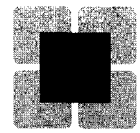

Assessment of the size of acute myocardial infarction I: biochemical methods

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■ Determination of infarction size is essential to evaluate interventions designed to salvage myocardium. Properly executed enzyme methods probably have the highest accuracy but can only be used in the early stage of infarction. Measurement of slowly catabolized enzymes such as LDH, LDH-1, and alpha-HBDH is recommended for routine purposes.

INDEX TERM: DIAGNOSIS, MYOCARDIAL INFARCTION CLEVE CLIN J MED 1990; 57:547–550

INFARCT SIZE is a major determinant of clinical outcome and long-term prognosis following acute myocardial infarction (MI).[1–3] Early interventions in acute MI, particularly thrombolytic therapy, primarily try to reduce infarction size.[3–8] Therefore, assessment of infarct size is a logical and direct way to determine and compare the efficacy of interventions such as thrombolytic agents.

See Bruschke and associates, part II (p 551–557).

This article reviews the uses, merits, and limitations of biochemical methods for assessing infarct size. A companion article deals similarly with nonbiochemical methods, including electrocardiography and various imaging methods.

RATIONALE FOR BIOCHEMICAL METHODS

The term "infarct size" refers to the volume or weight of the infarcted myocardium and factors that determine infarct size are closely related to the integrity of the plasma membrane. Metabolic derangements induced by ischemia lead to dissolution of transmembrane ionic gradients, depolarization and inexcitability, and, ultimately, to disruption of the plasma membrane—an unequivocal marker of cell death. The disintegration of the cell membrane allows efflux of intracellular and influx of extracellular macromolecules. Biochemical methods to assess infarct size may be based on both efflux and influx processes (the methods described in this article relate to the biochemical efflux process). Scintigraphy with radiolabeled antimyosin antibodies is an example of a method based on the influx process (see Part II). With methods based on the efflux process, damage may be assessed by measuring either the loss of intracellular macromolecules—most commonly myocyte proteins—from the myocardium, or their increase in the circulation.[9]

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ENZYME HISTOCHEMICAL METHOD

Among biochemical methods, the enzyme histochemical method continues to be considered by many investigators the gold standard for measuring infarct size. Yet, this method is inaccurate and cannot be performed in vivo. The procedure consists of cutting the heart in transverse slices and then staining for dehydrogenase. Noninfarcted myocardium stains blue within 15 minutes whereas in-

farcted myocardium, having lost its dehydrogenase, remains practically unstained. The visual assessment of the boundary between infarcted and noninfarcted myocardium is greatly hampered by regions with intermediate staining in which infarcted and noninfarcted myocytes may appear in an irregular and unpredictable pattern. After planimetry of the borders of the infarct in each slice, the "standardized" infarct size is calculated by summation, over all slices, of the product of the relative area infarcted and the slice weight. The assumption that the relative *area* infarcted times the slice weight equals the relative *volume* infarcted is an oversimplification that contributes to the inaccuracy of the method, especially if a small number of slices (five or fewer per heart) is used.

TIME-DEPENDENCY OF ENZYME ACTIVITIES

When proteins, including enzymes, are released from infarcted myocardium and enter the circulation, their concentrations or activities change over time. Apparently, the disintegration of the cell membrane of the infarcted myocyte enables the efflux of all cytoplasmic proteins which are not bound to structures such as the cytoskeleton and not confined to subcellular organelles such as mitochondria. Consequently, the different time-courses of the rise and fall of enzyme activities in plasma of a patient with acute MI are primarily caused by differences in clearance rates.

The infarction-induced increase in enzyme activity in plasma was first reported in 1954 by LaDue and coworkers.[10] They noted that the plasma aspartate aminotransferase (AST) activity in 16 patients with acute transmural MI rose to levels 2 to 20 times greater than normal within 24 hours, and then returned to normal range within 3 to 6 days.

Later reports indicated that other enzymes, such as lactate dehydrogenase (LDH), alpha-hydroxybutyrate dehydrogenase (alpha-HBDH), creatine kinase (CK), and the MB-isoenzyme of CK (CK-MB), as well as nonenzymatic proteins such as myoglobin and myosin light chain (LC-myosin) were also liberated by the acutely infarcted myocardium, although the time-dependency of their concentrations in plasma differed considerably. In many years of collaborative research with Hermens and colleagues, we have proven that the enzymes and proteins in the myocyte cytoplasm are liberated simultaneously and to the same relative degree, and are recovered completely in the circulation.[11]

If the plasma enzyme curve is corrected for the normal plasma enzyme level and for enzyme clearance, a cumulative enzyme curve is obtained which starts at 0 U/L at the onset of infarction (t=0) and rises along an S-shaped curve toward a plateau that is reached at about t=72 hours. The plateau level is the total activity of an enzyme released by the infarcted myocardium per liter of plasma. Subsequent multiplication by the patient's plasma volume and division by the enzyme activity per gram of normal myocardium gives the enzymatic infarct size in grams of myocardium.

TABLE 1
CRITERIA FOR ENZYME OR NONENZYMATIC PROTEIN ASSESSMENT OF INFARCT SIZE

High concentration in ventricular myocytes (sensitivity)
Absent in other cells (specificity)
Stable concentration in ventricular myocardium, independent of localization and concomitant abnormalities such as ventricular hypertrophy
Slow clearance from plasma

MEASUREMENT OF ENZYME ACTIVITY

The total activity of an enzyme that appears in plasma is equal to the total activity of that enzyme lost from the infarcted myocardium.[11,12] Theoretically, the quantity released can be calculated for each of the before-mentioned enzymes (CK, CK-MB, and LDH) and for nonenzymatic proteins such as myoglobin and LC-myosin. However, practical considerations limit the number of enzymes and other proteins suitable for this purpose (*Table 1*).

Since no enzyme, isoenzyme, or nonenzymatic protein meets all the criteria listed in *Table 1* the advantages and disadvantages of each choice must be considered. Standardization, accuracy, cost, and speed are all important considerations.

For routine purposes we advocate measurement of slowly catabolized enzymes and isoenzymes such as LDH, LDH-1, and alpha-HBDH. Alpha-HBDH is a measure of enzymatic activity for the five LDH isoenzymes, in decreasing order from LDH-1 to LDH-5. That is, the myocardial LDH isoenzymes, LDH-1 and LDH-2, have high alpha-HBDH activity, whereas the LDH isoenzymes of skeletal muscle and the liver, LDH-4 and LDH-5, have low alpha-HBDH activity. Blood samples are obtained for testing every 12 hours for 4 days after admission; in the event of reinfarction, the sampling protocol starts again.

Although we routinely use alpha-HBDH, the recently marketed LDH-1 test kits, based on immunoprecipitation of all LDH-isoenzymes except LDH-1, may be a good alternative.

After measurement of plasma enzyme activities, the cumulative activity release is calculated according to a

method based on a two-compartment model.[13,14] One compartment, the intravascular space, accepts the enzyme released from the infarcted myocardium. The enzyme is cleared from the intravascular space via an enzyme-dependent fractional catabolic rate (FCR). It may diffuse into the second compartment, the extracellular space, as well as backward into the intravascular space (plasma pool) via the enzyme-independent transcapillary escape rate (TER) constant and the extravascular return rate (ERR) constant, respectively.

The quantity of enzyme entering the plasma up to time t (Q[t]) equals the sum of the enzyme activity in the plasma at time t, the enzyme activity in the extravascular space at time t, and the activity that has been eliminated up to time t (*Figure 1*). To accurately determine the enzyme's FCR, one must start when the release phase has ended; ie, after the first 72 hours. Estimation of FCR for rapidly cleared enzymes and isoenzymes such as CK and CK-MB is difficult because their activities return to normal or near-normal after 72 hours. This phenomenon has led frequently to underestimations of infarct size.

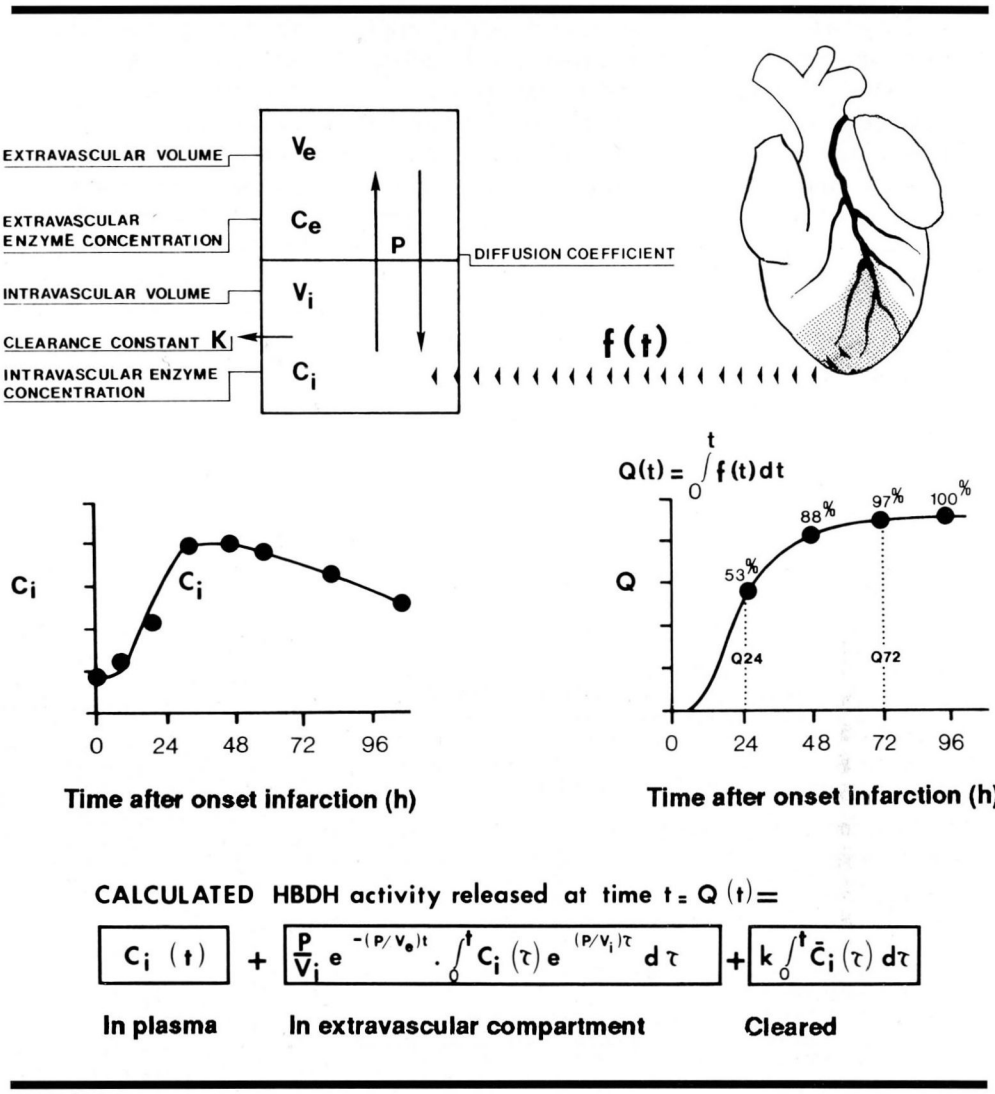

Figure 1. Two-compartment model to represent enzyme movements after release from infarcted myocardium. The integrated enzyme input function f(t) represents the cumulative quantity released from the infarct. This equals the sum of the quantity in plasma, the quantity in the extravascular compartment, and the quantity already cleared from the circulation (from van der Laarse[14] with permission).

THROMBOLYSIS AND ENZYME CURVES

Early thrombolytic recanalization of the infarct-related coronary artery has an effect on plasma enzyme curves.[15,16] Shortened time to peak plasma enzyme activity is often interpreted as washout of previously unperfused regions, but this interpretation is by no means convincing. In isolated perfused animal hearts, reoxygenation after a period of anoxia leads to accelerated enzyme release in the absence of any impairment of flow. Therefore, reperfusion-induced acceleration of enzyme release is merely a symptom of accelerated necrosis; the term "reperfusion damage" was coined to describe this phenomenon.[17]

Using alpha-HBDH to calculate enzymatic infarct size, it was shown that early thrombolytic therapy

limited infarct size by an average of 30%.[18] Alpha-HBDH release was accelerated in patients who received thrombolytic therapy, compared to the patients who received conventional treatment. However, rapid alpha-HBDH release proved to be an unreliable predictor of reperfusion since the relative release rate is inversely related to infarct size in patients assigned to conventional treatment; that is, the rate is high in small infarctions and low in large infarctions.[19]

It has been suggested that in reperfused infarcts, more enzyme activity is released per gram of infarcted myocardium than in unreperfused infarct.[19–21] In fact, however, the differences in quantities of enzyme released from reperfused and nonreperfused infarcts are based on "differences" in the apparent disappearance rates (Kd) of CK (or CK-MB). These Kd values do not represent the true FCR, as the clearance process overlaps with the release process.

De Zwaan and associates demonstrated that the parameters used in the two-compartment model, such as FCR of CK, CK-MB, and AST are identical in patients who receive thrombolytic therapy and in patients assigned to conventional treatment.[22] In addition, Hermens and colleagues showed that local inactivation of enzyme activity in the infarcted myocardium does not take place, since the activity of an enzyme lost from the infarcted heart is recovered completely in the circulation.[11]

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