activity was measured by Rosalki’s method, using a Unicam SP800 spectrophotometer at 340 nm fitted with an accessory SP850 scale expansion set to give a tenfold expansion of absorbance. A commercial test kit (Calbiochem) was used, and the reaction was carried out at 30°C.

Calculation of the Fractional Disappearance Rate (K0), and the Integrated Appearance Function (Et + K0ΣEdt)

The formula of Sobel and associates (Et + K0ΣEdt) was used in order to calculate the “integrated appearance function” for CPK. Preliminary theoretical analysis of the method of Sobel suggested that the accuracy of the method would be much improved if the “disappearance function” (termed K0) could be determined accurately for each individual patient and then used for the calculation of the integral for that patient. The following paragraph describes the present method for the calculation of K0, which is further illustrated by figures 1–3; reasons for the importance which we attach to accurate determination of this constant will be amplified in the Discussion.

Values for CPK activity from the peak down to near-normal levels were plotted against time on semi-logarithmic graph paper; as values were returning to normal (approximately 40 mIU/ml) rather than to zero levels, 40 mIU/ml was subtracted from each CPK value. The plot was then inspected to determine the value at which log CPK activity started to decline rapidly and uniformly towards near-normal levels, as it was inferred that at this stage enzyme release should be zero, enabling the CPK disappearance function to be studied independently of CPK release. In many cases, the semi-logarithmic plot showed a plateau occurring for 8–24 hr around the time of peak enzyme activity, which was followed by a rapid decline; in other cases, an initial slow phase of decline was followed by a more rapid fall.

In two atypical cases out of more than 50 examined, an initial rapid decline of activity was followed by a late slow disappearance phase; these cases were excluded from the study. Starting from the peak of the rapid phase of decline, the values were fed into a Hewlett Packard 9100B desk-top computer, programmed to calculate the fractional disappearance rate (K0), and the 95% confidence limits of the slope. By trial and error, five or more consecutive points were selected which gave the best fit, as judged by the lowest 95% confidence limits of the slope. As the error involved in the assumption that CPK activity is returning precisely to 40 IU/ml becomes greater the nearer the values return to normal, inclusion of values below approximately 100 mIU/ml was avoided whenever possible (figs. 1 and 2). Sometimes this was not possible, however, when a relatively low peak of activity was followed by a rapid decline. Since the error in calculation of the integral of CPK release (see Discussion) is identical with the error in determination of K0, cases in which confidence limits of K0 were greater than ± 15% were arbitrarily excluded from further study. For cases in which K0 could be determined within these confidence limits the integrated appearance function (Et + K0ΣEdt) was determined by the computer, subtracting 40 mIU/ml from each measured level, and including all points on the upslope and downslope of activity (figs. 1–5).

Figure 1
Serial CPK activity measurements in a male patient aged 51 years with inferior transmural infarction. Serial serum CPK activity levels (small closed circles), and a semi-logarithmic replot of the same levels from the peak of activity downwards (open circles) are shown, together with the calculated integral (Et = K0ΣEdt; large closed circles) for each measurement. An assumed normal value of 0.4 IU/ml (40 mIU/ml) has been subtracted from all measurements. K0 was calculated from nine points on the descending level of CPK activity, omitting the peak and succeeding four values on the assumption that the initial slow decline in activity (as judged from the semi-logarithmic plot) was influenced by continued release of CPK activity from the infarct. The last four points (.092 to .040 IU/ml) were not used for the calculation of K0, because error due to uncertainty about the precise normal level of CPK activity in this patient’s serum increases as the activity approaches normal. The value for K0 calculated from these nine points is .00096, with 95% confidence limits of ± .00002 (± 2%). The integral (integrated appearance function) reaches a plateau at the rapid phase of decline, but continues to increase during the early slow decline of activity; the slope of the integral curve at any time is proportional to the rate of release of CPK enzyme at that time.
Results

Decay rate of CPK Activity ($K_D$)

In early cases $K_D$ could often not be determined within 95% confidence limits of less than ±15%, the reason usually being failure to continue sampling for long enough during the phase of rapid decline in enzyme activity. In later cases the success rate improved to 22 (84%) of the last 26 cases. In all, acceptable values for $K_D$ were obtained in 33 patients. The mean number of values which were used to determine $K_D$ was seven (range 5 to 14), and the 95% confidence limits were less than ±5% in 16 cases (49%), less than ±10% in 27 (82%) and less than ±15% in 33 (100%). The mean value for $K_D$ in these 33 patients was 0.00109 (SD 0.0034, SEM 0.0006); this value agrees closely with that found by Sobel (0.001 ± 0.0001 SEM).

The possibility was considered that cases in which $K_D$ could not be calculated within the postulated confidence limits might have continued release of CPK due to recurrent infarction, obscuring the disappearance function of the enzyme. Inspection of the data showed that this was not the case; the rejected cases showed a continual decline in CPK activity, but at an apparently unsteady rate, while in several of the accepted cases a second peak of CPK release occurred due to a reinfarction, but with a subsequent steady decline in enzyme activity from which $K_D$ could be calculated.

Integrated Appearance Function ($E_t = K_0 \Sigma E_{id}$)

The integrated appearance function for CPK release was calculated for the 33 patients in whom $K_D$ was determined. In patients who had blood taken every one or two hours for the first 24 hours, all data were used for calculation of the integral. However, recalculation, omitting all but the four-hourly levels, showed an integrated appearance function which was on average, only 1% different (range 0–3%).

In ten patients who had subendocardial infarcts with a peak serum CPK activity of around 200 IU/ml, the fall in enzyme activity was not large enough for $K_D$ to be calculated according to our criteria. However, assumption of a mean figure of 0.00109 for these patients showed that the integrated appearance function (0.1–0.3 IU/ml) was low compared with that occurring in most patients with transmural infarction. The integrated appearance functions for these ten patients are included in the bottom right-hand portion of figure 4; although the 95% confidence limits of...
these integrals are wide (about ± 67%, see Discussion), they are included to show the lower order of magnitude of CPK release which occurs in most cases of subendocardial infarction.

Full data for three individual cases are displayed in figures 1–3, while values for all 43 cases are displayed in figure 4 in which patients are separated into groups having anterior, inferior, and subendocardial infarcts.

Correlation of the Magnitude of the Integrated Appearance Function with Clinical Indices of Severity of Infarction

Since determination of the integral of CPK release depends upon accurate determination of $K_D$, and hence sampling of venous blood for 3–4 days, patients dying early in hospital are excluded from the study. Only four (9%) of the 43 patients have died after a median follow-up period of six months (range 1–10 months). Three of these patients had anterior transmural infarction, and died suddenly without further chest pain at 8, 60, and 90 days after the infarct. The fourth patient had subendocardial infarction, and died after a further infarct. Two of the three patients who died following anterior infarction had the highest integrated appearance functions recorded (5.2 and 6.0 IU/ml, fig. 4). It was of particular interest that both of these patients had electrocardiographic evidence of acute right bundle branch block — a complication of antero-septal infarction which we have previously noted to be associated with clinical evidence of massive myocardial damage, and a high incidence of late sudden death from ventricular fibrillation.12–14

We have previously studied clinical factors influencing early and late survival after myocardial infarction,15,16 and have found the single most important prognostic factor to be the presence or absence of pulmonary venous congestion or pulmonary edema on a chest radiograph taken within 24 hours of admission to the hospital. Accordingly, patients having transmural infarction were separated into groups according to the presence or absence of pulmonary congestion or edema during the acute phase (fig. 5). Patients having congestion or edema had a mean integrated appearance function of 3.4 IU/ml, which was double that measured in the patients who were free from congestion (1.7 IU/ml; $P < 0.01$).

Discussion

Basis for Estimation of Infarct Size from Serial Changes in CPK Activity

Recent work by Sobel and colleagues6,17 has shown that a close relationship exists between the extent of CPK depletion from an experimental infarct and the total release of CPK activity into the circulation. A
method for calculating total CPK release (integrated appearance function) has been developed, based on the assumption that any instantaneous change in CPK activity must be due to the combined effects of an appearance function, i.e., release of CPK from the heart into the circulation, and a disappearance function due to removal of CPK from the circulation by a variety of mechanisms. The disappearance function was determined in conscious dogs\textsuperscript{6} by measuring the rate of decay of activity after intravenous injections of partially purified myocardial CPK, and in patients,\textsuperscript{7} by measuring the rate of decay as CPK activity was returning to normal after myocardial infarction. In both cases, the disappearance function was thought to be mono-exponential with respect to time, and was described as a fractional disappearance rate, having a mean value in dogs of 0.0048 ± 0.0003/min and in patients of 0.001 ± 0.0001/min. Knowing the value of the disappearance rate (K\textsubscript{D}), it was possible to calculate the integrated appearance function for CPK which was the total CPK activity per ml of serum released into the circulation by the myocardial infarct. Furthermore, by assuming values for CPK distribution space, for the proportion of released CPK activity which was degraded locally within the infarct, for the CPK activity of normal myocardium, and the amount by which this was depleted in the center of a myocardial infarct, it was possible to arrive at a figure for infarct size in grams, which would obtain if all the infarcted tissue were uniformly depleted of CPK.

Critical analysis of the method of Sobel and his colleagues shows that, in addition to the above four assumptions which are necessary in order to calculate infarct size from the integrated appearance function, calculation of the appearance function itself has an important potential source of error. The term used to express the integrated appearance function is E\textsubscript{t} + K\textsubscript{D}Edt, where E is the CPK activity at time t. As E declines exponentially after infarction, the integral approaches a constant (plateau), the height of which (integrated appearance function) is directly proportional to the value for K\textsubscript{D} which is used to calculate it.

By measuring the terminal slope of the decline in CPK activity in 24 patients, Sobel found a mean value for K\textsubscript{D} of 0.001/min. However, the standard error of the mean of these values was 0.0001, implying that the standard deviation was approximately 0.0005, or half as great as K\textsubscript{D} itself. Thus, assuming a mean value for K\textsubscript{D} in any particular patient must mean that CPK release is underestimated by 50% in patients in whom K\textsubscript{D} is 1 s/p above the mean, and by 100% in those in whom it is 2 s/p above the mean. Similarly, in patients with a low K\textsubscript{D}, CPK release will be overestimated to the same extent. For this reason, we considered it important to measure K\textsubscript{D} as accurately as possible in each patient, and to discard all data in which the constant could not be estimated within arbitrarily selected confidence limits.

Calculation of the Fractional Disappearance Rate (K\textsubscript{D})

Calculation of the integrated appearance function depends on the assumption that removal of CPK activity occurs exponentially. There is evidence that enzymes are cleared from the circulation at an exponential rate,\textsuperscript{15, 16} while a mono-exponential decay rate for CPK, following an initial mixing phase, has been shown to occur in conscious dogs.\textsuperscript{6} The assumption that the decay is also mono-exponential in patients recovering from myocardial infarction forms the basis for Sobel’s clinical method\textsuperscript{7} for calculation of total enzyme release, and for the present modification of this method.

There are at least three factors which might obscure a mono-exponential decline in CPK activity after myocardial infarction, however. First, myocardial CPK consists of two isoenzymes\textsuperscript{20, 21} and there is some evidence that the MB isoenzyme has a faster clearance constant than the MM form.\textsuperscript{7} If this occurred, the decline in total CPK activity would approximate to two exponentials, with an early rapid phase and later slow phase of decline. As already stated, we saw this pattern only twice in over 50 experiments, so it did not appear that there was an important difference between the K\textsubscript{D} of the MB and MM forms of CPK. No attempt was made in this study to separate these two isoenzymes of CPK, since precise quantitation of the MB form is uncertain using currently available methods of electrophoretic separation and fluorometric scanning.\textsuperscript{22} A second factor preventing a mono-exponential decline in activity would be the existence of a two-compartment distribution space for CPK; however, this should also be manifest by a rapid, followed by a slow, decline, and this did not occur in most experiments. Third, release of CPK activity from the myocardial infarct into the circulation might continue during the early phase of CPK decline; this almost certainly did occur in our patients, because a slow phase of decline nearly always preceded the more rapid phase as seen on the semi-logarithmic plot. Our method for calculation of the disappearance function from the start of the rapid decline to a level of 100–200 mIU/ml appears to avoid underestimation of K\textsubscript{D} due to nonrecognition of continued slow CPK release, while at the same time minimizing errors due to the assumption that CPK activity is returning to a level of precisely 40 mIU/ml. Moreover, computer-assisted calculation of the 95% confidence limits for K\textsubscript{D} allows for rejection of values which do not attain an arbitrarily selected confidence level.
Calculation of the Integral of Total CPK Release \((Et + K_p \Sigma Edt)\)

The main source of error in calculation of the integral of total CPK release is identical with the error involved in the calculation of \(K_p\); this has been reduced to less than \(\pm 15\%\) by the present method. Errors involved in the other terms used to calculate the integral, namely individual values for CPK activity \((E)\) up until the rapid decline phase, and the time between taking samples \((t)\), are likely to be of less importance, since errors in \(E\) are likely to cancel out by random distribution over 10–20 specimens, and errors in \(t\) can be minimized by punctual collection of blood samples. The potential error in measuring \(E\) at four-hourly rather than one or two-hourly intervals was studied, and was also found to be small (0–3%); the practical advantages of measuring fewer samples were thought to outweigh the slight loss in accuracy in sampling blood at four rather than one or two-hourly intervals.

Relation of Integrated Appearance Function of CPK Release to Myocardial Infarct Size

It has been shown in dogs that a close linear correlation exists between total CPK release, calculated from the integral \(Et + K_p \Sigma Edt\), and CPK depletion from the myocardium 24 hours later.\(^6\) However, only about one-third of enzyme activity lost from the myocardium could be accounted for by appearance in the circulation, so that it was assumed that two-thirds of the activity was degraded locally, presumably at its site of release from the myocardial infarct. If this proportion of local degradation is constant, the integral of CPK activity appearing in the blood should mirror reliably the loss of activity from the myocardium and, by inference, myocardial infarct size. The constancy or otherwise of local degradation of activity cannot be measured in man, and uncertainty on this point may constitute the most serious objection to the clinical use of total CPK release as a measure of infarct size.

No attempt has been made in the present study to absolutely quantitate total CPK release by calculating the product of CPK activity per ml of serum and the total distribution space for CPK (assumed to be a fixed proportion of body weight). In the first place, it is necessary for this and further calculations to assume figures not only for the CPK distribution space, but also for CPK activity in healthy myocardium, and for enzyme activity at the center of the infarct. None of these variables can be easily measured in individual patients. Furthermore, it is uncertain whether infarct size should be expressed in absolute terms in patients of varying body build and weight; a figure for total CPK release per unit of blood volume may mirror gradations of severity of infarction more reliably than a figure for total CPK release in absolute terms, since large patients are likely to have a high circulating blood volume, large hearts, and large myocardial infarcts.

On the other hand it must be remembered that the distribution space for CPK is likely to vary from time to time in the same patient, particularly when large changes in plasma volume occur as a result of diuretic therapy. This may cause unpredictable variations in enzyme activity levels which may affect calculation of \(K_p\), and of the integrated appearance function, whether or not the final result is related to an assumed distribution space.

Finally, total CPK release after myocardial infarction can be overestimated if the enzyme is released from striated muscle as well as from the myocardium. Intramuscular injections can cause a rise in CPK activity, but these were avoided in our patients, except in a few instances in which opiates were administered before admission to the coronary care unit. CPK release has also been described after cardioversion\(^{23}\) and in shock,\(^{24}\) due to poor perfusion of peripheral muscles. No cases of cardiogenic shock were included in our study, and collection of blood specimens was always abandoned following cardioversion.

Clinical Usefulness of the Measurement of Total CPK Release

There has been considerable interest recently in infarct size as a determinant of prognosis after myocardial infarction, but there is no firm agreement as to how this should be measured.\(^{25,26}\) Quantitation of total CPK release is a noninvasive method, needing only an intravenous line which can also be used for treatment. If four-hourly sampling is used, CPK release can usually be calculated from 18–24 samples of serum. Moreover, not only total CPK release but also the rate of release\(^6,27\) can be measured (figs. 1–3): it is likely that study of alterations in the rate of enzyme release following therapeutic interventions will throw light on the role of such agents in accelerating or retarding the rate of infarction. Disadvantages of the method are that it is applicable only for patients who survive long enough for \(K_p\) to be measured, and it is also unsuitable for patients who have had cardiac arrest or elective cardioversion.

The present study has shown an encouraging correlation between total CPK release and clinical indices of severity in infarction, and work is now proceeding on the effect of therapeutic interventions on the rate of CPK release.

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