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NOTE

Physiology

The profile of urinary lipid metabolites in cats with bacterial cystitis

Yui KOBAYASHI[1)#], Tatsuro NAKAMURA[1)#], Tomohiro YONEZAWA[2)], Koji KOBAYASHI[1)] and Takahisa MURATA[1)*]

[1)] Department of Animal Radiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

[2)] Department of Veterinary Clinical Pathobiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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ABSTRACT. Bacterial cystitis is one of the feline lower urinary tract diseases (FLUTDs). Polyunsaturated fatty acids, such as arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), are oxidized into various lipid mediators that modulate inflammation. Since the profile of lipid metabolites excreted in urine is useful for assessing inflammatory body conditions, we analyzed 126 types of urinary lipid metabolites in cats with bacterial cystitis. Using LC-MS/MS, we found that the levels of 11 metabolites were higher in the urine of cystitis cats than in the urine of healthy cats. In detail, the urinary levels of ARA, EPA, and DHA and eight of their metabolites were increased in cystitis cats. Focusing on the lipid oxidation pathway, the urinary levels of four cyclooxygenase-, three lipoxygenase-, and one cytochrome P450-dependent oxidated metabolites were increased in bacterial cystitis. These urinary lipid profiles can provide some insight into the pathology and future diagnosis of bacterial cystitis.

KEY WORDS: cat, cystitis, lipid metabolite, urine

Lipid mediators, such as prostaglandins (PGs) and leukotrienes, are crucial molecules that regulate inflammation. Since lipid mediators and their related metabolites are excreted mostly in the urine, their excretion profile can be indicative of the host pathophysiological condition.

Cats have a unique lipid metabolism. They have a limited delta 6 desaturase activity, which converts linoleic acid (LA) to arachidonic acid (ARA), the substrates of lipid mediators [3]. Therefore, cats need to obtain ARA from their diet. They also have limited activity of lipoxygenase (LOX) and cytochrome P450 (CYP), which oxidize LA or ARA and produce several types of lipid mediators [11, 12]. We previously found that ARA-derived cyclooxygenase (COX)-metabolites are mainly composed of the urinary metabolite profiles of lipid mediators in healthy cats [7]. However, it remains unknown whether inflammation changes the urinary lipid profile in cats.

Cats are susceptible to urinary diseases, such as urolithiasis and cystitis, because they excrete highly concentrated urine through the short urinary tract. It is estimated that bladder inflammation directly reflects changes in the lipid excretion profile into the urine. Based on this background, we simultaneously analyzed lipid metabolites in cat urine samples with bacterial cystitis.

Urine samples were collected from 10 cats through cystocentesis or catheter from June 2019 to July 2020 at Pigeon Animal Hospital, Tokyo, Koyama Animal Hospital, Tochigi and Veterinary Medical Center, The University of Tokyo, Tokyo. We used residual urine samples which collected for general medical purpose by veterinarians. Sample information is presented in Table 1. Six cats (five castrated males and one spayed female, 4.8 ± 0.9 years old) recruited in this study were diagnosed as healthy based on the results of the biochemical blood test, complete blood count test, and physical condition. These cats had no underlying diseases or intake of medications during the urine collection. Four cats (two castrated males and two uncastrated male, 3.3 ± 0.8 years old) were diagnosed with bacterial cystitis by urine slag test and gram staining following the International Society for Companion Animal Infectious Diseases (ISCAID) guidelines [17]. The urine samples were stored at −28°C for 3–11 months. Informed written consents were obtained from all the owners at admission.

After the urine samples were centrifuged at 20,000 × *g* for 5 min, 200 µl of supernatant was mixed with 350 µl 0.1% formic acid water and 50 µl internal standard solution (Table 2). The mixed solutions were applied to a solid-phase extraction cartridge (OASIS µElution plate, Waters, MA, USA) preconditioned with 200 µl methanol and distilled water (DW). After washing with 200 µl DW and 200 µl hexane, the lipid fractions were eluted with 100 µl methanol. The 5 µl sample solution was injected into

*Correspondence to: Murata, T.: amurata@mail.ecc.u-tokyo.ac.jp
#These authors contributed equally to this work.

Table 1. Gender and the results of clinical signs, blood test and urine test

	Number	Institution	Gender	Fix	Collecting method	Age (year)	TP (g/dl)	ALT (U/l)	Cre (mg/dl)	WBC (10^2/μl)	HCT (%)	pH
Healthy	1	Pigeon	M	C	Cystocentesis	2	7.5	50	0.96	82	36.1	6.70
	2	Pigeon	M	C	Cystocentesis	3	7.3	39	1.34	176	37.1	7.09
	3	Pigeon	M	C	Cystocentesis	8	6.7	41	1.7	71	44.3	6.12
	4	Pigeon	F	S	Cystocentesis	4	6.5	54	1.62	91	48.3	6.64
	5	VMCUT	M	C	Cystocentesis	6	5.5	14	0.81	115	-	6.80
	6	VMCUT	M	C	Catheter	6	7.0	57	1.22	100	-	9.13
Cystitis	1	Koyama	M	-	Catheter	2	-	-	-	-	-	7.67
	2	Koyama	M	C	Catheter	4	7.2	-	-	-	-	7.14
	3	Koyama	M	-	Catheter	2	7.0	-	2.47	-	-	7.80
	4	Koyama	M	C	Catheter	5	8.2	-	5.28	-	-	7.12

*Pigeon; Pigeon Animal Hospital, VMCUT; Veterinary Medical Center, The University of Tokyo; Koyama; Koyama Animal Hospital. M; male, F; female, C, castrated male; S, spayed female. TP, total protein; ALT, alanine transaminase. Cre, serum creatinin; WBC, white blood cell; HCT, hematocrit; pH, pH of urine.

Table 2. The list of internal standards (IS)

	Name	Concentration (ng/ml)
1	Tetranor-PGEM-d6	25.0
2	6-keto-Prostaglandin $F_{1\alpha}$-d4	25.0
3	Thromboxane B_2-d4 (TXB2-d4)	25.0
4	Prostaglandin $F_{2\alpha}$-d4 (PGF2α-d4)	25.0
5	Prostaglandin E_2-d4 (PGE2-d4)	25.0
6	Prostaglandin D_2-d4 (PGD2-d4)	25.0
7	Leukotriene C_4-d5 (LTC4-d4)	25.0
8	Leukotriene B_4-d4 (LTB4-d4)	25.0
9	5(S) HETE-d8	25.0
10	12(S) HETE-d8	25.0
11	15(S) HETE-d8	25.0
12	Oleoyl Ethanolamide-d4 (OEA-d4)	0.5
13	Eicosapentaenoic Acid-d5 (EPA-d5)	500.0
14	Docosahexaenoic Acid-d5 (DHA-d5)	50.0
15	Arachidonic Acid-d8 (ARA-d8)	500.0

a high-performance liquid chromatograph (Nexera 2, Shimadzu, Kyoto, Japan) equipped with a mass spectrometer (LCMS-8060, Shimadzu). Three types of polyunsaturated fatty acids (PUFAs) (ARA, EPA, and DHA), 123 types of metabolites, and 15 types of internal standards (Table 2) were measured and analyzed using the Method Package for Lipid Mediators Version 2 with LabSolutions software (Shimadzu). Each metabolite was identified by the retention time and selected reaction-monitoring ion transition. The change in each metabolite level between healthy and bacterial cystitis urine samples was assessed by comparing the peak area ratio calculated using the following formula: Peak area of each metabolite/peak area of internal standard. Each value was further corrected using the creatinine concentration measured using LabAssay™ Creatinine (Wako, Osaka, Japan). All data are shown as mean ± SEM. Statistical differences were determined by the Mann-Whitney *U* test for two-group comparisons. Statistical significance was at P-value <0.05.

In the present analysis, we focused on the lipid metabolites detected in all urine samples of either healthy or cystitis cats. Three PUFAs and 28 types of lipid metabolites were detected in cat urine samples. The detection rates of these lipid metabolites were similar between the healthy and cystitis cats (Table 3). In the current study, there were no PUFAs or lipid metabolites that were detected only in cystitis urine.

Next, we compared the levels of PUFAs and lipid metabolites in urine between healthy subjects and those with cystitis. First, we compared the levels of the three substrates, ARA, EPA, and DHA. As shown in Fig. 1, the levels of the three substrates were increased in cystitis urine compared to healthy ones. Epithelial and immune cells contain large amounts of PUFAs in the cell membrane. Bacterial infections cause the urinary bladder epithelium to abrade and induce leucocyte infiltration into the urinary bladder [6]. The excreted PUFAs in cystitis urine might be derived from the inflamed host epithelium and immune cells.

Figure 2 shows the ARA metabolites. In 15 types of ARA-COX metabolites detected in all the healthy or cystitis urine samples, the levels of three metabolites were increased and one metabolite (PGK_2) was decreased in cystitis urine. Although the urinary levels of major inflammatory mediators, PGE_2, $PGF_{2\alpha}$, PGD_2, or PGI_2 did not change in cystitis, the level of PGI_2 metabolites, 6-keto-$PGF_{1\alpha}$ and 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ and that of PGE_2 metabolite, PGB_2, were increased in the urine of cystitis cat. These changes seem to reflect bladder inflammation. Two cystitis cats represented elevated serum creatinine level suggesting decreased renal function (Table 1). We cannot exclude a possibility that decreased renal function also somehow affects the

Table 3. The list of lipid metabolites detected in urine of cats

PUFA	Enzyme	Name	Detection rate (%)	
			Healthy	Cystitis
LA	LOX	9-HODE	83.3	100.0
		9-KODE	66.7	100.0
		13-HODE	83.3	100.0
	CYP	9,10-DiHOME	100.0	100.0
		12,13-DiHOME	100.0	100.0
AA		Arachidonic Acid (AA)	100.0	100.0
	COX	11-dehydro-Thromboxane B_2 (TXB_2)	100.0	100.0
		11-HETE	50.0	100.0
		13,14-dihydro-15-keto-tetranor-Prostaglandin D_2 (PGD_2)	100.0	50.0
		13,14-dihydro-15-keto-tetranor-Prostaglandin E_2 (PGE_2)	100.0	100.0
		13,14-dihydro-15-keto-tetranor-$PGF_{1\beta}$	100.0	100.0
		6-keto-Prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$)	100.0	100.0
		6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$	100.0	100.0
		Prostaglandin A_2 (PGA_2)	100.0	100.0
		Prostaglandin B_2 (PGB_2)	50.0	100.0
		Prostaglandin D_2 (PGD_2)	100.0	100.0
		Prostaglandin E_2 (PGE_2)	100.0	100.0
		Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)	100.0	100.0
		Prostaglandin J_2 (PGJ_2)	100.0	100.0
		Prostaglandin K_2 (PGK_2)	100.0	75.0
		Tetranor-PGEM	100.0	100.0
	CYP	14,15-DHET	33.3	100.0
	non-OX	2,3-dinor-8-iso-$PGF_{2\alpha}$	100.0	100.0
		5-iPF2a-VI	100.0	100.0
		8-iso-15 (R)-$PGF_{2\alpha}$	100.0	100.0
		8-iso-$PGF_{2\alpha}$	100.0	100.0
DHA		Docosahexaenoic Acid (DHA)	100.0	100.0
EPA		Eicosapentaenoic Acid (EPA)	100.0	100.0
	COX	PGE_3	100.0	100.0
		PGD_3	100.0	100.0
	LOX	17,18-DiHETE	83.3	100.0

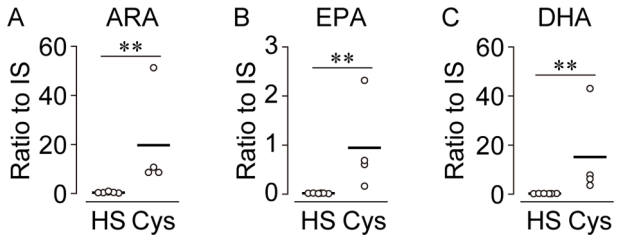

Fig. 1. The amounts of polyunsaturated fatty acids (PUFAs) detected in urine of cats. The amounts of arachidonic acid (ARA) **(A)**, eicosapentaenoic acid (EPA) **(B)** and docosahexaenoic acid (DHA) **(C)** detected in urine of healthy (HS) or bacterial cystitis (Cys) cats. A circle means the ratio of polyunsaturated fatty acids (PUFAs) detected in each cat. Black bar means average ratio of each group. IS: internal standard. **: $P<0.01$ compared with heathy and bacterial cystitis.

urinary levels of these metabolites. In four metabolites changed in cystitis urine, PGB_2 is a non-enzymatic dehydration product of PGE_2 in the presence of a strong base [13]. Urine pH increases to an alkaline pH (7.5–9) when urease-positive bacteria, such as *Staphylococcus* or *Proteus*, infected the cat [5, 10]. Thus, there is a possibility that the bacterial infection increased the urine pH to alkaline, which resulted in PGE_2 conversion into PGB_2. In contrast, the level of another PGE_2 metabolite, PGK_2, was decreased in the cystitis urine samples. The metabolic pathways of PGK_2 remain unclear. Further investigation is required to reveal the mechanism by which the level of PGK_2 is decreased in cystitis urine.

CYP2C and CYP2J convert ARA to several types of eicosatrienoic acids (EETs), such as 14,15 EETs. Epoxide hydrolase (EH) metabolizes EETs to dihydroxyeicosatrientic acid (DHET) [14, 16]. Some types of DHET ragioisomers including 14,15 DHET were detected in rat and human urine [9, 18]. In contrast, we found in the present study that 14,15 DHET was the only

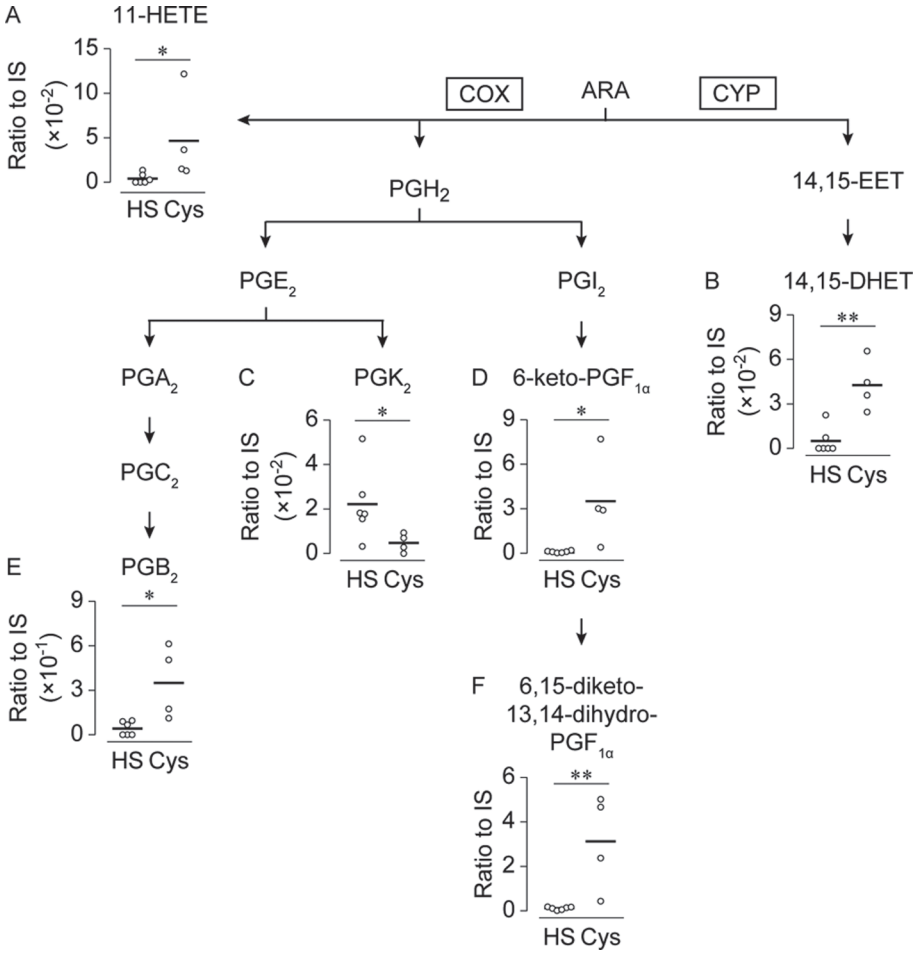

Fig. 2. Metabolites of arachidonic acid (ARA). Metabolites of ARA detected in urine of healthy (HS) and bacterial cystitis (Cys) cats. A circle means the ratio of lipid metabolites detected in each cat. Black bar means average ratio of each group. COX: cyclooxygenase, CYP: cytochrome P450, DHET: dihydroxyeicosatrienoic acid, EET: eicosatrienoic acid, HETE: Hydroxyeicosatetraenoic acid, PG: prostaglandin, IS: internal standard. *: $P<0.05$, **: $P<0.01$ compared with heathy and bacterial cystitis.

ARA-CYP metabolite detected in the urine of cats (Fig. 2B). Lautz *et al.* have shown that cats express relatively lower levels of CYP2C mRNA than humans and rats [8]. Thus, the lower expression and activity of CYP in cats might result in only 14,15-DHET detection in urine. Conversely, the levels of 14,15 DHET were increased in the urine of cystitis cats. Interestingly, commensal bacteria have been reported to have EH and produce lipid mediators in the host [2]. Some infected bacteria may be involved in the increased production of 14,15 DHET in the cat bladder.

In addition to the metabolites of ARA, we found that LA metabolites were also increased in the urine of patients with cystitis. LA is the dominant fatty acid, accounting for 40% of the total fatty acids in cat serum [4]. The levels of other PUFAs, EPA, and DHA were increased in cystitis urine, but the urinary levels of their metabolites did not change. LA and ARA may be consumed mainly in cats with bacterial cystitis. In particular, LOX metabolites of LA were significantly increased in cystitis urine (Fig. 3). Inflammatory stimulation enhances LOX expression and activity in various cell types [1, 15]. These phenomena may result in an increase of LA-LOX metabolites in cystitis urine.

In summary, we revealed that bacterial cystitis caused an increase in three types of PUFAs, the metabolites of PGE_2, PGI_2, and LA metabolites in the urine of cats. Our findings may help reveal the mechanism of bacterial cystitis and develop a new biomarker for this disease.

POTENTIAL CONFLICTS OF INTEREST. The authors have no conflicts of interest directly relevant to the content of this article.

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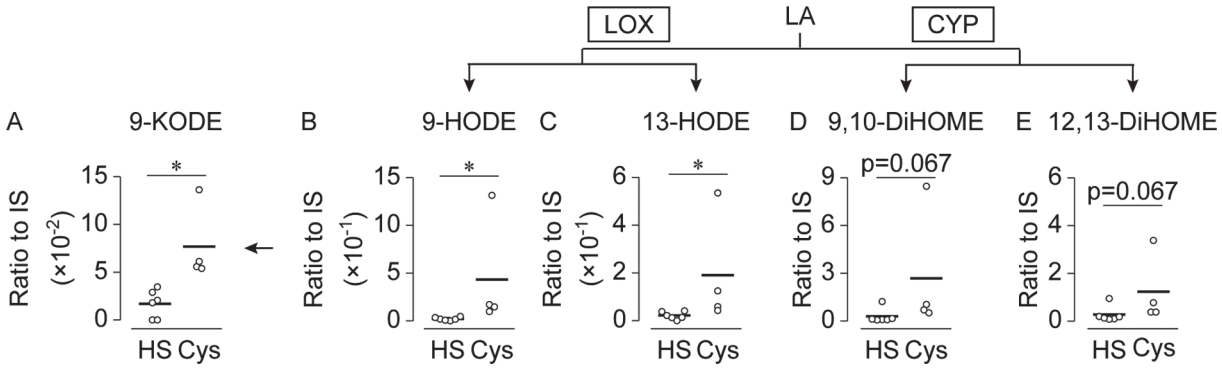

Fig. 3. Metabolites of linoleic acid (LA). Metabolites of LA detected in urine of healthy (HS) and bacterial cystitis (Cys) cats. A circle means the ratio of lipid metabolites detected in each cat. Black bar means average ratio of each group. CYP: cytochrome p450, DiHOME: dihydroxy-octadecenoic acid, HODE: Hydroxyoctadecadienoic acid, KODE: keto-octadecadienoic acid, LOX: lipoxygenase, PG: prostaglandin, IS: internal standard. *: $P<0.05$, compared with heathy and bacterial cystitis.

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