Clinical Studies on Bovine Leukemia in Japanese Black Cattle
III. Serum Lactate Dehydrogenase Activity and Its Isoenzyme Pattern in Groups of Leukemic Cattle and Those Negative or Positive for Antibody against Bovine Leukemia Virus

Katsuya ISHIHARA, Tsuyoshi OHTANI and Hitoshi KITAGAWA

Department of Veterinary Internal Medicine, Faculty of Agriculture,
Gifu University, Kakamihara-shi, Gifu 504

Misao ONUMA
Department of Epizootiology, Faculty of Veterinary Medicine,
Hokkaido University, Sapporo-shi, Hokkaido 060

(Received for publication September 7, 1979)

Abstract. Serum lactate dehydrogenase (S-LDH) activity was determined four times over a time course in 178 Japanese Black cattle of two groups which were positive and negative, respectively, for antibody against bovine leukemia virus. As a result, no significant difference in S-LDH activity was evidenced by the two groups at any time of testing. Seven to 12 months later, some cattle of the negative group were antibody-positive when examined by the immunodiffusion or complement fixation test. They failed to indicate any significant change in S-LDH activity, as compared with this activity shown when they were antibody-negative. A comparison was made between the zymograms of 19 antibody-positive cattle and antibody-negative cattle, both of which groups were randomly selected. They revealed significantly high LDH4 and LDH5 activity and markedly low LDH1 activity in the positive cattle. The mean S-LDH activity was significantly higher in 8 leukemic cattle than in both positive and negative groups, although no increase was found in 2 leukemic cattle. Moreover, the isoenzyme activity showed a significant increase in LDH4 and LDH5 even in cattle exhibiting no increase in total S-LDH activity.

Bovine leukemia is a lymphoproliferative disease associated with bovine leukemia virus (BLV) infection [5, 6, 18, 23]. Subclinical infection with this virus is conventionally diagnosed by the presence of serum antibody detected by the immunodiffusion test (ID) or complement fixation test (CF) [21, 22, 24–26, 29, 30, 33]. Human patients with malignant tumors and animals with carcinomatosis generally show an increase in serum lactate dehydrogenase (S-LDH) activity [1, 13, 28, 35, 37, 41]. This increase was reported to occur in bovine leukemia [2, 9]. Actually, nothing is known about the stage in which the S-LDH level begins to rise in bovine leukemia or about any isoenzyme pattern involved. Using cattle negative for antibody against BLV as controls, the authors investigated the S-LDH activity and zymogram of antibody-positive cattle and those with leukemia. A specific isoenzyme pattern was found to characterize the diseased cattle and those positive for antibody against BLV.

Materials and Methods
1. Animals
The animals used were 178 Japanese Black cows
Table 1. Results of blood and serum tests in leukemic cows

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4&lt;sup&gt;1)&lt;/sup&gt;</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>[years]</td>
<td>(9)</td>
<td>(7)</td>
<td>(9)</td>
<td>(8)</td>
<td>(9)</td>
<td>(11)</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte&lt;sup&gt;2)&lt;/sup&gt; (×10&lt;sup&gt;9&lt;/sup&gt;/μl)</td>
<td>654</td>
<td>63</td>
<td>76</td>
<td>141</td>
<td>73</td>
<td>103</td>
<td>60</td>
<td>307</td>
</tr>
<tr>
<td>RBC (×10&lt;sup&gt;6&lt;/sup&gt;/μl)</td>
<td>422</td>
<td>468</td>
<td>895</td>
<td>779</td>
<td>604</td>
<td>501</td>
<td>469</td>
<td>243</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>24.0</td>
<td>ND&lt;sup&gt;3)&lt;/sup&gt;</td>
<td>ND</td>
<td>37.0</td>
<td>34.0</td>
<td>25.0</td>
<td>24.0</td>
<td>21.0</td>
</tr>
<tr>
<td>A1-P (King-Armstrong unit)</td>
<td>12.6</td>
<td>7.2</td>
<td>3.9</td>
<td>2.9</td>
<td>ND</td>
<td>4.2</td>
<td>7.5</td>
<td>ND</td>
</tr>
<tr>
<td>GOT (Karmen unit)</td>
<td>122.0</td>
<td>62.0</td>
<td>87.0</td>
<td>68.0</td>
<td>ND</td>
<td>68.0</td>
<td>90.0</td>
<td>ND</td>
</tr>
<tr>
<td>ID&lt;sup&gt;4)&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1) Autopsied but no material sampling.
2) Absolute peripheral lymphocyte count.
3) Not done.
4) Immunodiffusion test for serum antibody against bovine leukemia virus.

Table 2. Serum lactate dehydrogenase activity in groups positive and negative for serum antibody against bovine leukemia virus over a time course

<table>
<thead>
<tr>
<th>Immunodiffusion test</th>
<th>Serum lactate dehydrogenase (LDH) activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1976</td>
</tr>
<tr>
<td></td>
<td>April</td>
</tr>
<tr>
<td>Negative</td>
<td>n=96</td>
</tr>
<tr>
<td>Positive</td>
<td>n=19</td>
</tr>
</tbody>
</table>

LDH activity: Wróblewski unit.

Table 3. Serum lactate dehydrogenase activity before and after positive conversion in immunodiffusion (ID) or complement fixation tests

<table>
<thead>
<tr>
<th>ID positive-conversion</th>
<th>Serum lactate dehydrogenase (LDH) activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With in 3 months</td>
</tr>
<tr>
<td></td>
<td>n=11</td>
</tr>
<tr>
<td>Before</td>
<td>2018.6±816.7</td>
</tr>
<tr>
<td>After</td>
<td>2354.6±377.0</td>
</tr>
</tbody>
</table>

LDH activity: Wróblewski unit.

from one to 14 years old raised in T Village in the H district of Japan, and 8 cows from 4 to 11 years old raised in H district in which a diagnosis of leukemia had been made clinically and at autopsy (Table 1). In April, July and November, 1976, and in April, 1977, a total of 336 blood samples were taken from the 178 cows, which were classified into negative and positive groups by an ID that had partially been run in conjunction with a CF.

2. Methods

The blood samples were centrifuged immediately after sampling. Serum was separated after fibrin had been allowed to clot. Serum samples were stored at −80°C and used as soon as possible. Total S-LDH activity was measured within 2 days, and isoenzyme analysis carried out from 2 days to about
Table 4. Serum lactate dehydrogenase activity in groups negative and positive for serum antibody against bovine leukemia virus and the probability of difference for negative group

<table>
<thead>
<tr>
<th>Serum LDH</th>
<th>Absolute serum lactate dehydrogenase (LDH) activity</th>
<th>Probability of difference for negative group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prop. of LDH isoenzyme</td>
<td>Activity</td>
</tr>
<tr>
<td>Serological group</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Total LDH</td>
<td>n=9</td>
<td>n=19</td>
</tr>
<tr>
<td>LDH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1279 ± 295</td>
<td>42.3 ± 6.9</td>
</tr>
<tr>
<td>LDH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>875 ± 237</td>
<td>28.5 ± 3.6</td>
</tr>
<tr>
<td>LDH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>600 ± 210</td>
<td>19.4 ± 4.4</td>
</tr>
<tr>
<td>LDH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>214 ± 94</td>
<td>6.9 ± 2.6</td>
</tr>
<tr>
<td>LDH&lt;sub&gt;5&lt;/sub&gt;</td>
<td>89 ± 57</td>
<td>2.9 ± 1.5</td>
</tr>
</tbody>
</table>

LDH activity: Wroblewski unit.
NS: Not significant.

3 weeks after sampling, ID and CF were performed in the same manner as described previously [30, 31]. Total S-LDH activity was measured by the phenazine methosulfate-nitro TB method (end-point assay, RaBA system). Nothing hindered reproductibility in the practical application of the RaBA system. Isoenzyme analysis was performed electrophoretically, with Cellogel (Chemtrum Ltd., Milan, Italy) used as supporting membrane. Electrophoresis was carried out in a pH 8.6 veronal-veronal Na buffer having I=0.06 at 4°C and at a current of 0.6 mA/cm. Staining was achieved by the method of Shioya et al. [39]. Densitometric determination of the stained isoenzyme fraction was made at 570 nm with an automatic microdensitometer. Isoenzyme activities were calculated from the total S-LDH activity and densitometrical relative ratios of the isoenzyme pattern.

Results

1. S-LDH activity in groups negative and positive for BLV antibody

Table 2 shows the S-LDH activity measured over a time course in the 178 cows divided into negative and positive groups. There was no significant difference in this activity of the two groups at any time of testing.

2. S-LDH activity before and after antibody-positive conversion

Table 3 shows the S-LDH activity before and after the appearance of positive ID or CF. Cattle which became positive for BLV antibody were grouped according to the time in months elapsing after the conversion. No significant differences were found in the activities of antibody-positive cows examined within 3 months to from 7 to 12 months after the conversion and the activities of the same cows before becoming positive.

3. S-LDH isoenzyme activity in groups negative and positive for BLV antibody

An S-LDH isoenzyme analysis was performed on 9 negative and 19 positive cows randomly selected from those used for serum sampling in November, 1976. The results obtained are shown in Table 4. The antibody-positive group displayed higher LDH<sub>2</sub> (p<0.02) and LDH<sub>3</sub> (p<0.01) activity and significantly lower LDH<sub>1</sub> activity (p<0.02) than the antibody-negative group. Moreover, LDH<sub>4</sub> activity tended to be low in the positive group.
4. S-LDH activity and its isoenzyme activity in leukemic cows

Table 5 indicates S-LDH activity in 8 cows of the leukemic group and its isoenzyme activity in six of these cows. Total S-LDH activity was significantly higher in the leukemic group than in the antibody-negative and positive groups (p<0.01), although it showed no increase in 2 cows (Nos. 2 and 6) of the leukemic group. Isoenzyme analysis also revealed significantly higher LDH₂ and LDH₃ activity in the leukemic group than in the negative group (p<0.01). Moreover, such a significant increase was observed even in cows in which total S-LDH activity did not increase. Correlations between peripheral lymphocyte count and S-LDH or isoenzyme activities were significant only in LDH₁ activity (r=0.83, p<0.05).

Discussion

LDH is distributed widely in tissue cells and body fluids. Actually, it is a cellular enzyme having a plasma of non-specific nature [28]. When cell disintegration and an increase in permeability of the cellular membrane or enzyme production take place, the enzymes produced flow into the bloodstream. Especially, when there is an injury in an organ or tissue with high enzyme activity and the enzymes produced are removed very slowly from the circulation [8], a marked increase is induced in LDH activity or isoenzyme activity in the blood plasma and can be used for diagnosis [1, 28, 42, 44]. Normal cattle [19, 32, 36, 38, 40] show a considerably higher S-LDH activity than any other mammal [4, 17, 42, 44, 45]. Moreover, S-LDH decreases physiologically with age [10, 14, 32, 36-38] and possibly with pregnancy [10, 38] and varies with the breed of cattle [38, 42]. On the other hand, LDH activity differs in level from one organ to another [4, 7, 10, 28, 45] and is known to be remarkably low in blood serum [4, 8, 28]. The 8 leukemic cattle were either mature or aged cows, but six of them showed a pronounced increase in S-LDH activity.

In bovine leukemia, S-LDH activity increases [2, 9], and LDH presents a relatively high activity in the cells of lymphosarcoma...
[9]. Autopsy disclosed tumorous changes in the heart in five of 6 leukemic cattle in which an increase of S-LDH activities had been found. LDH activity is high in the heart muscle [7, 20]. Although the zymogram shows a high rate of LDH_1 and LDH_2 activity [3, 20], the zymogram of the blood serum of leukemic cattle indicates high LDH_2 and LDH_3 activity even when there is no increase in total S-LDH activity. This finding is different from that from the heart muscle. In a useful differential diagnosis with S-LDH isoenzyme in cattle, it has been reported that no cardiac disease is involved [20, 34]. It is not considered that the increase in LDH_2 activity found in diseased cattle is merely attributable to a deviation from the heart muscle.

As mentioned above, a high LDH activity has been reported in cattle with lymphosarcoma [9]. In animals with a transplanted tumor, S-LDH is said to increase with lapse of time [12, 35, 41], and excision or regression to cause the level to drop [11, 12, 35]. Moreover, the zymogram of blood plasma is reported to be the same as that of carcinoma cells [16].

In the light of the findings mentioned above, the increase in S-LDH activity in the leukemic cattle may be interpreted in the following manner. This activity may have been diverted into the blood through the initially cited mechanism by which lymphosarcoma cells produced isoenzymes of their own [8, 41]. Moreover, in this disease an increase in total S-LDH activity is considered to appear in blood plasma when accompanied by lymphoproliferative changes in the advanced stage of the disease [15, 33], judging from the findings shown in Tables 3 and 5. There was no significant correlation between peripheral lymphocyte count and S-LDH activity.

Analysis of S-LDH and its isoenzyme is a very useful auxiliary means for the diagnosis of leukemia in cattle. The antibody-positive group does not obviously differ from the antibody-negative group in clinical routine testing [27]. It has, however, a zymogram with the characteristics shown in Table 4. LDH_2 and LDH_3 activity are significantly high and LDH_1 activity is significantly low. The serum LDH zymogram reflects an isoenzyme pattern with injured tissue features. Thus, it is highly rated in terms of clinical enzymology [8, 28, 42]. The zymogram from the antibody-positive group yields findings somewhat similar to those in leukemic cattle.

The significance of the zymogram cannot be established on the basis of the present experiment. However, if the findings of the antibody-positive group reflect enzymological changes in the subclinical case (in which no onset of disease is clinically evident), the zymogram will be of both great clinical and pathogenetic significance. There are many cattle which become positive for BLV antibody at different times after infection. For these cattle it is advisable to examine S-LDH zymograms closely in relation to those for lymph nodes in order to clarify the cause of enzymological changes in the serum.

References


[31] Onuma, M., Olson, C., and Driscoll, D. M.


