A Study of Metabolites Isolated from the Urine Samples of Cats and Dogs Administered Orbifloxacin

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ABSTRACT. Urine samples of cats and dogs collected for 24 hr after a subcutaneous injection of orbifloxacin (OBFX) were analyzed. The metabolites were examined using HPLC. In the dog urine, 87% of total was the parent compound and 13% glucuronide compound of OBFX and 96% was parent and 4% metabolite in the cat urine. The metabolite of cat urine was identified as N-hydroxy OBFX, determined by comparison of the extraction of urine with chloroform with the standard compound of N-hydroxy OBFX, using LC/APCIMS. N-hydroxy OBFX had a weaker antibacterial activity against fluoroquinolone sensitive bacteria than the parent compound.
—KEY WORDS: canine, feline, orbifloxacin metabolite.

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Orbifloxacin (OBFX: 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid), a fluoroquinolone antibacterial agent, has been developed especially for use in veterinary medicine. We have previously studied the pharmacokinetics of OBFX in cats and dogs and found that the urinary recoveries of OBFX in the first 72 hr urine of cats and dogs after a single subcutaneous dose (5.0 mg/kg) of OBFX were 28% and 45%, and fecal recoveries were 15% and 18%, respectively [4]. In the present study, we have examined the urinary metabolites in these species after OBFX administration.

An injectable form of OBFX (Victas ®Injection 5 %, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) was used. Two male and 2 female cats (from 5 to 8 months old) and 5 male dogs (from 10 to 11 months old) were used. The first 24 hr urine samples were collected from cats and dogs receiving a single subcutaneous dose(5.0 mg/kg) of OBFX, and kept frozen at −20°C until analysis. The urinary metabolites were determined using HPLC and liquid chromatography/atmospheric pressure ionization mass spectrometry (LC/APCIMS). A 10 μl urine sample filtered with a millipore filter (0.2 μm) was injected into HPLC apparatus (LC-6AD, Shimadzu Co., Ltd., Kyoto, Japan). A portion of the urine sample was treated with β-glucuronidase (Type B, 6,000 units/m*l*, Sigma Chemical Company, St. Louis, MO, U.S.A.) at 37°C for 24 hr to examine for the presence of the glucuronide compound of OBFX and analyzed using HPLC. An analytical column (YMC Pack A-312, ODS, 150 × 6 mm, Yamamura Chemical Co., Ltd., Tokyo, Japan) and a guard column (TSK-gel, ODS-80 TM, 3.2 × 15 mm, Touso Co., Ltd., Tokyo, Japan) were used for determination of OBFX and its metabolites with a column temperature of 32°C, a mobile phase containing 0.2% SDS (0.15 M citrate buffer (pH 5.0): methanol-dimethyl formamide (9:1 v/v)=43.5: 56.5 v/v), and a flow rate of 1 ml/min. The UV detection wavelength was 340 nm.

M-2 was extracted by chloroform from the first 24 hr urine of a cat receiving a single subcutaneous dose of OBFX. The organic layer was concentrated to dryness under reduced pressure, and the residue was dissolved in methanol. A portion (10 μl) of the resulting solution was subjected to LC/APCIMS. A Hitachi Model M-1000 LC/APCIMS spectrometer (Hitachi Co., Ltd., Tokyo, Japan) attached to a Model L-6200 HPLC system (Hitachi Co., Ltd., Tokyo, Japan) was used, with the following measurement conditions: drift voltage 40 V, focus voltage 113 V, vaporization temperature 270°C and desolvation temperature 399°C. The HPLC conditions were almost the same as those described above, except for the composition of mobile phase (methanol: 0.01% trifluoroacetic acid (42: 58 v/v)).

The antibacterial activities of metabolites of OBFX were determined by the method of the Japanese Society of Chemotherapy for assay of minimum inhibitory concentration (MIC) [1].

The elution patterns of HPLC chromatograms of urine samples were almost the same in the 4 cats. A typical pattern (cat no. 601) is shown in Fig. 1. (a, b). Two peaks (M-2, M-3) were detected at retention times (t_R) of about 10 min and 23 min. When the chloroform extract of the urine sample which including M-2 was analyzed by LC/APCIMS, a protonated molecule (MH^+) appeared at m/z 412 in the mass spectrum of M-2 (Fig. 2), suggesting that the molecular weight of M-2 was 411. The mass spectrum of N-hydroxy OBFX corresponded to that of M-2 (Fig. 2). The mass spectrum of M-3 corresponded to that of standard OBFX. On HPLC analysis, standard OBFX and standard N-hydroxy OBFX were eluted at t_Rs of 23 min and 10 min, respectively. These findings suggested that M-3 and M-2 were OBFX and N-hydroxy OBFX, respectively. Two peaks (M-1, M-3) were detected in dog urine at t_Rs of 5 min and 23 min. A typical pattern (dog no. 111) is shown in Fig. 1. (A-C). After the treatment of the urine sample of each dog with β-glucuronidase, the peak of M-1 disappeared and the peak of M-3 on the chromatograph increased.

The ratio of each metabolite was calculated on the basis of the peak area on HPLC and the molecular absorption coefficient of each metabolite. In the urine of cats, the ratios of OBFX and N-hydroxy OBFX were 0.96 ± 0.004

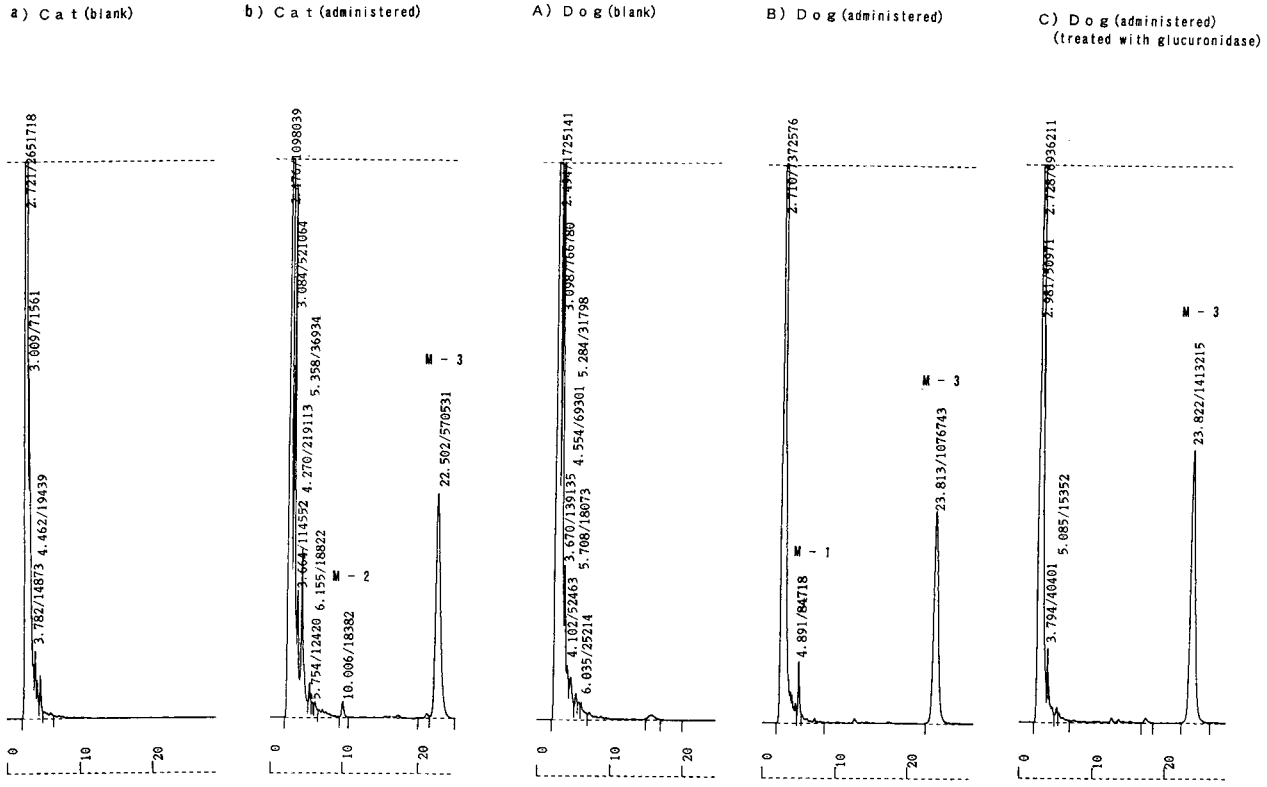


Fig. 1. HPLC chromatograms of urine and urine treated with glucuronidase of a cat (cat no. 601) and a dog (dog no. 111) receiving a single subcutaneous dose of OBFX (5.0 mg/kg). M-1 is thought to be the glucuronide of OBFX. t_R of M-2 corresponded to that of N-hydroxy OBFX. t_R of M-3 corresponded to that of unchanged OBFX. HPLC conditions are described in the text.

(mean ± S.E.) and 0.04 ± 0.004, respectively. In the urine of dogs, the ratios of OBFX and the glucuronide compound of OBFX were 0.87 ± 0.008 and 0.13 ± 0.008, respectively. These findings suggest that OBFX is difficult to be metabolized in cats and dogs. Sparfloxacin (SPFX), which has a 3, 5-dimethyl piperazine ring at position 7, being similar to OBFX, is known to be metabolized only a little [6].

Although the glucuronide compound of OBFX was not detected in cat urine, it was detected at 13% in dog urine after the OBFX administration. This is caused by the species difference of drug metabolism [2]. It has been reported that the glucuronide compounds of temafloxacin and lomefloxacin are principal metabolites in dogs [3, 5]. Yamaguchi *et al*. [6] has reported that only the glucuronide compound of SPFX was detected as a metabolite in the urine sample of dogs administered SPFX.

The *in vitro* antibacterial activities of N-hydroxy OBFX and OBFX are shown in Table 1. The MIC ranges of N-hydroxy OBFX against Gram-positive and negative bacteria varied from 0.39 to 6.25 μg/m*l* and from 0.05 to 25 μg/m*l*, respectively. The *in vitro* antibacterial activity of N-hydroxy OBFX was weaker than that of OBFX.

In the present study, N-hydroxy OBFX and the glucuronide compound of OBFX can be detected as metabolites of OBFX in the urine of cats and dogs, using HPLC apparatus. The existence of other metabolites may be undeniable.

Table 1. *In vitro* antibacterial activities of OBFX and N-hydroxy OBFX.

Organism	MIC: μg/m*l*	
	OBFX	N-hydroxy OBFX
Gram-positive:		
Staphylococcus aureus 209 JC-1	0.78	0.39
Staphylococcus epidermidis 8	0.39	0.39
Streptococcus pyogenes A65	3.13	6.25
Enterococcus faecalis 2473	6.25	6.25
Gram-negative:		
Escherichia coli JC-2	0.05	0.78
Escherichia coli Kp	0.0125	0.05
Salmonella typhimurium S-9	0.1	0.78
Salmonella typhi 901	0.05	0.78
Salmonella enteritidis 1891	0.025	0.39
Shigella flexneri 2a EW 10	0.1	1.56
Proteus mirabilis IFO 3849–4	3.13	12.5
Klebsiella pneumoniae 13	0.2	3.13
Enterobacter aerogenes ATCC 13048	0.2	3.13
Pseudomonas aeruginosa Tsuchijima	3.13	25

The method for assay of minimum inhibitory concentrations (MIC) is described in the text.

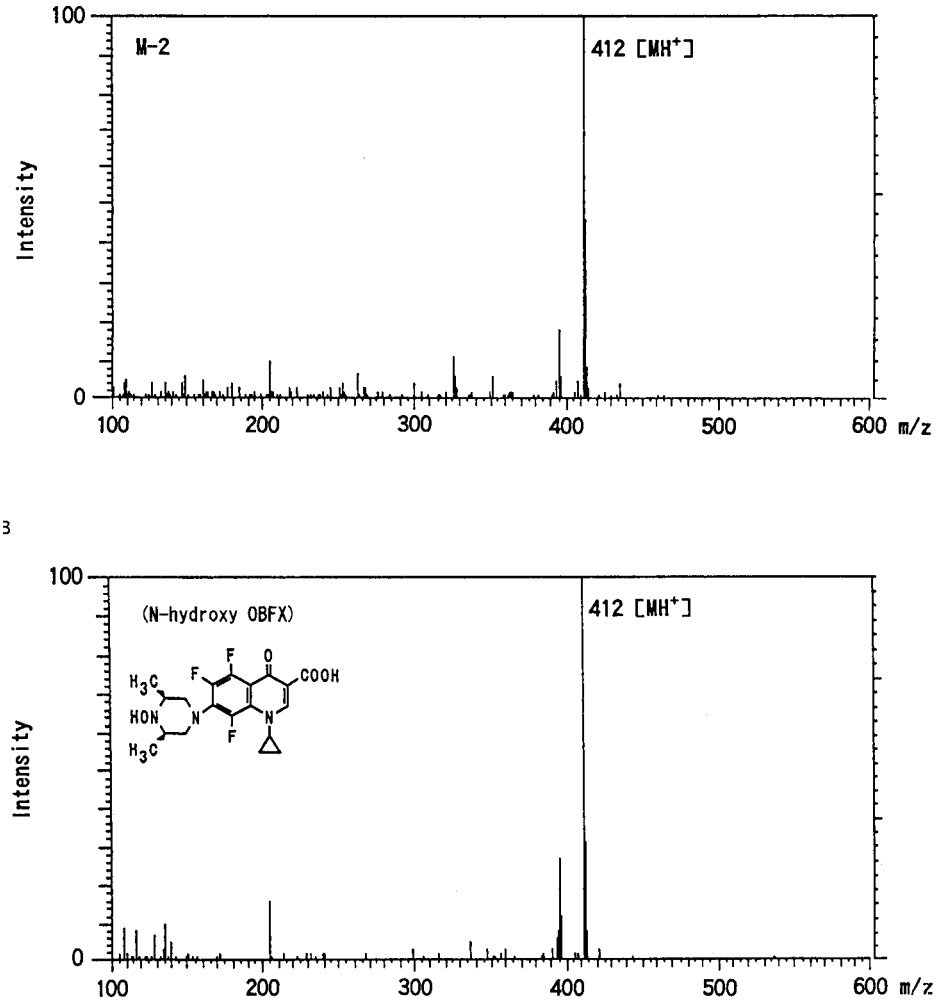


Fig. 2. Mass spectra of M-2 (A) and N-hydroxy OBFX (B) on LC/APCIMS. M-2 was extracted by chloroform from the urine of a cat administered OBFX. The molecular weight of M-2 is thought to be 411. The molecular weight of N-hydroxy OBFX is thought to be 411. LC/APCIMS conditions are described in the text.

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