NOTE *Clinical Pathology*

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.

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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), piruvate 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.

Popliteal lymph nodes of normal dogs were obtained surgically under general anesthesia with isoflurane (Rhodia, England). Lymphoma cells were collected by fine needle aspiration biopsy from enlarged lymph nodes of the dogs with lymphoma before the initiation of chemotherapy and put in RPMI 1640 medium. Lymphocytes and lymphoma cells were further purified by gradient centrifugation with a commercially available mononuclear cell isolating solution (Histopaque-1077, Sigma-Aldrich Co., St. Louis, MO, U.S.A.), followed by three washes with cold phosphate-buffered saline (0.15 M NaCl, pH 7.2).

Washed lympyocytes were resuspended in 2 m*l* 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 m*l* 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 franctions 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.

Table 1. Signalment of lymphoma cases

Breed	Age(yrs)	Sex
Labrador retriever	6	male
Labrador retriever	6	female
Labrador retriever	7	male
Labrador retriever	7	male
Labrador retriever	10	female
Golden retriever	7	female
Golden retriever	8	female
Golden retriever	10	male
Welsh corgi	3	female
Shetland sheepdog	8	male

Table 2. Enzyme activities[a] and cytsolic M/L ratio in normal canine lymphocytes and lymphoma cells

	Normal lymphocytes	Lymphoma cells
Cytosol		
HK	7.0 ± 3.6	11.3 ± 5.1
PK	211.6 ± 56.8	501.4 ± 202.6[b]
LDH	1554.6 ± 402.5	920.7 ± 351.0[b]
MDH	1355.5 ± 280.5	1181.7 ± 211.8
AST	90.2 ± 32.7	102.2 ± 28.5
M/L ratio	0.9 ± 0.2	1.4 ± 0.3 [b]
Mitochondria		
MDH	2064.0 ± 200.3	3621.5 ± 1181.8[b]
AST	663.0 ± 101.8	534.6 ± 141.1

a) The activities (mean ± SD) are expressed as nmol/min per mg protein.
b) Significantly different ($p<0.01$) from the value for normal lymphocytes.
The data were analyzed statistically using Student's *t*-test.

The protein concentrations were determined by the method of Bradford [5] with bovine serum albumin as the standard.

Table 2 shows the activities of cytosolic and mitochondrial enzymes and the ratio of the cytosolic MDH/LDH (M/L ratio) in normal lymphocytes and lymphoma cells. Cytosolic PK and mitochondrial MDH activities in lymphoma cells were significantly higher than those in normal lymphocytes, whereas cytosolic LDH activity was significantly lower in lymphoma cells. There were no significant differences in the activities of cytosolic HK, MDH, and AST, and mitochondrial AST between normal lymphocytes and lymphoma cells. The M/L ratio was significantly higher in lymphoma cells compared with normal lymphocytes.

It has been reported that the enzyme activities related glucose metabolism in tumors are significantly different from normal tissues [2, 6, 7] and glucose transport activities in canine mammary tumor cells were markedly elevated compared with normal mammary tissues [3]. Cytosolic LDH activity was significantly lower in lymphoma cells compared with normal lymphocytes in this study. The M/L ratio, defined as the MDH activity divided by the LDH activity in cytosolic fractions, was significantly higher in lymphoma cells than in the normal lymphocytes. Cytosolic MDH transfers cytosolic NADH into mitochondria through the malate-aspartate shuttle, thus, tissues with a high M/L ratio are considered to have an efficient energy production system [4]. Moreover, mitochondrial MDH activity was significantly higher in lymphoma cells. Mitochondrial MDH is directly involved in energy production through the reduction of NAD+ to NADH [13]. Elevated mitochondrial MDH activities are observed in leukocytes of race horses after continuous hard training [11] and tumor cells [1], which need excess glucose as an energy source. Therefore, increases in M/L ratio, cytosolic PK and mitochondrial MDH activities are considered to reflect higher energy production in the lymphoma cells.

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

1. Arai, T., Ogawa, T., Nakamura, M., Hosoya, M. and Ohnishi, T. 2002. *J. Vet. Med. Sci.* **64**: 1065–1067.
2. Arai, T., Ogawa, T., Hosoya, M., Nakamura, M. and Kawakami, E. 2002. *Vet. Res. Commun.* **26**: 347–352.
3. Arai, T., Sugawara, M., Sako, T. and Motoyoshi, S. 1997. *J. Equine Sci.* **8**: 113–116.
4. Arai, T., Takahashi, M., Araki, K. and Washizu, T. 2001. *Vet. Res. Commun.* **25**: 577–583.
5. Bradford, M. M. 1976. *Anal. Biochem.* **72**: 248–254.
6. Brocklehurst, D., Champion, A. E., Creek, T. R. J. and Dewhurst, D. G. 1986. *Tumor* **7**: 99–104.
7. Deshpande, N., Mitchell, I. and Millis, S. 1981. *Eur. J. Cancer* **17**: 443–448.
8. Garrett, L. D., Thamm, D. H., Chun, R., Dudley, R. and Vail, D. M. 2002. *J. Vet. Intern. Med.* **16**: 704–709.
9. Greenlee, P. G., Filippa, D. A., Quimby, F. W., Patnaik, A. K., Calvano, S. E., Matus, R. E., Kimmel, M., Hurvitz, A. I. and Lieberman, P. H. 1990. *Cancer* **66**: 480–490.
10. Hess, B. and Wieker, H. J. 1974. pp. 778–783. *In*: Methods of Enzymatic Analysis, vol. 2 (Bergmeyer, H.U. ed.), Verlag Chemie Weinheim Academic Press, New York.
11. Hosoya, M., Inoue, A., Kimura, N. and Arai, T. 2004. *Res. Vet. Sci.* **77**: 101–104.
12. Kaloustian, H.D., Stolzenbach, F.E., Everse, J. and Kaplan, N.O. 1969. *J. Biol. Chem.* **244**: 2891–2901.
13. Lane, R. H., Flozak, A. S., Ogata, E. S., Bell, G. I. and Simmons, R. A. 1996. *Pediat. Res.* **39**: 390–394.
14. Moulton, J. E. and Harvey, J. W. 1990. pp. 231–307. *In*: Tumors of Domestic Animals, 3rd ed. (Moulton, J. E. ed.), University of California Press, California.
15. Ogilvie, G. K., Ford, R. B., Vail, D. M., Walters, L. M., Salman, M. D., Babineau, C. and Fettman, M. J. 1994. *J. Vet. Intern. Med.* **8**: 62–66.
16. Ogilvie, G. K., Vail, D. M., Wheeler, S. L., Fettman, M. J., Salman, M. D., Johnston, S. D. and Hegstad, R. L. 1992. *Cancer* **69**: 233–238.
17. Ponce, F., Magnol, J. P., Ledieu, D., Marchal, T., Turinelli, V., Chalvet-Monfray, K. and Fournel-Fleury, C. 2004. *Vet. J.* **167**: 158–166.
18. Rej, R. and Horder, M. 1983. pp. 416–433. *In*: Methods of Enzymatic Analysis, 3rd ed., vol. 3 (Bergmeyer, H.U. ed.), Verlag Chemie Weinheim Academic Press, New York.
19. Siegel, A. and Bing, R.J. 1956. *Proc. Soc. Exp. Biol. Med.* **91**: 604–607.
20. Vail, D. M., Ogilvie, G. K., Wheeler, S. L., Fettman, M. J., Johnston, S. D. and Hegstad, R. L. 1990. *J. Vet. Intern. Med.* **4**: 8–11.
21. Vinuela, E., Salas, M. and Sols, A. 1963. *J. Biol. Chem.* **238**: 1175–1177.