Condensing Enzyme Levels in the Serum After Experimental Myocardial Infarction

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Following tissue damage several tissue enzymes are released into the serum. On this basis, changes in serum glutamate-oxalacetate transaminase and lactate dehydrogenase have been used as indicators of the occurrence and severity of myocardial infarction. These tests lack specificity because damage to liver and other organs also causes an increase in the serum levels of these enzymes. Recently we observed that heart tissue contains singularly high levels of condensing enzyme (see reaction 2 below). We wondered whether a rise in serum condensing enzyme would prove to be an indicator of myocardial damage more specific than either glutamate-oxalacetate transaminase or lactate dehydrogenase. The present study demonstrates the release of condensing enzyme into serum following experimental myocardial infarction in dogs.

Methods

One acute and 5 chronic myocardial infarction preparations were studied. Dogs were anesthetized by intravenous injection of sodium pentobarbital, 30 mg. per Kg. Myocardial infarcts were then induced by tying the anterior descending coronary artery according to the procedure of Harris. The animals with chronic infarctions were studied for one week. Periodic venous blood samples were taken, allowed to clot, and centrifuged. The serum was analyzed for condensing enzyme, and glutamate-oxalacetate transaminase. Both of these enzymes were stable in serum at -20 C. At the time blood samples were taken, standard lead II electrocardiograms were recorded on a direct-writing Cardiotron machine.

Condensing enzyme was assayed by measuring the rate of increase of absorbency at 340 m/ due to DPNH* formation in the following reactions:

1. L-malate = + DPN+ OAA= + DPNH + H+

2. AcCoA + OAA= + H2O citrate= + CoA + H+

A cuvette having a light path of 1.0 cm. and 1.0 ml. volume was used. Aliquots of 0.1 ml. of serum were assayed. To show that the enzyme assayed was condensing enzyme, the formation of DPNH

*The following abbreviations are used in this text: DPNH, reduced diphenylpyridine nucleotide; DPN+, diphenylpyridine nucleotide; AcCoA, acetyl coenzyme A; CoA, coenzyme A; OAA=, oxaloacetate.
was shown to depend upon the presence of serum, L-malate, and acetyl CoA. The rate of DPNH formation was shown to depend upon the amount of serum used in the assay. The average rate per minute in the interval from 3 to 10 minutes was used in the calculation. This rate obtained at room temperature (24 to 27) was corrected to correspond to the rate at 25.0 C. The correction of 10 per cent per degree was determined with purified condensing enzyme from pig heart. The unit of activity is defined as the amount of enzyme forming one micromole of DPNH per minute, and the enzyme concentration is expressed as the number of units per L. of serum. The number of micromoles of DPNH formed has been found to be equivalent to the number of micromoles of citrate produced. The units are calculated using the factor 6.22 absorbency units as equivalent to 1 micromole of DPNH formed.

Glutamate-oxaloacetate transaminase was assayed by measuring the rate of decrease of absorbency at 340 m\textmu due to the disappearance of DPNH in the reactions:

3. \textit{\alpha-ketoglutarate$^+= +$ aspartate$^-$}\textit{\rightleftharpoons L-glutamate$^-$ +$ OAA$^+$}.

4. \textit{OAA$^-$ +$ DPNH +$ H$^+$}\textit{\rightleftharpoons L-malate$^-$ +$ DPN$^+$.}

In this case, the unit is similarly defined as the amount of enzyme causing 1 micromole of DPNH to be utilized per minute, and the concentration is expressed again as the number of units per L. of serum.

Heart tissue extracts were prepared by homogenizing 5.0 Gm. of the heart muscle with 2 successive portions of 45.0 ml. of 0.4 M KCL in 20 per cent alcohol in a water-cooled Waring Blender for 10 minutes. The homogenates were centrifuged and the supernatant fluid assayed for condensing enzyme.

Results

No condensing enzyme activity was detected in normal dog serum by the method used. The enzyme was not detected in the serum of dog no. 1 with acute coronary occlusion in an open chest preparation, nor in the serum of animals nos. 2 and 4 in which only a minor branch of the coronary vessel was occluded. These animals serve as a control for the effect of the operative procedure. In dogs nos. 3, 5, and 6, condensing enzyme was detected in the serum about 7 hours after occlusion and reached a peak value about 21 hours after occlusion (fig. 1); the activity disappeared within a few days.

The serum glutamate-oxaloacetate transaminase values for dogs nos. 4, 5, and 6 are
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plotted in Figure 1. In terms of pyridine nucleotide reacted, the total transaminase activity was about 10 times the total condensing enzyme activity at the 21-hour peak, when both these values are maximal. Glutamate-oxalacetate transaminase was found in the normal serum at approximately 8 units per L. and rose to 24 units per L. of serum as a result of surgery alone (i.e., in dog no. 4 in which a ligation of a minor branch of the coronary vessel failed to produce an infarction).

One week after the coronary occlusion, each dog was anesthetized with sodium pentobarbital and the heart removed. Infarcted and corresponding normal areas were extracted and assayed for total units of condensing enzyme. Dogs nos. 1, 2, and 4 contained no infarcted areas. The extractable condensing enzyme from infarcted areas of dogs nos. 3, 5, and 6 is compared in Table 1.

Serial electrocardiograms showed changes characteristic of myocardial infarction in dogs nos. 3, 5, and 6, and essentially no changes in dogs nos. 2 and 4. Dogs nos. 3, 5, and 6 showed T-wave inversion and progressive Q-wave enlargement over the 7-day period. Ventricular ectopic beats appeared at about 6 hours postocclusion and persisted until 48 to 72 hours after surgery. The maximum rate of ventricular ectopic activity was observed from 18 to 24 hours. The sequence of electrocardiogram changes in dog 6 is shown in Figure 2.

In addition to the studies on the dogs, we have examined the sera of 4 patients who suffered myocardial infarctions. Condensing enzyme could be distinctly demonstrated in these sera, although the activity was lower than that found in the dogs studied. No activity could be detected in normal human serum.

Discussion

The expected positive correlation between the amount of enzyme lost from the infarcted area and the amount appearing in the blood was noted in the 3 dogs with significant infarctions. The animal that showed the highest concentration of serum condensing enzyme was dog no. 3, which had the largest infarct with the lowest residual condensing enzyme concentration in the infarcted area. Further, there is a time correlation between the serum levels of condensing enzyme and of transaminase, with both reaching peak serum concentrations at about 21 hours after occlusion. Finally, there is a rough correlation between condensing enzyme level in the serum and the ventricular ectopic activity recorded on the electrocardiogram. By the time the condensing enzyme level had fallen to very low levels (60 to 90 hours), ectopic activity had disappeared, and the heart again had a normal sinus rhythm.

Because the concentration of condensing enzyme is relatively low in other tissues (especially in liver) and high in heart muscle, the appearance of condensing enzyme in the serum may give a more specific indication of heart muscle damage than the serum transaminase activity. We are now studying this possibility.

Summary

Increased levels of condensing enzyme are shown to occur in the sera of dogs after experimental myocardial infarction. The animals with the elevated levels also showed characteristic changes of the electrical activity of the heart and associated increases in the glutamate-oxalacetate transaminase activity of serum.

Condensing enzyme has been detected in the sera of patients who suffered myocardial infarctions.

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Summario in Interlingua

Esseva demonstrate que elevationes del nivellos de enzyme condensatori occurre in le sera de canes post infarcimento experimental del myocardio. Le animales que exibivin le elevate nivellos sera de enzyme condensatori otiam exibivin alterationes caracteristic del activitate electric del corde et le associate augmentos del activitate de transaminase glutamico-oxalacetetic in le sera.
Enzyma condensatori esseva detegite in le sero de patientes qui habeva suffrite infarcimentos myocardial.

References
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