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Clinical Applications of Lactate Dehydrogenase Isoenzymes

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ABSTRACT

A quantitative electrophoretic test for the determination of lactate dehydrogenase isoenzymes was applied to the analysis of human tissues, cells and fluids in order to obtain their normal isoenzyme patterns and to form a reference record. The same test was employed in the analysis of serum samples from patients with defined pathological conditions. The abnormal serum isoenzyme patterns were correlated with the tissue patterns, thus indicating the origin of the abnormality. This type of correlation, together with the clear demonstration of the actual isoenzymes and their quantitation, improves diagnostic discrimination and enhances the early detection of a biochemical abnormality that aids in the prevention of disease.

Introduction

The glycolytic enzyme lactate dehydrogenase (LD) is found in human tissues, cells and fluids. It exists in five molecular forms or subunits which have been named isoenzymes or isozymes. The quantitative distribution of the five LD-isoenzymes in various tissues and fluids is different and characteristic for each. The release of LD-isoenzymes from a tissue into the adjacent fluid owing to abnormal cellular metabolic activity inflammatory conditions, degenerative processes, toxicity, or injury causes a change of the normal fluid pattern. The resulting pattern more nearly resembles that of the particular tissue affected, thus indicating the possible site of origin of the abnormality. This correlation forms the basis for the use of the isoenzymes in diagnostic applications.

Methods

There are several electrophoretic methods available for the determination of LD-isoenzymes. These methods utilize either cellulose acetate membranes, agar, agarose or acrylamide gels as support media. Although the LD-isoenzyme patterns of tissues and fluids obtained by the various methods are qualitatively comparable, quantitative differences exist. For this reason, it is important to apply the same technique for the determination of LD-isoenzymes originally in tissues, to obtain a reference record, as well as in routine analysis of fluids, in order to make meaningful com-

parisons and to enhance diagnostic specificity.

Our contribution in this field has been the development of a method for the quantitative determination of LD-isoenzymes[1] as well as its application for the detection of biochemical abnormalities and to aid the diagnosis of disease.[2,3,4]

The main features of our technique[1] are the use of agar gel on a microscope slide as a support medium for the separation of the LD-isoenzymes, the application of a measured amount of serum in a narrow slit that enables the production of clear LD-isoenzyme patterns and the use of barbital buffer of low ionic strength which allows the application of high voltage and reduces the time of electrophoresis to 13 minutes. Following electrophoresis, the slide is incubated in a buffered mixture of substrate and co-factors, which also contains the colorless tetrazolium salt iodonitrotetrazolium (INT). During the incubation, INT is reduced to formazan, producing pink zones on the sites of the isoenzymes. At the end of the incubation, an instant photograph of the isoenzyme pattern is obtained which allows evaluation by visual inspection and forms a permanent record. Finally the intensities of the zones are quantitated by densitometry and their percent distributions are calculated. The details of the procedure for measuring lactate dehydrogenase isoenzymes are given in the paper by Papadopoulous and Kintzios.[1]

Applications

The agar gel electrophoretic method has been applied to (1) the determination of the LD-isoenzyme patterns of normal human fluids, tissues and cells in order to establish a reference record of the normal patterns and (2) the analysis of serum samples from various pathological cases in order to correlate abnormal isoenzyme patterns with defined pathological conditions. This information is used for interpretation of the results obtained in routine determinations of unknowns.

In Fluids

Blood is allowed to clot at room temperature. The serum is separated by centrifugation and analyzed the same day or a day later after storage at 4°C. The biological fluids cerebrospinal and aqueous, are centrifuged after removal and analyzed the same day. Anticoagulants and preservatives are not added to avoid spurious results. As shown in a typical example (figure 1), five LD-isoenzymes are determined in normal human serum which are clearly separated and delineated on the microscope slide. They are numbered one to five in order of decreasing mobility toward the anode. The average percent dis-

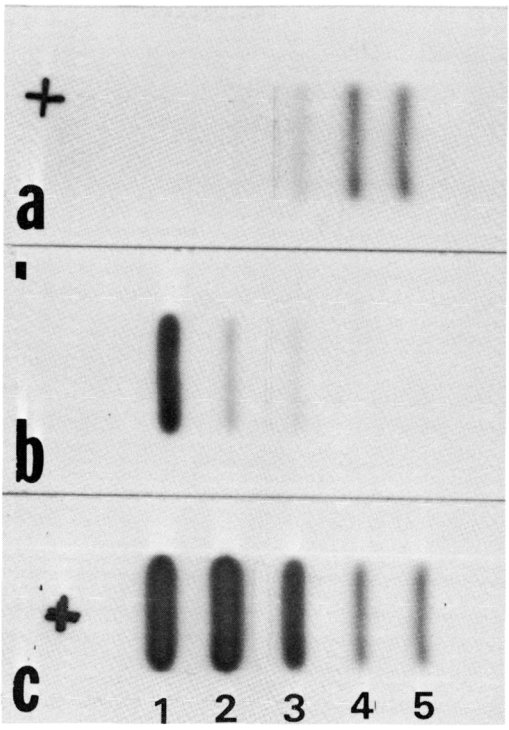

FIGURE 1. Electrophoretic patterns of lactate dehydrogenase isoenzymes in normal human fluids where A = aqueous, B = cerebrospinal and C = serum.

tribution of the LD-isoenzymes in normal serum by this technique is 30-40-20-6-4 for isoenzymes one to five, respectively.

The LD-isoenzyme patterns of typical normal samples of cerebrospinal fluid (CSF) and aqueous humor are also included in the same figure. When compared with the serum these patterns are significantly different. In CSF, LD-1 predominates; in the aqueous humor LD-5, 4 and 3 are more prominent. The determination of LD-isoenzymes in CSF may be useful for the detection of brain tissue abnormalities and their origin since there is a different distribution of the isoenzymes in the gray and white matter of the brain. The LD-isoenzyme patterns of aqueous humor and ocular tissues have been determined.[2] This determination may find useful application in

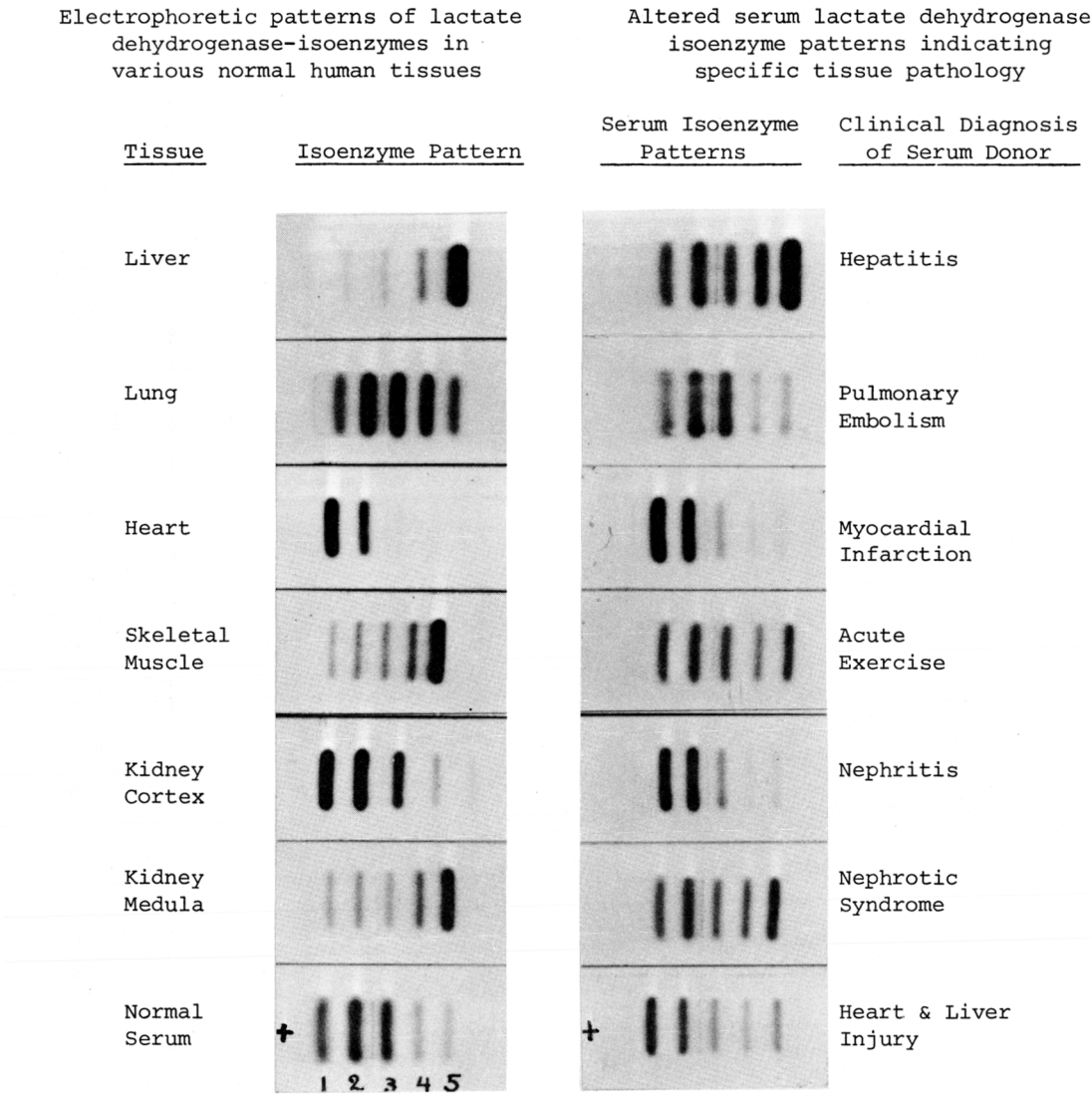

FIGURE 2. Representative examples of the patterns of various normal tissues obtained by the author's technique.

the detection of eye tumors which is difficult by present methods.

In Tissues

The LD-isoenzyme patterns of normal human tissues were determined in biopsy and autopsy specimens which were homogenized in a phosphate buffer 0.1 M, pH 7.4. Representative examples of the patterns of various normal tissues thus obtained by our technique are shown in figure 2. Typical examples of abnormal serum LD-isoenzyme patterns from patients with various pathological conditions are also included in the same figure for associations and comparison with those of the normal tissues.

Several observations are apparent from this figure. (1) The distribution of LD-isoenzymes in various tissues is different and characteristic for each. (2) In homogeneous tissues such as the heart, only one pattern can be demonstrated. In heterogeneous tissues such as the kidney, two or more different patterns are found. (3) A correlation can be made between abnormal serum LD-isoenzyme patterns from patients with pathological conditions with the patterns of normal human tissues from which they may be derived. (4) Multiple tissue injury can be detected as illustrated by the increased densities of LD-1 and 5 in heart and liver injuries. (5) The LD-isoenzyme patterns in certain tissues are sharply different from each other, e.g., the heart and the liver, while the differences in others are not as obvious. Following the detection of an abnormal LD-isoenzyme pattern, its interpretation may require determination of other enzymes and isoenzymes specific for the tissues potentially involved and the clinical assessment of the patient.

Cells

Normal human blood cells were separated and isolated by centrifugation over a Ficoll-Isopaque separating medium* and lysed in a hypotonic solution. The LD-isoenzyme patterns of erythrocytes, lymphocytes and granulocytes are shown in figure 3. In the same figure are also included the LD-isoenzyme patterns of (1) a normal blood hemolysate, (2) a serum sample from a patient with lym-

* A lymphocyte separating medium Litton Bionetics, Kensington, MD.

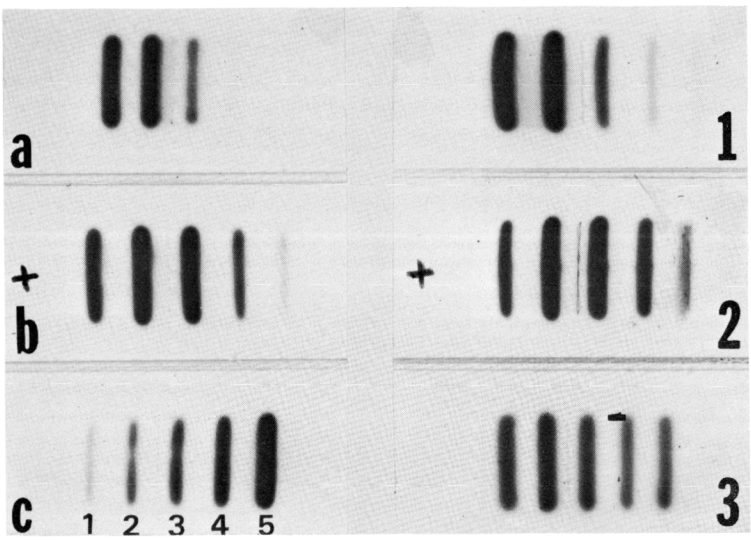

FIGURE 3. Electrophoretic patterns of lactate dehydrogenase Isoenzymes in normal human blood cells (A, B, C) and abnormal serum samples (1, 2, 3) where A = erythrocytes, B = lymphocytes, C = granulocytes, 1 = hemolysate, 2 = lymphocytic leukemia and 3 = granulocytic leukemia.

phocytic leukemia and (3) a serum sample from a patient with granulocytic leukemia for diagnostic comparisons.

It is apparent in this figure that the LD-isoenzyme patterns of the various normal human blood cells are distinctive for each type of cell. Their characteristic patterns may serve as markers to identify disproportionate amounts of these cells in a mixed cell population. Their proliferation in leukemic blood is evident from the shift in LD-isoenzymes compared to those in normal sera. Furthermore these characteristic patterns of LD-isoenzymes may serve as an indication of the target cell involved in the malignancy.

The reference library of the LD-isoenzyme patterns of normal tissues and their comparison to the sera patterns of patients with defined pathological conditions form the basis for interpreting abnormal LD-isoenzyme patterns. This information in turn is used to aid in the diagnosis of disease. An interpretation may be simple when the test is confirmatory e.g., the increase of LD-5 in a patient with jaundice or an increase of LD-1 and 2 and reversal of their ratio in a patient with acute myocardial infarction. The similarity of patterns between certain tissues and the potential contribution of multiple factors to an abnormal isoenzyme pattern require the employment of additional enzymatic and other laboratory tests to facilitate the interpretation of the isoenzyme pattern. Examples are the determination of creatine kinase and glutamic pyruvic transaminase to distinguish an increase of LD-5 between liver and skeletal muscle or the determination of creatine kinase to distinguish between hemolysis and heart muscle injury when LD-1 and 2 are increased. The laboratory information is then correlated with a careful clinical assessment of the patient for the final diagnosis of disease.

Conclusion

The determination of LD-isoenzymes is a useful test for clinical and research applications because of their presence in all tissues and cells, their relative stability and consistently measurable activity. Methodological details such as the clear demonstration of the LD-isoenzymes on a microscope slide and the use of the same technique to determine normal and abnormal patterns further enhance its clinical usefulness.

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