Changes in Activities of Enzymes Related to Energy Metabolism in Canine Lymphoma Cells

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ABSTRACT. Alterations in the activities of enzymes related to energy metabolism in canine lymphoma cells were investigated. Cytosolic pyruvate kinase (PK) and mitochondrial malate dehydrogenase (MDH) activities in lymphoma cells were significantly higher than those in lymphocytes obtained from lymph nodes of healthy dogs, whereas cytosolic lactate dehydrogenase (LDH) activity was significantly lower in lymphoma cells. The cytosolic M/L ratio (MDH activity/LDH activity), which is considered to be a good indicator of energy metabolism related to glucose utilization in animal tissues, was significantly higher in lymphoma cells than in the normal lymphocytes.

KEY WORDS: canine, lymphoma, malate dehydrogenase.

Lymphoma is one of the most common neoplasms observed in the dog and primarily affects middle-aged to older dogs [14]. The most common form of canine lymphoma is the multicentric form, which usually presents with superficial lymphadenopathy. Morphology, immunophenotypes, responsiveness to chemotherapy, and correlations between morphology and survival time of canine lymphoma have been reported [8, 9, 17]. Several reports have discussed the metabolic alterations of dogs with lymphoma [15, 16, 20], however, the enzyme activities of lymphoma cells per se have not been investigated. In the present study, lymphoma cells and normal lymphocytes from the lymph nodes of healthy dogs were obtained, and the activities of the enzymes related to energy metabolism including hexokinase (HK), pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and aspartate aminotransferase (AST) were measured.

Five healthy mixed breed dogs (2 males and 3 females; 3–6 years old) maintained for research in our laboratory and fed on a commercial diet (Hill’s Pet Products, Topeka, KS, U.S.A.) were used as control dogs. Their hematological and serum chemistry values were within the normal ranges. In addition, ten dogs referred to our Veterinary Medical Teaching Hospital and diagnosed as multicentric, stage III lymphoma were used. Signalment of the ten dogs are shown in Table 1.

Table 1. Signalment of lymphoma cases

<table>
<thead>
<tr>
<th>Breed</th>
<th>Age (yrs)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labrador retriever</td>
<td>6</td>
<td>male</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>6</td>
<td>female</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>7</td>
<td>male</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>7</td>
<td>male</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>10</td>
<td>female</td>
</tr>
<tr>
<td>Golden retriever</td>
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<td>Golden retriever</td>
<td>8</td>
<td>female</td>
</tr>
<tr>
<td>Golden retriever</td>
<td>10</td>
<td>male</td>
</tr>
<tr>
<td>Welsh corgi</td>
<td>3</td>
<td>female</td>
</tr>
<tr>
<td>Shetland sheepdog</td>
<td>8</td>
<td>male</td>
</tr>
</tbody>
</table>

Washed lymphocytes were resuspended in 2 ml ice-cold STE solution (0.25 M sucrose, 10 mM Tris-HCl buffer, pH 7.5, containing 2 mM EGTA), followed by homogenization with an ultrasonic processor (VP-5, Taitek, Koshigaya, Japan) for 5 sec. The homogenate was centrifuged at 100 × g for 1 min to remove nuclei and cell debris, and the supernatant was centrifuged at 8,000 × g for 25 min at 4°C and the resulting supernatant was saved as the cytosolic fraction. The pellet was resuspended in 0.5 ml of 50 mM Tris-HCl, pH 7.5 containing 2 mM 2-mercaptoethanol and 1 mM EDTA, homogenized as above for 5 sec, and centrifuged at 14,000 × g for 30 min at 4°C. The supernatant was used as the mitochondrial fraction. Both cytosolic and mitochondrial fractions were stored at –80°C until assayed.

The activities of the enzymes in the cytosolic and mitochondrial fractions were measured by previously reported methods: HK [21], PK [10], LDH with pyruvate as substrate [12], MDH [19] and AST [18]. All enzyme assays were performed using the spectrophotometric absorbance of solutions in cuvettes at 340 nm. Changes of NADH to NAD+(PK, LDH, MDH and AST) and NADP+ to NADPH (HK) were used to calculate enzyme activities. All enzymatic activities were determined at 24–26°C and expressed as nmol of substrate degraded per min per mg of protein.
The enzyme activities and cytosolic M/L ratio in normal canine lymphocytes and lymphoma cells are shown in Table 2. The activities (mean ± SD) are expressed as nmol/min per mg protein. The data were analyzed statistically using Student’s t-test.

This is a preliminary study and the results suggest that more energy is produced in mitochondria of lymphoma cells than in normal lymphocytes.

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REFERENCES