Changes in plasma free amino acids concentration in dogs with cancer

Summary

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Cancer is the leading cause of death in humans and dogs in Japan. Up to the present date, a great deal of clinical examination and therapy of cancer has been investigated. In particular, early detection and treatment of cancer are recognized as important challenges. Recently, analysis of plasma free amino acids (PFAAs) using liquid chromatography-mass spectrometry (LC/MS) had been developed in human medicine. PFAAs enables risk assessment of multiple types of cancer with only one blood sampling. However, PFAAs have not ever been clinically applied in veterinary medicine. Furthermore, there are few studies investigating the relationship between cancer and PFAAs using exhaustive analysis. Purpose of the current study was to investigate the changes in PFAAs in dogs with cancer and to obtain new findings showing the relationship between cancer and PFAAs.

Chapter 1 Basic research on measurement of canine PFAAs using LC/MS.

First, we evaluated accuracy and reproducibility of the PFAAs assay using LC/MS in dogs. We looked for appropriate blood sampling times, since the concentration of PFAAs could be changed by food intake. In the current study, 39 types of PFAAs were measured by LC/MS.

Intra-assay reproducibility of PFAAs was estimated with pooled plasma samples of healthy dogs. Intra-assay coefficients of variation (CV) did not exceed the commonly accepted 15% limit. The samples were used for evaluation of the inter-assay imprecision too, by 8 independent runs over 1 month. The CV of PFAAs besides Cystine did not exceed 15% limit, but the CV of Cystine was 66%. We suggest that it would be better to treat the specimen immediately after blood collection and measure PFAAs within one

week, since Cystine did not show stable results during 8 weeks. Dilution linearity was assessed by serially diluting a pooled plasma sample. Excellent linearity was demonstrated in many kinds of PFAAs, up to 16-fold dilution ($P < 0.05$). However some kinds of PFAAs did not show good linearity since these PFAAs concentrations were lower than the detection limit value under the dilution condition, but the P value did not exceed 0.05. From these results, excellent accuracy and reproducibility of PFAAs using LC/MS were confirmed.

We also investigated the changes of PFAAs before and after food intake in healthy dogs. Except for α-aminobutyric acid, dietary effects were not observed in PFAAs after postprandial 14 hours. Furthermore, different daily fluctuation between daytime and nighttime was observed in some kinds of PFAAs. As such, we standardize time of taking blood sampling for measuring PFAAs as the morning with fasting over 14 hours.

From the above results, PFAAs in dogs can be assayed by LC/MS. Furthermore, we recommend that dogs should be fasted over 14 hours, and blood sampling for PFAAs measurement should be performed in the morning. And the assay should be made as soon as possible after blood collection.

Chapter 2 The changes in PFAAs concentrations in dogs with different types of cancer.

The concentrations of PFAAs in 39 cancer dogs and 20 healthy dogs were compared. Cancer dogs were categorized by the types of cancer such as transitional cell carcinoma (n = 8), mammary gland tumor (n = 3),

hepatocellular cell carcinoma (n = 8), malignant melanoma (n = 6) and thyroid carcinoma (n = 4).

Cancer dogs had significantly higher Threonine, α-aminoadipic acid, Cystine, Cystathionine, Phenylalanine, 3-Methylhistidine, 1-Methyhistidine, Tryptophan and Aromatic amino acid concentrations and had significantly lower Glycine, Histidine and Fischer ratio than healthy dogs. The PFAAs that significantly decreased in cancer dogs might be ingested by cancer cells aggressively, and the PFAAs that significantly increased might be produced by protein catabolism, but not used in cancer cells, or produced in cancer cells and secreted into circulation. These PFAAs are possible markers of cancer in dogs. Significant increase in some PFAAs related to skeletal muscle metabolism might reflect catabolism of muscle proteins in cancer dogs. In particular, 3-Methylhistidine is applied as a biomarker of muscle protein catabolism in dogs as the case with human medicine.

Taurine and Arginine were significantly lower in transitional cell carcinoma. Glutamine was significantly lower in malignant melanoma. Glycine was significantly lower and Tyrosine, Tryptophan and Phenylalanine were significantly higher in malignant breast cancer. Many kinds of PFAAs changed in the same way with other types of cancers, but branched chain amino acids and aromatic amino acids reflecting liver function were significantly higher in hepatocellular carcinoma. Threonine and Proline were significantly higher in thyroid cancer. Isoleucine was significantly lower in thyroid cancer and significantly higher in hepatocellular carcinoma. Collectively, specific changes in PFAAs were observed in different types of cancer. In addition, some PFAAs, such as Glycine, Methionine, 3-Methylhistidine and 1-Methylhistidine showed same changes in different

types of cancer. These results may reflect altering amino acid metabolism in different type of cancer.

Chapter 3 Concentrations of PFAAs before and after chemotherapy in canine transitional cell carcinoma.

PFAAs are reported to change in human patients with cancer before and after chemotherapy. However, it is unclear in cancer dogs. In this chapter, we investigated the change of PFAAs after chemotherapy in dogs, focusing on transitional cell carcinoma (TCC) because chemotherapy is recommended as treatment for TCC. Significant changes were observed in mean plasma Cystathionine concentrations between before and after chemotherapy. Cystathionine gradually decreased 1 - 3 weeks after chemotherapy, however, it increased again 6 weeks after chemotherapy. Plasma Cystathionine in TCC dogs could be taken up into cancer cells and utilized to synthesize glutathione for drug detoxification. Since the clinical symptoms improved and the tumor size did not enlarge, Cystathionine might not be used for glutathione synthesis after the second administration, so the anticancer drug effectively acted on cancer cells. From these results, we conclude that chemotherapy influenses the PFAAs concentrauions in cancer dogs.

In conclusion, we performed an exhaustive measurement of PFAAs with difference types of cancer and their changes before and after chemotherapy in dogs. We obtained new findings on the relationship between cancer and PFAAs in dogs. The results indicates that the PFAAs assay is

useful for early detection and risk assessment of cancer, and the monitoring of treatment in dogs.