Therapeutic and Adverse Effects of Flunixin-Meglumine in Adult and Young Cats

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ABSTRACT. In this study, we elucidated the difference in nonsteroidal anti-inflammatory drug sensitivities between young and adult cats on therapeutic and adverse effects. In the prevention of lipopolysaccharide (LPS)-induced hyperthermia using flunixin-meglumine, young (<3 months old) and adult (>12 months old) cats of both sexes were given LPS (0.3 μg/kg, i.v.), and body temperature was measured 24 hr later. Flunixin (1 mg/kg, s.c.) was administered 30 min before the LPS injection. LPS-induced hyperthermia was almost completely inhibited by pre-treatment with flunixin in both adult and young cats. In addition, flunixin showed almost the same antipyretic effects in both young and adult cats. The animals were administered flunixin (1 mg/kg, s.c.) once a day for 3 days, and sacrificed 24 hr later to examine the gastrointestinal mucosal lesions. In adult cats, flunixin caused many severe lesions in the small intestine. In contrast, very few gastrointestinal lesions were produced by flunixin in young cats. In the pharmacokinetics of flunixin, plasma concentrations of flunixin between young and adult cats from 0.5 to 4 hr after the injection. These results demonstrated that NSAIDs could be used more safely in young than in adult cats from the points of gastrointestinal adverse effects. Furthermore, this difference in gastrointestinal lesions between adult and young cats was not related with the plasma concentration of flunixin.

KEY WORDS: flunixin-meglumine, gastrointestinal effect, lipopolysaccharide, nonsteroidal anti-inflammatory drug, pharmacokinetics.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics in veterinary and human medicine. In most species, they are very effective in acute and chronic pains. For example, NSAIDs are often the first drugs used for treatment of pain caused by osteoarthritis in veterinary medicine [20]. In the analgesic study of cats, the effect of flunixin-meglumine (FNX) has been reported in 40 cats undergoing a variety of surgical procedures [9]. It is also well known that NSAIDs cause some adverse effects such as gastrointestinal side-effects in the veterinary medicine. In rats, many kind of NSAIDs have been reported to cause gastrointestinal lesions because of the inhibition of prostaglandins synthesis even new NSAIDs [1]. However, the data on the usage of NSAIDs in cats are less complete than in dogs and, to date, there are no published data about gastrointestinal side-effects in cats.

Most NSAIDs are cleared from the body through hepatic metabolism comprising primarily glucuronidation followed by excretion of the resultant polar metabolites via bile and/or urine. Flunixin is well absorbed from the gastrointestinal tract and undergoes enterohepatic circulation, resulting in a bioavailability of >100% in dogs and cats [16]. At least, two other active transport pathways are involved in the pharmacokinetics of flunixin-meglumine in cats. In the primary pathway, flunixin is actively transported into liver cells glucuroconjected, and then excreted into bile. The second pathway, renal tubular secretion, is a minor pathway of excretion [10]. For many NSAIDs, glucuronidation is an important pathway. For example, the metabolism of ketoprofen is dominated by glucuronidation reactions in dogs [25]. However, many studies have reported that glucuronidation in cats is limited [5, 7, 11, 18, 19, 23, 24, 30]. Therefore, NSAIDs may induce more severely adverse-effects in cats compared with other species.

On the other hand, in human beings, NSAIDs are widely used in children. Adverse gastrointestinal or renal events from short-term use of either ibuprofen or acetaminophen appear to be quite rare in children [14]. Furthermore, in vasopressin-dependent rats, it is reported that lesion area of old rats caused by indomethacin is significantly severer than lesion area of young rats [8]. However, there is little data comparing the use of NSAIDs in young and adult cats. There are many NSAIDs including the FNX in veterinary medicine market. In U.S.A. and European countries, the FNX is commonly used in horses and dogs. Experimentally, the FNX did not produce a remarkable adverse effect on the gastrointestinal tract in calves [13]. In a comparative study of the postoperative analgesia of phenylbutazone, FNX and carprofen, the FNX showed an analgesic effect of the longest duration (12.8 hr), compared to phenylbutazone (8.4 hr) and carprofen (11.7 hr) in horses [12]. However, there are no available data about the therapeutic and adverse effects in cats.

The purpose of the present study was to elucidate the difference on therapeutic and adverse effects of flunixin-meglumine between adult and young cats. As well as we know, this is the first study about that.
MATERIALS AND METHODS

Animals: This study compared effects of flunixin administration (flunixin, 1 mg/kg, s.c.) in young and adult cats in terms of antipyretic, pharmacokinetics and gastrointestinal adverse effects. Young (<3 months, 0.4–0.9 kg body weight) and adult (>6 months, >3 kg body weight) cats of both sexes were used (4 animals in each group). The cats were kept in the experimental room more than 7 days before the start of the experiment. During experiments, the animals were housed individually at an ambient temperature of 25 ± 1°C with a 12 hr light/dark cycle, with the lights being switched on at 07:00. All of cats received commercially available dry cat food containing >30% protein, >9% fat, <4% fibre, <10% mineral and <10% water. Water was allowed ad libitum. Experiments were conducted between 08:30 and 17:00. Study protocols were approved by the Animal Research Committee of Tottori University, Tottori, Japan.

Drugs: Flunixin-meglumine (Banamin®, flunixin 50 mg/ ml, Dainippon Sumitomo Pharmaceutical Co. Ltd., Osaka, Japan), xylazine (Ceractal®, 2% solution, Bayer, Osaka, Japan), sodium pentobarbital (Nembutal, Dainippon Sumitomo Pharmaceutical Co. Ltd.), and lipopolysaccharide (LPS, Lot no.1163540JC, W. E. Coli, Wako Pure Chemical Industries Ltd., Osaka, Japan) were used in this study.

Design for prevention of LPS-induced hyperthermia using flunixin-meglumine: The experiment consisted of five treatment groups in both young and adult cats. The four cats were assigned to each of the five treatment groups. For the induction of pyrexia, LPS (0.3 μg/kg) was administered intravenously (i.v.). Flunixin-meglumine were administered subcutaneously (s.c.) 0.5 hr before LPS injection. In both young and adult cats, the control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was injected PSS before LPS injection. The cats in the other groups received 0.25, 0.5 or 1 mg/kg flunixin (s.c.), and LPS (0.3 μg/kg, i.v.). Those groups are hereafter referred to as CONT, LPS, FNX0.25, FNX0.5 and FNX1.0. Body temperature was measured at 0.5 hr before and 0, 0.5, 1, 2, 4, 8 and 24 hr after the LPS injection.

Design for gastrointestinal lesions by flunixin: This experiment consisted of non-injected groups (young and adult groups) and flunixin-injected groups (young and adult groups). To determine gastrointestinal lesions, 1 mg/kg flunixin was administered (s.c.) once a day after the morning meal for 3 days. In both groups, the animals were sacrificed using xylazine (1.0–3.0 mg/kg, s.c.) and sodium pentobarbital (50–75 mg/kg, i.v.) 24 hr after the final injection of flunixin. Mucosal lesions in the stomach and intestine were examined using a stereomicroscopy. The percentage of the lesion area was calculated as 100% of total small intestine area.

Design for pharmacokinetics of flunixin: Flunixin-meglumine were administered 1.0 mg/kg subcutaneously (s.c.) in young and adult cats. The pharmacokinetics of flunixin were analysed using a high-performance liquid chromatography (HPLC). The HPLC analysis was performed by using a model L-62000 (Hitachi Co. Ltd., Tokyo, Japan). For pharmacokinetic analysis, blood samples were collected from the jugular vein at 0, 0.5, 1, 2 and 4 hr after flunixin injection. The plasma was separated and stored at 30°C for 1 week, after which it was analysed by HPLC. Chromatographic separations were performed on Ashaipak ODS-3 (4.6 mm ID × 250 mm L; Asahi Kasei Co. Ltd., Japan). The mobile phase was composed of acetonitrile–methanol water (40:40:20, v/v/v), with 0.04% glacial acetic acid. The solution was filtered through a 0.45 μm membrane prior to use. The flow rate was 1.1 ml/min, and column temperature was maintained at 40°C. The channel on the UV detector was configured at 330 nm. The volume injection was 20 μl.

Statistical analysis: All values are expressed as means and standard error. In the data of body temperature, one-way analysis of variance for repeated measures was used to examine the time effect within each group and the four groups’ effect at each time point. When a significant difference was found, a least significant difference (LSD) test was used to compare the means. The other data were subjected to an analysis of variance. When F value was not significant, differences between two groups were analyzed by Student’s t-test. When a significant F value was found, a Wilcoxon-Mann-Whitney test was used for the statistical evaluation. The level of significance in all tests was set at \(P<0.05\).

RESULTS

Prevention of LPS-induced hyperthermia using flunixin-meglumine: As shown in Fig. 1, in young cats, the mean body temperature before LPS injection was 38.5 ± 0.1°C (n=4). In the LPS group, body temperature at 1, 2 and 4 hr was significantly higher compared to at –0.5 hr (\(P<0.05\)). After LPS injection, body temperatures increased and reached a maximum 2 hr after the injection (39.35 ± 0.15°C). After the peak of LPS-induced hyperthermia at 2 hr, body temperature decreased. Body temperature was significantly higher in the LPS group compared to CONT at 0.5, 1, 2, 4 and 8 hr (\(P<0.05\)).

As shown in Fig. 2, flunixin suppressed hyperthermia induced by LPS in a dose-dependent manner. In the FNX0.25 group, body temperature at 0.5, 1, 2 and 6 hr after LPS injection was significantly higher than at –0.5 hr (\(P<0.05\)). In the FNX0.5 and FNX1.0 groups, body temperatures did not significantly change at each elapsed time compared with –0.5 hr. Two hours after LPS injection, body temperature was significantly lower in cats of FNX0.25 group than in the LPS group (\(P<0.01\)). In the FNX0.5 and FNX1.0 groups, body temperatures at 1, 2 and 4 hr after LPS injection were significantly lower than in the LPS group (\(P<0.05\) to 0.01).

As shown in Fig. 3, in adult cats, mean body temperature in the LPS group was significantly (\(P<0.05\) to 0.01) higher at 1, 2, 4 and 8 hr compared with –0.5 hr. Body temperature increased to a maximum (39.6 ± 0.27°C) at 2 hr after the
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LPS injection, and then decreased gradually. Body temperature was significantly ($P<0.01$) higher at 2, 4 and 6 hr in the LPS group than the CONT group.

As shown in Fig. 4, flunixin suppressed hyperthermia caused by LPS injection in a dose-dependent manner in adult cats. In the FNX0.25 group, body temperature at 4 and 8 hr after LPS injection was significantly ($P<0.05$ to 0.01) higher than at −0.5 hr value. In the FNX0.5 and FNX1.0 groups, body temperatures did not significantly change at each elapsed time compared with −0.5 hr. At 1 and 2 hr after LPS injection, body temperature was significantly ($P<0.01$) lower in the FNX0.25 group than in the LPS group. Body temperature in the FNX1.0 group was significantly ($P<0.05$ to 0.01) lower from 1 to 8 hr compared to the LPS group.

In both young and adult cats, body temperature increased significantly from 2 hr after LPS injection. In both age groups, higher dosing levels of flunixin greatly suppressed the hyperthermia induced by LPS.

**Gastrointestinal side-effects:** In both adult and young cats, lesions were observed in the duodenum and lower small intestine following repeated doses of flunixin. In the stomach, some lesions were observed in adult cats, but not in young cats. As shown in Fig. 5, the lesion area (cm²) caused by flunixin in both duodenum and small intestine were significantly less in young cats compared with adult cats. In addition, the lesion rate (%) of small intestine was significantly less in young cats than adult cats (Fig. 6). In both adult and young cats of non-injected groups, gastrointestinal lesions were not observed.

**Pharmacokinetics of flunixin:** As shown in Fig. 7, in both adult and young cats, blood levels of flunixin reached a maximum concentration at 0.5 hr after a subcutaneous injection. One hr after injection, flunixin concentration...
decreased to approximately half of the maximum level, and then decreased gradually. The area under time curve (AUC) of the plasma flunixin concentration was not significantly different between adult and young cats.

DISCUSSION

Flunixin is one of the traditional NSAIDs, and this drug has the strong anti-cyclooxygenase (COX) activity [17]. COX has been recognised as the principal enzyme catalyzing the synthesis of prostanoids from arachidonic acid. The COX has the two isoforms, COX-1 and COX-2 [21, 22]. COX-1 is constitutively expressed in almost of cells, while COX-2 is induced in inflammatory conditions. Much effort has gone into developing NSAIDs that selectively inhibit COX-2 rather than COX-1, so as not to affect the homeostasis functions of the prostanoids preferentially syn-
thesised by COX-1, and in particular to reduce the gastrointestinal bleeding caused by COX-1 inhibition [6, 13]. In a vitro study, the FNX has a lower COX-2 selectivity in comparison with carprofen and meloxicam [2]. LPS from Gram-negative bacteria, stimulates host defence cells to release several endogenous pyrogens. Many evidences show that fever induced by LPS is mediated by a number of endogenous pyrogenic cytokines produced [4, 21, 22, 27]. These pyrogenic cytokines are transported to the thermostegulatory center in the preoptic area, and they stimulate the production of COX-2-dependent prostaglandin (PG) E2, the putative final mediator of the febrile response [15].

The present study demonstrated that LPS caused hyperthermia in both young and adult cats, and that flunixin suppressed dose-dependently hyperthermia induced by LPS. This study also revealed that flunixin at the dose of more than 0.5 mg/kg (s.c.) can significantly suppress hyperthermia. Then, veterinarians may be use flunixin in doses higher than 0.5 mg/kg for treatment of bacterial febrile conditions. In a study, the FNX has been administered at 1 mg/kg/day for 7 consecutive days in cats [28]. In this study, the biochemical and haematological variables did not significantly alter in cats [28]. Therefore, it is conceivable that the dosage of 0.5 to 1 mg/kg FNX may be safely used for cats.

The present study further demonstrated that in both adult and young cats, lesions were observed in the duodenum and lower small intestine following repeated doses of flunixin. In the stomach, some lesions were observed in adult cats, but not in young cats. Since it could be a result of the difference in small intestinal area between adult (387 ± 24.7 cm²) and young cats (234 ± 15.7 cm²), we calculated the rate of erosions relative to the surface area of the intestinal lumen. As shown in Fig. 6, in adult cats, the erosion area was 1.52 ± 0.89% of total surface area, while in the young cats it was 0.09 ± 0.04%, which was significantly lower than adult cats. These results revealed that gastrointestinal lesions were less in young than in adult cats. There are some possible explanations about those results. Firstly, we examined about the plasma concentration of flunixin in this study. Pharmacokinetic data of flunixin using HPLC in young cats were similar to those in adult cats. In the pharmacokinetics of flunixin, Tₘᵡₐₓ after oral doses of flunixin is approximately 1.3–2 hr in cats [29]. The elimination half-life has been found to be 1–1.5 hr, using an assay with a limit of 0.25 μg/ml [28, 29]. In our study, Tₘᵡₐₓ after subcutaneous injection was 0.5–1 hr, and the elimination half-life was around 1 hr in both young and adult cats. Therefore, our study demonstrated that plasma concentration of flunixin was not different between young and adult cats.

An another possible explanation is that there may be significant age-based differences in bile acids, enterobacteria, and mucosal defense. Alterations of intestinal glycolcalyx have been reported in rats after indomethacin administration, suggesting that changes in epithelial mucin content may contribute to NSAID-induced deleterious effect on bowel. Inflammation and ulceration are the ultimate result, once the mucosal barrier has been disrupted by the local and systematic effects of damaging therapeutic agents [3]. Intraluminal factors including bacteria may be key elements in the initiation of damage in NSAID-induced mucosal erosions. On the other hand, the administration of indomethacin has been reported to induce an increase in bacterial counts in the mucosa [1]. There is a study suggesting that bacterial flora may play a role on the pathogenesis of NSAIDs bowel injury. This study has demonstrated that antimicrobials attenuated NSAIDs induced enteropathy in rats [26]. From these observations, it may be possible that gastrointestinal bacterial counts may be lower in young cats than in adult cats. Therefore, some factors described above may be involved in the reasons why NSAID-induced erosion is milder in young than in adult cats. However, it is unknown why the gastrointestinal effects in the young cat are milder than those in the adult cat.

In conclusion, this study demonstrated that flunixin suppressed dose-dependently hyperthermia induced by LPS in both young and adult cats, and that flunixin-induced gastrointestinal lesions were less in young than in adult cats. This difference between young and adult cats on gastrointestinal adverse effects was not related with the plasma concentration of flunixin. In the present study, as the FNX caused severe gastrointestinal adverse effects in adult cats, it may not be suitable for the use to adult cats. Although the essential causes of the difference are unknown, the present results suggest that NSAIDs could be safer in young than in adult cats, with respect to gastrointestinal adverse effects.

REFERENCES