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SOME NOTES ON SERUM LDH ANOMALY

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Abstract

Seven examples of LDH isozyme anomaly were encountered for the past three years through screening 2,572 patient's sera submitted to our laboratory for routine chemical study. The sera with abnormal LDH isozymes were from the patients with multiple myeloma (3), chronic hepatitis (2), encephalomalacia (1) and coronary insufficiency (1). The abnormality (electrophoretically slow, fast, deleted or split) was related to LDH-2, LDH-3, or LDH-4 isozymes, and immunoelectrophoresis combined with LDH-staining revealed its cause to be conjugation of immunoglobulin (Ig-G or Ig-A) of κ-type with the relevant LDH isozymes (LDH-2, LDH-3, LDH-4). Participation of immunoglobulins of λ-type in formation of abnormal LDH isozyme was never encountered. κ-type light chain may constitute an arm which grips LDH subunit when immunoglobulin conjugates with LDH. IgM was not concerned with LDH anomaly, probably because it is too large or inadequate for conjugation with LDH molecule.

INTRODUCTION

It has been established that serum LDH isozyme test is a useful measure for the diagnosis of neoplastic diseases, myocardial infarction and hepato-biliary disorders. Relative intensity of stained electrophoretic stripes of individual LDH isozymes (LDH-1～LDH-5) show various patterns which are pathognomonic of these diseases when viewed en bloc. However, rarely some single isozyme stripe is deleted or shifted, giving rise to an unusual electrophoretic pattern. This is called "LDH anomaly"[1,2,3], and the cause of its appearance has hitherto been attributed to either

1) abnormal structural gene of subunits (H, M) of the molecule of LDH[4].
2) acquired plasma protein abnormality such as conjugation of a particular LDH isozyme with an immunoglobulin[5~14], etc.

For the recent 3 years, during the course of routine LDH isozyme examination we experienced seven examples of LDH-anomaly and we made

efforts to elucidate the characters of the anomaly through careful observation of thermostability test, dissociation test with urea, mixing with normal or pathological serum and immunoelectrophoresis. This paper aims to present the results obtained in our study.

MATERIAL AND METHOD

1. Examination of LDH Isozymes

A modified method of Wieme's[15] agar gel electrophoresis[16] was employed. An aliquot of 15 μl of patient's serum was applied to the surface of agar gel layer laid on a microscopic slide glass (pH 8.6). The slide glass was cooled in petroleum ether and ice at 5°C in the electrophoretic apparatus. Electrophoresis (pH 8.6) was carried out for 60 minutes at a voltage gradient of 100 V/cm. At the end of electrophoresis the slide was stained by Van der Helm's tetrazolium method[17]. Agar grains of Difco Special Noble, Eiken (Japan) or Nakarai (Japan) was put in Veronal-HCl buffer solution (pH 8.6, $\mu=0.04$) and boiled until they were dissolved in order to make warm 0.85 % agar solution.

2. Thermostability Test of Isozymes

LDH isozyme thermostability test described previously[13,14] was carried out on patient's sera by incubating them for 20 minutes at 45, 55 and 60°C to see the change in electrophoretic isozyme patterns.

3. Denaturation Effect of Urea

Dilution series of urea solutions (5M~0.5M) were prepared. Serum specimens (0.1 ml) were mixed with an equal volume of the urea solutions and allowed to stand for 20 minutes at room temperature. Electrophoretic isozyme tests were carried out in the same manner as described in 1.

4. Observation of the Changed Isozyme Pattern Caused by Mixing Patient's Serum with Normal Serum

Changed isozyme patterns were observed electrophoretically after the specimens (0.1 ml) were mixed with an equal volume of normal serum.

5. Immunoelectrophoretic Test

Scheidegger's agar gel immunoelectrophoresis[18] was employed to demonstrate the lines of precipitation of each protein ingredient of patient's serum with anti-A, anti-G, and anti-M sera. LDH was stained in the same way as described in 1 after washing to detect stained precipitin arcs in comparison with normal specimens. Similar tests were also done with anti-κ and anti-λ sera.

RESULT

We found seven examples of "anomaly" out of 2,572 patient's sera through our routine laboratory work (Table 1). These abnormal LDH sera were from multiple myeloma (3 out of seven cases), chronic hepatitis (2 cases) encephalomalacia (one case) and coronary insufficiency (one case). Interestingly, deviation of serum protein concentration was not so evident in the multiple myeloma cases: hyperproteinemia was seen in only one and in the other two cases the serum protein was within the normal range. No abnormal LDH isozyme was found in 524 examples of hyper-γ-globulinemia specimens. The result of our examination on the seven examples of abnormal LDH isozymes is shown in Table 2. As seen from this table, the majority of abnormality is concerned with LDH-2. LDH-2's in case 1, 2 and 4 were slower in migration to the anode (denoted by 2s) while that in case 3 faster than the normal LDH-2 (denoted by 2f). In case 6 a broad trailing of LDH-3 and the absence of LDH-5 stripe were visualized. In case 7, LDH-4 migrated close to LDH-3 because of slower mobility of LDH-3 (3s) and faster mobility of LDH-4 (4f) to the cathode. In case 5, LDH-5 stripe was abnormal, being split into two peaks. Sometimes these abnormal isozymes showed relatively strong resistance to the deleterious effects of temperature and urea (Table 2). The LDH stain of the precipitin arcs produced by immunoelectrophoresis revealed positive staining of abnormal isozymes with Ig-G-κ (case 1), Ig-A-κ (cases 2 and 4) and Ig-A-κ plus Ig-G-κ (case 7).

DISCUSSION

A considerable number of communications have been published in the literature on abnormal LDH isozymes (Kreutzer[1], Lundh[5], Voigt[6], Ganrot[7], Kindmark[8], Biewenga[9], Nagamine[10], Takatsuki[11], Kano[12] and Shoda[13,14]. Most of the authors who investigated the abnormal LDH isozyme attributed the abnormality to the change in electric charge caused by conjugation of the relevant LDH isozyme with immunoglobulin. It seems that the other physicochemical properties of LDH isozyme are also influenced by the conjugation with immunoglobulin, one of which is represented by the altered thermostability. It has been said by some authors that thermostability is variously altered in abnormal isozymes. In our experience there was no example which showed lower thermostability. In case 1, we found an interesting finding. We could not find any LDH-2 isozyme corresponding to that of normal sera, but LDH-2s appeared when the equal volumes of patient's and normal sera were mixed. It is thought that this is accounted for by the assumption that

TABLE 1

Seven Cases of Serum LDH Isozyme Anomaly

Case	Age	Sex	Diagnosis	LDH activity (Wroblewski u.)	Serum Protein (g/dl)	Serum Zn TT (Kunkel u.)	Serum protein fractions Alb	α_1	α_2	β	γ	Ig (mg/dl) A	G	M	Blood Hb (g/dl)	Blood WBC (g/mm^3)
1	81	F	Myeloma	290	7.8	37.2	30	2	5	6	57	90	2350	74	9.6	3950
2	65	M	Encephalo-malacia**	630	7.3	15.5	52	3	12	10	22	150	1330	81	12.0	8100
3	61	M	Myeloma	450	6.9	5.9	49	4	7	10	30	130	2600	45	9.6	8600
4	44	M	Chronic hepatitis	1100*	7.2	6.9	69	4	6	8	13	150	1400	92	15.5	5500
5	73	M	Myeloma	270	10.8	—	23	4	1	21	43	480	3200	120	6.8	3000
6	77	F	Coronary insufficiency	480	7.8	11.5	50	5	8	9	28	56	3320	105	12.5	6200
7	41	F	Chronic hepatitis	650	7.8	10.9	65	3	9	8	15	140	2100	85	12.3	8700

* Serum GPT was in normal range.

** Slight azotemia (BUN 21.5 mg/dl) was noted.

*** Multiple myeloma cases were diagnosed on the basis of increased plasma cell percentage in the bone marrow aspirate as well as by the electrophoretic demonstration of M protein in serum.

TABLE 2 Laboratory Data of the Seven Cases of LDH Isozyme Anomaly

Case	LDH Activity (Wroblewski u.)	Isozyme Pattern	Abnormal Isozyme: Thermo-stability	Abnormal Isozyme: Resistance to Urea	Abnormal Isozyme: Conjugation with normal serum ingredient	Conjugation with L Chain of Ig (+)	Conjugation with L Chain of Ig (−)
Normal	100-400	(+) 1 2 3 4 5 (−)					
Case 1	290	1 2s 3 4 5	2s higher than normal	2s stronger than normal	2s +	Ig G-κ	
Case 2	630	1 2s 3 4 5	2s much higher than normal	2s much stronger than normal	2s −	Ig A-κ	
Case 3	450	1 2f 2 3 4 5	2f equal to normal	2f equal to normal	2f −		Ig G-κ myeloma
Case 4	1100	1 2s 3s 4 5	2s 3s much higher than normal	2s 3s much stronger than normal	2s 3s −	Ig A-κ	
Case 5	270	1 2 3 4 5f 5	5f equal to normal	5f equal to normal	5f −		Ig G-κ myeloma
Case 6	480	1 2 3 4 5	3s equal to normal	3s equal to normal	3s −		Benign Ig G-κ monoclonal gammopathy
Case 7	650	1 2 3s 4f 5	3s 4f equal to normal	3s 4f equal to normal	3s 4f −	Ig A-κ Ig G-κ	

massive conjugative Ig-G existing in patient's serum (See table 1) changed LDH-2 of normal sera to LDH-2s by conjugation. Similar phenomenon was, however, not observed in other cases. The positive LDH staining of the precipitin arcs of abnormal isozymes immunoelectrophoretically demonstrated with anti-Ig-G or anti-Ig-A connotes that the relevant isozymes are conjugated or coupled with immunoglobulins (Ig-G or lg-A). There was no example in which Ig-M precipitin arc was stained by LDH staining. Probably, LDH isozyme may not conjugate with Ig-M. In addition, precipitin arcs related to the isozymes were all of κ-type when examined with anti-light chain serum : precipitin arcs produced by anti-κ serum was stainable with LDH-staining, but the arcs formed with anti-λ serum faild to stain. This suggests that only the immunoglobulins of κ-type conjugates with the abnormal LDH isozymes. This is an interesting fact which has never been mentioned in the literature. We examined additional 8 cases of λ-type multiple myeloma in the same way cautiously, and got no LDH-stainable immunoglobulin precipitin arcs. In case 4, only the precipitin arc of anti-κ serum could be stained, although both of anti-κ and anti-λ serum produced the precipitin arcs. This provides further supports to the view that only the immunoglobulin (Ig-G or Ig-A) with κ-type light chain can conjugate with LDH isozyme. It may be supposed that the κ chain makes an arm which grips LDH subunit, probably more easily than λ chain does.

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