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Original research paper

Decreased plasma amino acid concentrations in cats with chronic gastrointestinal diseases and their possible contribution in the inflammatory response

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ABSTRACT

In humans, plasma amino acids (AAs) levels are used as dynamic nutritional markers. Moreover, some AAs are associated with chronic inflammation. In this study, we analyzed plasma AA profiles in cats with chronic gastrointestinal (GI) diseases. Eight healthy controls (HCs) and 12 client-owned cats with chronic GI diseases including chronic enteritis (n = 8) and neoplasms (n = 4) were recruited. Plasma albumin, total protein, and 22 AAs (11 essential and 11 non-essential AAs) levels were estimated. There was no significant difference in plasma albumin and total protein concentrations between the cats with chronic GI diseases and HCs. The plasma concentrations of 7 essential AAs (arginine, histidine, lysine, methionine, phenylalanine, taurine, and tryptophan) and 7 non-essential AAs (asparagine, aspartic acid, glutamic acid, glycine, hydroxyproline, proline, and serine) were significantly decreased in the cats with chronic GI diseases ($P < 0.05$). Moreover, plasma histidine and tryptophan levels were inversely correlated with severity of symptoms (histidine: $r_s = -0.7781$, $P < 0.005$; tryptophan: $r_s = -0.6040$, $P < 0.05$). To examine the contribution of altered AAs levels in the inflammatory response, feline macrophages were stimulated by lipopolysaccharides (LPS) with or without histidine, and the expression of interleukin-8 (IL-8) mRNA was quantified. The expression of IL-8 mRNA was significantly increased in the LPS-stimulated feline macrophages ($P < 0.05$). Histidine almost suppressed the LPS-induced IL-8 expression in the feline macrophages ($P < 0.05$). Our findings suggest that plasma AAs levels are more sensitive nutritional markers than albumin and total protein levels in cats with chronic GI diseases. There is a possibility that the decrease of histidine levels in cats with GI diseases is associated with chronic inflammation.

1. Introduction

Plasma albumin and total protein are used as conventional markers of nutritional status. These parameters are called static nutritional markers, which reflect average nutritional status in the past several weeks. In humans, plasma amino acids (AAs) have been used as dynamic nutritional markers to reflect short-term change (previous 24 h) of nutritional status. Moreover, previous studies have shown that some AAs such as histidine and tryptophan were associated with chronic enteritis (Hisamatsu et al., 2012; Mine and Zhang, 2015; Son et al., 2005).

Although plasma AA concentrations are constantly maintained in healthy cats, alteration of AAs is observed in cats with chronic wasting diseases. A previous study has shown that glutamine concentrations were significantly lower in cats with hyperthyroidism compared with healthy cats (Sabatino et al., 2013). In cats with chronic renal failure, plasma levels of arginine, tryptophan, tyrosine, alanine, glutamate, glycine, hydroxyproline, and proline were significantly decreased, whereas 1-methylhistidine, 3-methylhistidine, asparagine, citrulline, and ornithine were increased (Goldstein et al., 1999). Therefore, as in humans, plasma AA concentrations have potential as nutritional assessment markers in cats.

Cats with chronic gastrointestinal (GI) diseases have clinical signs such as vomiting, diarrhea, anorexia, or weight loss, and result in malnutrition. However, there has been no report investigating plasma AA concentrations in cats with chronic GI diseases. The aims of this study were to analyze plasma AA profiles in cats with chronic GI diseases and to investigate the association of altered AAs with

Abbreviations: AA, amino acid; GI, gastrointestinal; HC, healthy control; IL, interleukin; FIV, feline immunodeficiency virus; FeLV, feline leukemia virus; FCEAI, feline chronic enteropathy activity index; SAA, serum amyloid A; BCS, body condition score; EAA, essential amino acid; NEAA, non essential amino acid; HBSS, Hanks' balanced salt solution; cDNA, complementary deoxyribonucleic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RPS7, ribosomal protein S7

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pathogenesis.

2. Materials and methods

2.1. Animals

Cats with persistent GI clinical signs (at least 3 weeks) were recruited from those presenting to the Veterinary Medical Center of the University of Tokyo from April 2015 to March 2016. The chronic GI diseases in this study were defined as chronic gastroenteritis and intestinal neoplasms. The diagnoses were based on clinical signs, complete blood count, biochemistry, fecal examination, urinalysis, radiography, ultrasonography, endoscopy, cytology, histopathology, and response to treatment. Kittens (less than 1 year old), intact cats, and cats with anorexia and concomitant diseases were excluded because plasma AA concentrations are influenced by age, neuter status, appetite, and concomitant diseases (Goldstein et al., 1999; Heinze et al., 2009). Individual client was requested to sign a research consent form and was asked to provide clinical data including signalment, diet, infection of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), and history of treatment with steroids. Based on the feline chronic enteropathy activity index (FCEAI; Jergens et al., 2010), severity of symptoms was scored (range, 0–19). Plasma serum amyloid A (SAA) concentration was measured by latex photometric immunoassay as an inflammatory index (LSI Medience Corporation, Tokyo, Japan).

Eight healthy controls (HCs) were recruited. This group included 4 male and 4 female cats (all neutered), which were aged 4–9 years (median, 7.5 years). The cats belonged to two breeds including domestic shorthair (n = 6) and Scottish fold (n = 2). The median body weight was 4.12 kg (range, 3.50–5.45 kg), and the body condition score (BCS) was 3/5 in all of the cats. Six cats were fed Gastrointestinal™ formula (ROYAL CANIN JAPON, Inc., Tokyo, Japan), and 2 cats were fed Sensitivity Control (ROYAL CANIN JAPON, Inc.). These cats did not receive any drugs, and were healthy with no clinical signs of GI diseases such as vomiting, diarrhea, anorexia, or weight loss. No abnormalities were observed in blood examinations, fecal examination, urinalysis, radiography, and ultrasonography.

2.2. Sample collection

From 9 to 12 a.m., fresh EDTA blood samples (1–1.5 mL) were collected from cats that had been fasted for more than 12 h. The samples were immediately centrifuged (1200 × *g*, 15 min, 4 °C). Plasma samples were then transferred to individual plastic tubes and stored at −30 °C until analyses. The median intervals between sample collection and analyses were 1.5 days (range, 1–10 days) in HCs and 48 days (range, 3–90 days) in the cats with chronic GI diseases, respectively. All procedures in this study were conducted in accordance with the guidelines of the Animal Care Committee of the Graduate School of Agricultural and Life Sciences, the University of Tokyo.

2.3. Plasma albumin, total protein, and AA analyses

Plasma albumin and total protein concentrations were measured by using DRI-CHEM 7000 (FUJIFILM, Tokyo, Japan). The reference ranges of plasma albumin and total protein were 2.3–3.5 g/dL and 5.7–7.8 g/dL, respectively. Eleven essential AAs (EAAs; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, taurine, threonine, tryptophan, and valine) and 11 non-essential AAs (NEAAs; asparagine, aspartic acid, alanine, cysteine, glutamine, glutamic acid, glycine, hydroxyproline, proline, serine, and tyrosine) levels were measured by liquid chromatography/mass spectrometry (SRL, Inc., Tokyo, Japan).

2.4. Cell preparation

Feline macrophages were prepared according to previously described protocols (Tamamoto et al., 2012). Briefly, EDTA blood from 4 healthy cats was overlaid onto Histopaque®-1077 (Sigma-Aldrich, St. Louis, MO, USA), and centrifuged at 800 × *g* for 30 min at room temperature. PBMC at the interface were resuspended in Hanks' balanced salt solution (HBSS; Sigma-Aldrich), and centrifuged at 200 × *g* for 10 min to remove contaminating platelets. PBMCs were resuspended in RPMI 1640 (Sigma-Aldrich) supplemented with 10% FBS (Gibco, Carlsbad, CA, USA) and 1% penicillin–streptomycin (Gibco). The cells were plated on a 24-well tissue culture-treated plate (Corning, Lowell, MA, USA) at a concentration of 1×10^6 cells/well, stimulated with 10 ng/mL PMA (Sigma-Aldrich), and cultured at 37 °C in 5% CO_2. Monocytes were allowed to attach to the plastic bottom of the plate for 24 h, and the plate was subsequently washed with HBSS to remove non-adherent cells. PMA-free culture medium (2 mL) was added to the culture plate, and the cells were cultured for 6 days to induce macrophage differentiation.

2.5. Feline macrophages stimulation

The cells were cultured in RPMI 1640 without FBS and antibiotics for 12 h prior to pretreatment. After washing, the medium was replaced with Dulbecco's modified Eagle medium without AAs (WAKO, Osaka, Japan). The cells were cultured with or without L-histidine (Sigma-Aldrich) at concentrations of 2 and 20 mM for 2 h prior to stimulation with 10 ng/mL LPS from *Escherichia coli* 0111: B4 (Sigma-Aldrich) for 2 h. We chose the stimulation condition according to a previous study (Hasegawa et al., 2011) and our preliminary examinations.

2.6. Measurement of interleukin-8 (IL-8) mRNA in feline macrophages

Total RNA was extracted from the cells using NucleoSpin RNA (TAKARA BIO INC., Shiga, Japan). After treatment with deoxyribonuclease, total RNA was converted into complementary DNA (cDNA) using a SuperScript III First-Strand Synthesis System for reverse transcription PCR (Invitrogen, Carlsbad, CA, USA). Transcriptional expression of IL-8, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and ribosomal protein S7 (RPS7) were quantified by StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) using Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan). Conditions for PCR cycles were as follows: preheating at 95 °C for 10 min; 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Each PCR reaction included a no-template control with sterile distilled water instead of a cDNA template to test for contamination of assay reagents or primer dimers. All the samples were examined in duplicate, and the mean ΔCt value was calculated. Each sample had only a single peak in the melting curve.

The following primer pairs were used: IL-8 forward, 5′-gtggcccaca ctgtgaaaact-3′, IL-8 reverse, 5′-cacaaccttctgcacccactt-3′; GAPDH forward, 5′-gagctgaatgggaagctcac-3′, GAPDH reverse, 5′-cgtatttggcagctttc tcc-3′; RPS7 forward, 5′-gtcccagaagccgcactttgac-3′, RPS7 reverse, 5′-ctcttgcccacaatctcgctcg-3′. These primers were chosen according to previous studies (Penning et al., 2007; Dolieslager et al., 2013; Straubinger et al., 2003). GAPDH and RPS7 were used as reference genes.

2.7. Statistical analyses

The Mann-Whitney *U* test was used for comparisons between two groups. The Dunnett's test was used for multiple comparisons of IL-8 expression in the feline macrophages. The Spearman rank correlation was used to test the associations of individual AA concentrations with FCEAI scores in the cats with chronic GI diseases. The Fisher's exact test was used to determine the sex differences between the cats with chronic GI diseases and HCs. Statistical analyses were performed using Prism software, version 5.0.1 (Graph Pad Software, San Diego, CA, USA). Statistical significance was defined as $P < 0.05$.