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Selective Determination of Lactate Dehydrogenase Isoenzyme 1 in Serum after Inhibition by 1,6-Hexanediol

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Summary: A rapid selective method for measuring the activity of lactate dehydrogenase isoenzyme LD-1 in serum by using 1,6-hexanediol as an inhibitor of the M-subunit was developed.

Hexanediol was added to serum at a final concentration of 0.7 mol/l. After incubation at 30 °C for 15 min, the activity was measured with an automatic analyser. The inter-assay coefficient of variation was 6.9% for the lactate dehydrogenase isoenzyme LD-1 measurement. The results obtained from the sera of 100 patients analysed by the proposed selective method and by the conventional electrophoretic method, respectively, showed an excellent correlation. This selective method was used to determine the lactate dehydrogenase isoenzyme LD-1 activity of sera from patients with acute myocardial infarction, and the results were correlated well with those obtained by the immunological, Roch Isomune method. Addition of 1,6-hexanediol did not affect the measurement of activities of other enzymes such as alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase and alanine aminotransferase.

Introduction

Recently, many investigators have reported the use of lactate dehydrogenase (EC 1.1.1.27) isoenzymes and creatine kinase (EC 2.7.3.2) isoenzymes in suspected myocardial infarction (1–3), and the precise, accurate and automated determination of lactate dehydrogenase isoenzyme activities in serum has been discussed (4–6).

We previously reported a manual method for measuring the activities of lactate dehydrogenase isoenzymes LD-1 and LD-2 in serum, involving 1,6-hexanediol as an inhibitor of the M-subunit (7). The LD-1 and LD-2 isoenzymes were well discriminated from the other three isoenzymes (LD-3, LD-4, LD-5) by this method. Analytical recovery tests and correlation experiments with the conventional electrophoretic method supported the validity of the analysis with this inhibitor.

We applied this method to the assay of LD-1 isoenzyme activity in serum, using an automatic analyser.

Materials and Methods

We used serum samples taken for routine analysis in the Central Clinical Laboratory of our University Hospital. Total enzyme activity was measured by the continuous method with Concmitrate-LDH reagent (Chugai Pharmaceutical Co. Ltd., Japan) by the routine procedure using the Clinalyzer JCA-MS 24 (Nippon Denshi Electric Co. Ltd., Japan). This reagent contains 100 mmol/l phosphate buffer, pH 7.4, 0.69 mmol/l pyruvate and 0.217 mmol/l NADH. Decrease of absorbance at 340 nm was read at 37 °C. Samples were read 2 min after starting the reaction.

We added one part of hexanediol stock solution to four parts of serum to yield a final appropriate concentration. After incubation at 30 °C for 15 min, we measured the enzyme activities in sera with the automatic analyser described above.

Manual measurement of the enzyme activity and electrophoresis of isoenzymes and subsequent staining of enzyme activity on "Microzone plus" electrophoresis membranes (Beckman Instruments, Inc., Fullerton, CA; Cat. No. 655420) were carried out according to the methods reported previously (7). The inhibitor, 1,6-hexanediol was of extra pure grade from Aldrich Chemical Co., Inc., Milwaukee, WI, USA.

Immunochemical determination of lactate dehydrogenase isoenzyme LD-1 activity was carried out by using the Roche Isomune-LD method (Roche Diagnostic Systems, Division of Hoffman-La Roche Inc., Nutley, NJ, USA).

Results

Procedure optimization

To determine inhibition as a function of time and temperature, we measured manually lactate dehydrogenase activities in normal sera after inhibition with 1,6-hexanediol at several temperatures and after several different periods of incubation. From figure 1, it is evident that the residual catalytic concentration of lactate dehydrogenase remains constant after incubation for 15 min at 30 °C at hexanediol concentrations of 500 to 700 mmol/l, and remains constant for at least a further 15 min. These levels after incubation at 30 °C corresponded well to levels of LD-1 isoenzyme determined in normal serum by electrophoresis. At 25 °C, the time needed to attain these levels was increased. On the other hand, the activity was inhibited more rapidly and markedly at 37 °C.

In order to determine an appropriate concentration of inhibitor, we measured lactate dehydrogenase activities with the automatic analyser in normal sera and sera from patients with liver disease and myocardial infarction in the presence of different concentrations of 1,6-hexanediol; the resulting activities were compared with those obtained by the electrophoretic method (tab. 1). It is evident that the final concentration of hexanediol necessary to obtain the true value for LD-1 isoenzyme activity alone is 700 to 750 mmol/l. This hexanediol concentration of 700 mmol/l was used in the proposed selective method for measuring LD-1 isoenzyme activity.

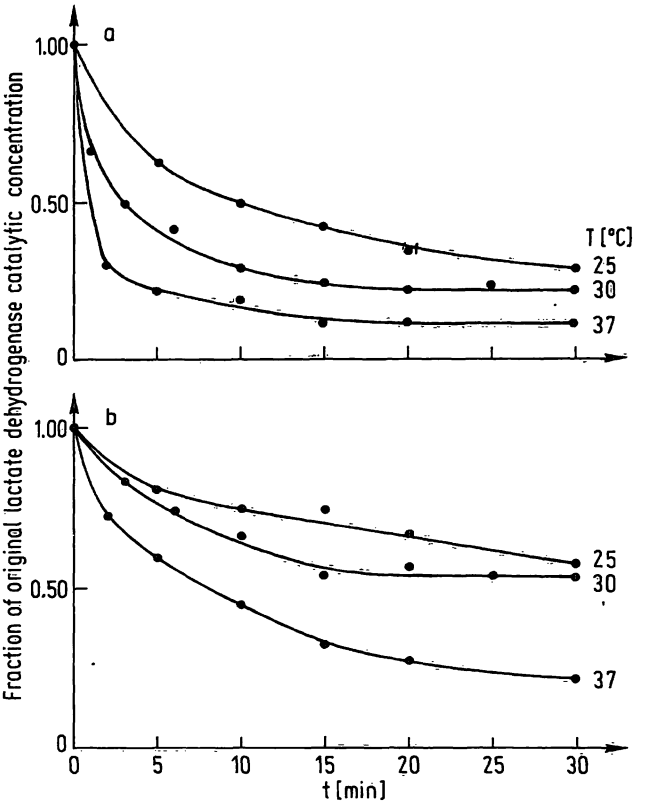

Fig. 1. Inhibition of lactate dehydrogenase activity by 1,6-hexanediol as a function of time and temperature.
Lactate dehydrogenase activities in normal sera were measured manually after incubation with 1,6-hexanediol at
a) 700 mmol/l
b) 500 mmol/l
and at several time intervals.

Precision study

We studied the precision on the proposed method by using normal human serum, containing a total lactate dehydrogenase activity of 232 U/l and in which LD-1 isoenzyme represented 25.5% of the total activity as determined by the electrophoretic method. Mean ± standard deviation from 8 measurements of LD-1 isoenzyme activity in this serum was 25.8 ± 1.8%, with an inter-assay coefficient of variation of 6.9%.

Method comparison

The results obtained from the sera of 100 patients analysed by the proposed method and by a conventional electrophoretic method, respectively, were compared. LD-1 isoenzyme activities as measured by the former method (y) and by latter (x) correlated well (fig. 2). Twenty seven of these 100 patients had liver disease and 9 had myocardial infarction.

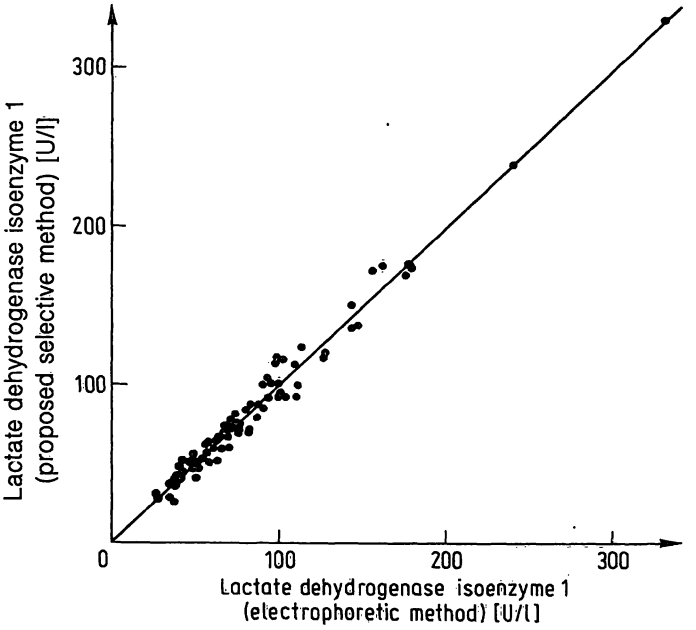

Fig. 2. Catalytic concentrations of lactate dehydrogenase isoenzyme LD-1 by the proposed selective method vs those measured by the electrophoretic method.
$y = 1.019x - 1.54$
$r = 0.9958$
$n = 100$
$\bar{x} = 91.82$
$\bar{y} = 92.10$
$s_{yx} = 8.08$

Tab. 1. Effect of 1,6-hexanediol concentrations on the measurement of the activity of lactate dehydrogenase isoenzyme LD-1.

	After inhibition by 1,6-hexanediol at a final concentration of				After electrophoresis LD-1 isoenzyme %
	650 mmol/l	700 mmol/l	750 mmol/l	800 mmol/l	
Normal sera (n = 7)	37.9 ± 3.0	26.7 ± 2.5	25.8 ± 3.2	24.9 ± 3.3	26.3 ± 2.7
Patients' sera (liver disease n = 7)		15.9 ± 6.4			15.5 ± 3.8
Patients' sera (heart disease n = 7)		32.7 ± 10.6			33.7 ± 8.4

Numerals are lactate dehydrogenase % total serum activity (mean ± 2 SD, standard deviation).
n: sample size

Other enzyme activities in the presence of hexanediol

We also measured activities of alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase and creatine kinase after addition of hexanediol solution (final concentration of 700 mmol/l). From the least-squares and *Student*'s t-test parameters, essentially no differences between data of each enzyme activity measured before and after the addition of inhibitor were observed ($p < 0.025$, $df = 74$), but creatine kinase activities were about 10% inhibited by 700 mmol/l of hexanediol.

Practice in acute myocardial infarction

As we were able to obtain sera from patients with acute myocardial infarction, we measured their LD-1 isoenzyme activities by the proposed selective method, by the electrophoretic and by the immunochemical, Roche Isomune method (tab. 2). Creatine kinase isoenzyme MB activity in each patient was 26.0% in case M.T. and 7.6% in case Y.K. at the onset. The results obtained by these three different methods during the first 3 days or 2 days were in close agreement.

Discussion

Measurement of the lactate dehydrogenase isoenzyme LD-1 level is required in the diagnosis of suspected myocardial infarction and in the use of thrombolytic therapy (3, 8), and in the diagnosis of renal disease, etc. (9–11).

We previously described a manual inhibition method, using 1,6-hexanediol as an inhibitor of the M-subunit (7). In the present study we have developed a rapid method to replace the manual inhibition method.

In the manual method, we measured enzyme activities after incubation of serum with the inhibitor for 15 min at 30 °C and immediately after dilution with the reaction medium. In the analysis by the Clynalyzer, it is difficult to ensure an incubation time of precisely 15 min before analysing each sample, but no changes of activities were observed after incubation for longer than 15 min as shown in the figure 1. Furthermore, when the samples are diluted in the reaction medium, the inhibition is completely abolished. However, it is desirable that the activities are measured automatically throughout the whole process under the same conditions as in the manual method. Another type of automated system, for example, the centrifugal

Tab. 2. Activity of serum lactate dehydrogenase isoenzyme LD-1 in acute myocardial infarction.

Patients		Roche Isomune method LD-1 isoenzyme	Electrophoretic method LD-1 isoenzyme	Inhibition method by 1,6-hexanediol LD-1 isoenzyme
M.T.	At the onset of chest pain	59.4%	57.5%	56.8%
	2 days after the onset	56.8%	53.9%	53.2%
	4 days after the onset	59.4%	52.7%	50.4%
Y.K.	At the onset of chest pain	61.5%	51.9%	51.9%
	The 2nd day after the onset	48.7%	51.7%	50.8%

Procedures for the Isomune, electrophoretic and inhibition methods are given in the text.

system, in which the incubation period and temperature can be adjusted as necessary for the inhibition, might be more suitable.

The minimal concentration of 1,6-hexanediol necessary in the selective method for measurement of LD-1 isoenzyme was found to be 700 mmol/l. An essentially acceptable inter-assay coefficient of variation was obtained in precision studies under these conditions. An excellent correlation was obtained between the selective method and the conventional electrophoretic method, when patients' sera with various levels of lactate dehydrogenase activities were used. We found this method was reliable for the rapid quantification of LD-1 isoenzyme activity in serum. However, it was difficult for us to distinguish a leukaemia case from liver disease or normal subjects, for example, the case with low LD-1 and high LD-3 isoenzyme activities (isoenzymes %: LD-1 12.7, LD-2 39.3, LD-3 34.8, LD-4 11.7, LD-5 1.5) from the case with low LD-1 and high LD-5 isoenzyme activities (LD-1 19.8, LD-2 22.3, LD-3 10.9, LD-4 3.9, LD-5 43.1). This proposed selective method seems to be suitable for the determination of the activity of lactate dehydrogenase isoenzyme LD-1 in the diagnosis of suspected myocardial infarction.

The addition of 1,6-hexanediol as an inhibitor did not interfere with the measurement of other enzyme activities, but we must report a slight decrease of creatine kinase activities caused by hexanediol.

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