Abnormal Lactate Dehydrogenase Isoenzyme in Serum and Tumor Tissue of a Patient with Neuroblastoma

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Serum and tumor tissue of a patient with neuroblastoma contained an abnormal isoenzyme of lactate dehydrogenase (LDH; EC 1.1.1.27), which, on agarose gel electrophoresis, migrated between LDH-2 and LDH-3 with a mobility the same as that of the extra LDH isoenzyme found in normal human erythrocytes. Upon surgical removal of the tumor, the high total LDH activity (775 U/L) in the serum of the patient rapidly decreased to normal (70–220 U/L), and the abnormal LDH isoenzyme was no longer detected. The total LDH activity of the abnormal LDH isoenzyme per gram of hemoglobin in the tumor tissue was 26 times that of erythrocytes, suggesting that the abnormal isoenzyme originated mainly from the tumor cells themselves rather than the erythrocytes contained in the tumor tissue. This first report on the appearance of the abnormal LDH isoenzyme in a patient with neuroblastoma suggests that this abnormal LDH isoenzyme may have some significance as a marker enzyme for neurogenic tumors.

Lactate dehydrogenase (LDH; EC 1.1.1.27) is a tetramer composed of two types of subunits, H and M, and has five distinct isoenzymes, designated LDH-1, -2, -3, -4, and -5 according to the composition of their subunits. The proportion of these isoenzymes differs in various organs, and the human erythrocytes usually contain an extra LDH isoenzyme, which migrates between LDH-2 and LDH-3 in gel electrophoresis. This additional LDH isoenzyme, designated "y" by Zail and Van der Heo (1), is not detected in the normal serum, but can be detected in the serum of patients with paroxysmal nocturnal hemoglobinuria or hemolytic anemia.

Here we report on an abnormal LDH isoenzyme that we detected in the serum of a patient with neuroblastoma. It co-migrates with the LDH isoenzyme "y" on agarose gel and evidently originates in the neuroblastoma cells.

Case History

A six-year-old boy complaining of abdominal pains was hospitalized on May 28, 1982. He was diagnosed as having an abdominal tumor, which was removed on June 8, 1982. The 530-g tumor was identified histopathologically as a neuroblastoma.

Materials and Methods

Sample preparation. Serum, erythrocytes, and tumor tissue from the patient were evaluated for LDH isoenzymes. Erythrocytes of a normal subject were used as the control. Serum, hemolysates, and tumor extracts were either analyzed on the same day or kept at −80 °C until analysis.

Hemolysates were prepared by adding two volumes of physiological saline to the clot, collecting the suspended erythrocytes by centrifugation (3000 rpm, 5 min), washing them three times with 10 volumes of physiological saline, and suspending them in nine volumes of distilled water containing 10 mL of Triton X-100 surfactant per liter to hemolyze them. After 30 min, the mixture was again centrifuged (3000 rpm, 10 min) and the supernate was used.

To prepare the tumor extract, we homogenized the removed tumor, using a Willems Polytron (Kinematics GmbH, Luzern, Switzerland) for four 30-s intervals, with nine volumes of 0.05 mol/L Tris HCl buffer (pH 7.2) containing 10 mL of Triton X-100 per liter. The homogenate was centrifuged (25 000 × g, 30 min) and the supernatant fraction was used as the tumor extract.

Assay of the enzyme activity. Total LDH activity was determined in an aca discrete analyzer (Du Pont, Wilmington, DE 19898) according to the modified method of Wacker et al. (2). We measured LDH in each fraction obtained by gel filtration (with Sephadex G-200 chromatography, see below), using a Model 716 discrete analyzer (Hitachi Co., Tokyo, Japan) and an ultraviolet method (3) (Daiichi Chemicals, Tokyo, Japan).

Isoenzyme analysis. LDH isoenzymes, separated by agarose gel electrophoresis (Corning Medical, Medfield, MA 02052), were detected on the electrophoretic plate by use of an LDH staining kit containing nicotinamide adenine dinucleotide, phenazine methosulfate, Nitro Blue Tetrazolium (p-nitro tetrazolium blue), and sodium lactate (Muto Pure Chemicals, Tokyo, Japan). The amounts of each isoenzyme were determined by densitometry, by scanning the gel film at 570 nm.

Gel filtration with Sephadex G-200 column. Tumor extract and hemolysate, 2 mL of each, were applied to a 1.6 × 90 cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Uppsalas, Sweden) that had been equilibrated with 0.1 mol/L phosphate buffer, pH 7.2. The proteins were eluted with the same buffer, and 2.1-mL fractions were collected. The protein concentration in the eluent was monitored by measuring the absorbance at 280 nm.

Determination of hemoglobin concentration. Hemoglobin in the tumor extract and hemolysate was measured by a benzidine method (4).

Results

Table 1 summarizes laboratory data on the patient at admission. We observed remarkably high values for serum LDH activity (775 U/L), urinary dopamine (4100 µg/24 h), and leukocytes (17 700/mm³). Serum LDH isoenzymes as evaluated by gel electrophoresis showed increased LDH-1 and the appearance of the abnormal LDH isoenzyme, accounting for 4.7% of the total LDH activity and being detected between LDH-2 and LDH-3 on the zymogram.

The tumor was surgically removed on the 12th day after hospitalization. All the serum LDH activity dramatically decreased to 269 and 221 U/L on the 3rd and 13th day after the operation, respectively. Moreover, as shown in Figure 1, the abnormal isoenzyme pattern of LDH returned to normal and concomitantly the abnormal band also disappeared.

Total LDH activity of the tumor tissue was 32.5 U/g of wet tissue. When the tumor extract was analyzed by gel electrophoresis at the same concentration of Triton X-100 as used for the serum, the pattern of the abnormal isoenzyme was identical to that of the serum. On agarose gel electrophoresis, the abnormal isoenzyme was not detected in the serum of the patient, while the isoenzyme "y" was detected in the serum of the control. The tumor extract and hemolysate of the patient showed increased LDH-1 and the appearance of the abnormal isoenzyme, which migrated between LDH-2 and LDH-3 on gel electrophoresis. However, the tumor extract and hemolysate of the control showed only the normal isoenzymes.

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Table 1. Laboratory Data on the Patient at Admission

<table>
<thead>
<tr>
<th>Biochemical examination of serum</th>
<th>Percentage of LDH isoenzymes in serum</th>
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<tbody>
<tr>
<td>TP</td>
<td>LDH-1 66.1</td>
</tr>
<tr>
<td>T-Bil</td>
<td>LDH-2 16.0</td>
</tr>
<tr>
<td>D-Bil</td>
<td>Extra isoenzyme 4.7</td>
</tr>
<tr>
<td>AST</td>
<td>LDH-3 9.1</td>
</tr>
<tr>
<td>ALT</td>
<td>LDH-4 1.7</td>
</tr>
<tr>
<td>LDH</td>
<td>LDH-5 2.1</td>
</tr>
<tr>
<td>ALP</td>
<td>LDH-2 16.0</td>
</tr>
<tr>
<td></td>
<td>Extra isoenzyme 4.7</td>
</tr>
</tbody>
</table>

Urinary catecholamine  
E 6.8 µg/24 h  
NE 30.7 µg/24 h  
DA 4100 µg/24 h  
Hematological examination  
RBC 3.69 x 10^6/mm³  
WBC 17.7 x 10^3/mm³  
Hb 103 g/L  
Ht 29.9 %

Abbreviations: TP, total protein; T-Bil, total bilirubin; D-Bil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; E, epinephrine; NE, norepinephrine; DA, dopamine; RBC, red blood cells; WBC, white blood cells; Hb, hemoglobin; Ht, hematocrit. In parentheses: normal reference intervals.

Fig. 1. Serum LDH activities after admission  
Broken line: upper limit of the normal reference interval

Fig. 2. LDH isoenzyme pattern of serum collected from the patient pre- and post-operatively  
a. Before operation (at admission, LDH 775 U/L); b. the third day after the operation (LDH 269 U/L)

Fig. 3. LDH isoenzyme pattern  
A. Patient's serum at admission; B, tumor extract; C, patient's hemolysate; D, hemolysate of a normal subject

The retic mobility of this abnormal isoenzyme was the same as that of the abnormal isoenzyme found in the serum (Figure 3A) and that of the isoenzyme "Y" in erythrocytes (Figure 3B). The fractional value of the activity of "Y" in the erythrocytes of the patient was almost the same as that of the normal subject (6.6%). Also, the LDH isoenzyme pattern of the erythrocytes of the patient did not differ from that of the erythrocytes of the control subject (Figure 3D).

When the tumor extract was chromatographed on the Sephadex G-200 column, LDH was eluted with a single peak (Figure 4), in which the abnormal LDH isoenzyme was also contained, indicating that the tumor tissue did not contain LDH having a different molecular mass.

It was found by microscopic examination that the tissue contained a considerable amount of erythrocytes, as hemorrhages were observed in some area of the tumor tissue. To exclude the possibility that the abnormal LDH isoenzyme originated from the erythrocytes but not from the tumor cells, we compared the activity of the abnormal isoenzyme per gram of hemoglobin in tumor tissue with that in erythrocytes. As shown in Table 2, the former activity was 26 times that of the latter, suggesting that the abnormal isoenzyme found in tumor tissue may originate from the tumor cells.

Discussion

There have been several reports of anomalies of LDH isoenzymes, such as immunoglobulin-linked LDH (5-8), LDH subunit variant (9, 10), and LDH subunit deficiency (11, 12). The present patient with neuroblastoma, whose serum contained an abnormal LDH isoenzyme migrating between LDH-2 and LDH-3 on gel electrophoresis, also had abnormal LDH isoenzyme in the tumor tissue. According to the report of Kinumaki et al. (13) on serum LDH of patients with neuroblastoma, the total activity of serum LDH increased in 20 of 26 patients and the proportion of total LDH that was LDH-2 increased in some patients. In our case, the isoenzyme pattern differed entirely from the cases of Kinumaki et al. (13), since the content of LDH-1 increased remarkably and an abnormal LDH isoenzyme co-migrating with "Y" appeared (Table 1 and Figure 3). As for an interpretation of the appearance of the abnormal LDH isoenzyme in the serum of the patient, the following possibilities are to be considered: (a) the abnormal isoenzyme...
originate from erythrocytes; (b) there is complex formation of LDH with some protein such as immunoglobulin; or (c) the abnormal isoenzyme originates from the tumor.

Although erythrocytes reportedly contain an extra LDH isoenzyme in addition to the ordinary LDH isoenzymes on the zymogram, there have been no detailed reports on its biochemical properties or on the mechanism by which it appears. By electrophoresis, we examined the occurrence of the extra LDH isoenzyme in various tissues (liver, kidney, pancreas, lung, spleen, lymph node, skeletal muscle, adrenal gland, thyroid gland, cerebrum, cerebellum, and sciatic nerve) obtained at necropsy. Because the extra LDH isoenzyme was not detected in these tissues, we presumed that the erythrocyte is the only cell that normally contains the extra LDH isoenzyme. On the occurrence of an abnormal LDH isoenzyme in pathological organs, there has been only one report on patients with a brain tumor, that of Soetens et al. (14). On the other hand, Zail and Van den Hoek (1) analyzed LDH of human erythrocytes by polyacrylamide gel electrophoresis and reported that the LDH isoenzyme "y" activity made up 2% of the total LDH activity. Our data on analysis of LDH isoenzymes of erythrocytes showed the occurrence of the LDH isoenzyme "y" in all of 16 normal subjects and the activity of "y" accounted for 7.1 ± 0.9% (mean ± SD) of the total LDH activity. Although the activity of "y" in erythrocytes of the patient with neuroblastoma accounted for 6.6% of the total LDH activity, it would be possible that "y" of the erythrocytes could appear in serum if there were violent hemolysis in the blood vessels. In this patient, however, there was no symptom indicating hemolytic disorders, although anemia was recognized. For these reasons, we believe we can assume that the abnormal LDH isoenzyme found in the serum of the patient with neuroblastoma did not originate from erythrocytes, an interpretation supported by the observation that the serum LDH activity dramatically decreased in a short time after removal of the tumor (Figure 1), accompanied by normalization of the isoenzyme pattern of serum LDH and the disappearance of the abnormal LDH isoenzyme (Figure 2).

Possibility a (above) may be excluded, because the molecular mass of the abnormal LDH isoenzyme in the tumor was identical with the ordinary LDH isoenzymes. Therefore, we believe that possibility c best explains the occurrence of the abnormal LDH isoenzyme in the serum of the patient.

However, it is not clear whether the abnormal LDH isoenzyme found in the tumor tissue is biochemically or immunochemically identical with "y" of erythrocytes.

References
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