pISSN 1229-845X, eISSN 1976-555X
J. Vet. Sci. (2012), **13**(4), 433-436
http://dx.doi.org/10.4142/jvs.2012.13.4.433
Received: 6 Jul. 2011, Revised: 6 Dec. 2011, Accepted: 4 Apr. 2012

JOURNAL OF
Veterinary
Science

Short Communication

Plasma free amino acid profiles of canine mammary gland tumors

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The purpose of this study was to elucidate the relationship between plasma free amino acid (PFAA) levels and the clinical stages of mammary gland tumors (MGT) in dogs. PFAA levels in canines with malignant mammary tumors were decreased compared to those of healthy animals. The levels of aspartate and ornithine, in the dogs with tumor metastasis were significantly decreased when compared to those of dogs that did not have metastases. Results of this study indicate that PFAA levels could be a risk factor or biomarker for canine MGT metastasis.

Keywords: dog, mammary gland tumor, plasma free amino acid

Amino acids play important roles as both substrates and regulators in many metabolic pathways [3]. Measurements of free amino acid concentrations in body fluids and individual tissues can provide useful information regarding the biochemical and nutritional status associated with various diseases [15]. In humans, it is known that the levels of plasma free amino acids (PFAAs) are altered in patients with breast, lung and colorectal cancer [12]. These amino acid imbalances are caused by changes in protein metabolism [9]. A previously conducted isotope-labeled amino acid analysis demonstrated that tumor-bearing rats have higher liver fractional synthetic rates, greater muscle catabolic rates with lower skeletal muscle fractional synthetic rates, and higher whole-body protein turnover rates than normal controls [6]. These changes could be seen before a negative nitrogen balance, weight loss, or decreased food intake was observed [6].

Mammary gland tumors (MGTs) very commonly develop in female dogs, and 40～50% of canine mammary tumors are malignant [8]. Histopathological diagnosis of canine MGTs is based on TNM (T: size of the primary tumor, N: condition of the regional lymph nodes, and M: absence/presence of distant metastasis) staging [16]. A few studies have described methods for determining the prognosis and staging of canine MGTs using blood samples [5,10]. For example, circulating vascular endothelial growth factor is clinically available and useful for determining the prognosis of canine MGTs [5]. In addition, serum C-reactive protein concentrations are elevated in dogs with metastasis [14].

The relationship between PFAA levels and canine MGT prognosis has not been previously investigated. Therefore, the aim of the current study was to compare the levels of PFAAs in normal dogs and ones with MGTs (with or without metastasis).

We evaluated a total of 16 dogs with malignant MGTs brought to the Animal Medical Center of Tottori University (Japan) from March 2009 to November 2010 (Table 1). All cases were classified according to TNM staging and divided into two groups: non-metastasis (NM: n = 10) and

Table 1. Comparison of age, gender, body weight, tumor staging, total plasma protein levels, plasma albumin concentrations, and histological type among control dogs, dogs with non-metastatic malignant mammary tumors, and dogs with metastatic mammary tumors

	Control	NM group	M group
Numbers	6	10	6
Female/Male	3/3	10/0	6/0
Age (y)	2～6	6～13	8～12
Body weight (kg)	7.0～12.0	3.1～12.7	3.3～28.3
TNM stage	–	T1: 4, T2: 3, T3: 3	N1: 2, M1: 4
Total plasma protein (g/dL)	6.6～7.8	7.0～9.1	5.3～8.1
Plasma albumin (g/dL)	2.8～3.2	3.0～4.1	2.9～3.9
Histological diagnosis	–	–	–
Complex adenocarcinoma	–	8	–
Simple adenocarcinoma	–	–	6
Papillary adenocarcinoma	–	2	–

NM: non-metastasis, M: metastasis.

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metastasis (M: n = 6). Dogs in the NM group included two miniature dachshunds, two maltese, and one each of the following: a mixed breed, bichon frise, toy poodle, beagle, pembroke welsh corgi, and shiba inu. The M group consisted of two miniature dachshunds, two golden retrievers, a Yorkshire terrier, and a pug. We used six healthy beagles (three males and three females) that were maintained at Tottori University as control animals. In this study, we used three males and nineteen female dogs.

The tumor size was measured at the largest diameter. The condition of the regional lymph nodes and presence of distant metastases were evaluated based on physical examination results, radiographic findings, and/or computed tomography data. All dogs were fasted for 10 h before blood collection. Blood collections and analysis of amino acids were performed according to our previous methods [1]. Briefly, blood was collected in tubes containing heparin and immediately centrifuged at 2,000 × g for 5 min at 4°C. After centrifugation, the plasma was promptly removed and frozen at −20°C until the PFAAs were measured. The plasma was mixed with an equal volume of 3% (w/w) sulfosalicylic acid and incubated at 4°C for 1 h. Next, the samples were centrifuged at 4°C for 15 min at 2,000 × g, and the precipitated proteins were removed.

An automatic amino acid analyzer (JLC-500/V2, AminoTac; JEOL, Japan) was used to measure the concentrations of 22 different amino acids. The essential amino acids (EAAs) measured were threonine (Thr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe),

Table 2. Plasma free amino acid levels (nmol/mL) in control dogs, canines with non-metastatic malignant mammary tumors, and animals with metastatic mammary tumors

	Control	NM group	M group
Thr	110.8 ± 12.6	91.0 ± 14.3	77.0 ± 12.6
Val	93.8 ± 11.5	73.3 ± 13.9	90.3 ± 22.4
Met	35.1 ± 1.4	20.2 ± 2.9†	19.5 ± 4.0†
Ile	32.2 ± 8.8	30.7 ± 4.7	30.3 ± 3.7
Leu	66.3 ± 6.7	60.5 ± 9.7	55.9 ± 6.3
Phe	34.4 ± 1.6	30.5 ± 3.0	32.5 ± 3.8
Lys	122.6 ± 11.2	89.0 ± 13.9	75.8 ± 11.6*
His	46.3 ± 2.8	37.9 ± 5.6	25.1 ± 2.6†
Trp	35.2 ± 3.0	34.9 ± 8.2	34.8 ± 11.8
Arg	64.1 ± 9.0	50.5 ± 6.0	34.0 ± 4.2*
Asp	4.4 ± 0.3	4.6 ± 0.5	2.3 ± 0.4†‡
Ser	99.5 ± 8.4	60.4 ± 6.6†	54.2 ± 10.3†
Asn	36.0 ± 2.5	20.7 ± 2.1†	19.4 ± 2.9†
Glu	25.6 ± 2.8	19.8 ± 1.9	15.5 ± 2.5*
Gln	493.2 ± 35.3	327.4 ± 30.9†	261.6 ± 32.2†
Gly	107.6 ± 6.7	99.8 ± 11.5	90.3 ± 15.6
Ala	346.8 ± 28.9	222.9 ± 26.2†	193.1 ± 21.8†
Tyr	25.1 ± 1.5	23.3 ± 2.6	20.8 ± 2.2
Pro	66.1 ± 4.5	70.8 ± 7.3	61.0 ± 10.8
Tau	101.6 ± 12.7	62.1 ± 8.3*	54.5 ± 12.4*
Cit	53.7 ± 3.2	31.5 ± 6.5*	21.1 ± 2.9†
Orn	12.8 ± 2.8	9.6 ± 1.5	5.0 ± 0.8*‡
BCAAs	192.2 ± 52.4	164.4 ± 91.9	176.5 ± 77.3
Fischer's ratio	3.2 ± 0.7	3.0 ± 0.8	3.1 ± 1.1
EAAs	640.6 ± 35.9	518.0 ± 72.6	475.2 ± 82.5
NEAAs	1,373.5 ± 74.8	952.8 ± 75.6†	798.8 ± 68.6†
TAAs	2,014.1 ± 101.0	1,471.2 ± 145.5*	1,274.0 ± 145.1†

Thr: threonine, Val: valine, Met: methionine, Ile: isoleucine, Leu: leucine, Phe: phenylalanine, Lys: lysine, His: histidine, Trp: tryptophan, Arg: arginine, Asp: aspartic acid, Ser: serine, Asn: asparagine, Glu: glutamic acid, Gln: glutamine, Gly: glycine, Ala: alanine, Tyr: tyrosine, Pro: proline, Tau: taurine, Cit: citrulline, Orn: ornithine, NM: non-metastasis, M: metastasis, EAAs: plasma essential amino acids, NEAAs: plasma non-essential amino acids, TAAs: plasma total amino acids. Data are expressed as the mean ± SD. *Significantly different ($p < 0.05$) from the control group value. †Significantly different ($p < 0.01$) from the control group value. ‡Significantly different ($p < 0.05$) from the values for the NM group.

lysine (Lys), histidine (His), tryptophan (Trp), and arginine (Arg). The non-essential amino acids (NEAAs) assayed included aspartic acid (Asp), serine (Ser), asparagine (Asn), glutamic acid (Glu), glutamine (Gln), glycine (Gly), alanine (Ala), tyrosine (Tyr), proline (Pro), taurine (Tau), citrulline (Cit), and ornithine (Orn). The plasma branched chain amino acid (BCAA) levels were calculated as the sum of the values for Val, Leu, and Ile concentrations. The plasma total amino acid (TAA) levels were the sum of the levels for all 22 amino acids. Fischer's ratio was used as an indicator of liver function [13] and calculated as BCAA/(Tyr + Phe). PFAA levels were expressed as nmol/mL. Statistical analyses were carried out using a one-way ANOVA and Tukey-Kramer's test. All data are presented as the mean ± SD. p-values < 0.05 were considered to be statistically significant.

Results of the analyses are shown in Table 2. The levels of TAAs were significantly decreased in the NM and M groups compared to those of the control dogs. TAA levels of the M group were lower than those of the NM group. In both the NM and M animals, plasma EAA concentrations were lower than those of the control group, and the EAA levels of the M group were lower than those of the NM group. The levels of plasma NEAAs were significantly decreased in the NM and M groups compared to those of the control dogs. NEAA levels of the M group were lower than those of the NM group. However, EAA and NEAA levels of the three groups were not significantly different. BCAA levels of the NM and M groups were lower than those of the control group. BCAA levels of the M group were lower than those of the NM group; however, the BCAA levels of the three groups were not significantly different.

In all three groups, Gln, Ala, Lys, Thr, Gly, and Val were the most abundant plasma amino acids. In the NM group, the plasma levels of Met, Ser, Asn, Gln, Ala, Tau, and Cit were significantly decreased compared to those of the control. In the M group, Met, Lys, His, Arg, Asp, Ser, Asn, Glu, Gln, Ala, Tau, Cit, and Orn concentrations were significantly decreased relative to the control. The levels of each PFAA in the M group were lower than those of the NM group. In particular, the Asp and Orn concentrations in the M group were significantly decreased compared to those of the NM group. In the NM group, the Met, Ser, Asn, Gln, and Ala levels were significantly decreased compared to those of the control animals. In the M group, the Met, Lys, His, Arg, Asp, Ser, Asn, Glu, Gln, and Ala concentrations were significantly decreased compared to those of the controls.

Various amino acids are associated with cancer metabolism in humans. For example, Ala and BCAAs are needed for carbohydrate and protein synthesis, Gln is required for adenosine triphosphate synthesis by cancer and immune cells, Gln and Asp are essential for nucleic acid synthesis, Arg and Orn are needed for polyamine synthesis, Arg is necessary for nitric oxide synthesis in immune cells, and Met is needed for transmethylation [15]. In human colon and stomach cancer patients, the levels of all amino acids except for Gln are significantly higher in tumor tissues than normal colon and stomach tissues [4]. It has previously been demonstrated that Gln is an amino acid preferred for energy generation by cancer cells [11]. Previous reports indicated that the demands for amino acids in tumor tissues are greater than those of normal tissues. Our results demonstrated that PFAA imbalances also occur in dogs with MGTs.

Lower plasma total protein and albumin levels were not observed in the NM and M groups compared to the control dogs. Aside from cancer-dependent malnutrition, significant decreases in PFAA concentrations and other indicators of nutritional status, such as body mass index and serum albumin levels, are seen in subjects with cancer-independent cachexia [2]. On the other hand, Miyagi *et al*. [12] determined that PFAA imbalances result from changes in whole-body metabolism due to cancer rather than nutritional status. In the present study, the Asp and Orn plasma levels in the M group were significantly decreased compared to those of the NM group. Our results suggest that the PFAA levels in dogs with MGTs might also be affected by cancer progression. Therefore, measuring plasma Asp and Orn concentrations might be useful for predicting cancer metastasis.

No changes in Fischer's ratio were observed among the different groups in our study. Fischer's ratio is one parameter for evaluating liver function. This ratio is lower in patients with hepatic dysfunction than healthy subjects [15]. In breast cancer patients, Fischer's ratio is higher than in healthy individuals [7]. Our results suggested that Fischer's ratio is not affected in dogs with MGTs.

In conclusion, results of the current study clearly demonstrated that imbalanced PFAA levels exist in dogs with MGTs. The concentrations of aspartate and ornithine were particularly decreased in the dogs with metastasis compared to the control and NM groups. Our findings indicate that PFAA levels may be used as a factor for evaluating the prognosis of canine MGTs.

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