Therapeutic and adverse effects of non-steroidal anti-inflammatory drugs in adult and young cats

（幼猫と成猫における非ステロイド性抗炎症薬の薬効と副作用）

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Contents

General Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics in veterinary and human medicine. In most species, NSAIDs are very effective in alleviating acute and chronic pain. For example, NSAIDs are often the first line of drugs used in managing the pain caused by osteoarthritis in veterinary medicine [26]; however, NSAIDs also cause some adverse effects, including gastrointestinal adverse effects. In rats, even the newly developed NSAIDs have been reported to cause gastrointestinal lesions because of the inhibition of prostaglandin synthesis [21]. However, to our knowledge, no studies reporting the gastrointestinal adverse effects of NSAIDs in cats have been published yet.

Most NSAIDs are cleared from the body through hepatic metabolism, which primarily comprises glucuronidation followed by excretion of the resultant polar metabolites through the bile and/or urine. Glucuronidation is an important pathway in many NSAIDs; for example, in dogs, the metabolism of ketoprofen is dominated by glucuronidation reactions [14]. However, many studies have reported that glucuronidation is limited in cats [7, 10, 15, 25, 34]; therefore, NSAIDs may induce more severe adverse effects in cats than they do in other species.

In humans, NSAIDs are widely prescribed to children; however, in children, gastrointestinal or renal adverse effects from the short-term use of ibuprofen or acetaminophen are quite rare [3]. Moreover, vasopressin plays an important role in

microcirculation and may be responsible for the resistance to gastric mucosal lesions [11]. In vasopressin-deficient rats, gastric lesions caused by indomethacin were significantly more severe in older rats than in younger rats [35]. These findings suggest that the age-dependent severity of indomethacin-induced gastric injury is not related to vasopressin activity. However, to our knowledge, no studies comparing the effects of NSAIDs between young and adult cats have been reported yet.

Flunixin-meglumin, which is one of the traditional NSAIDs, was used in chapter 1. In the USA and European countries, flunixin-meglumin is used in horses and dogs. The analgesic effect of flunixin-meglumin in horses persists for a long duration [2, 16]. However, the data regarding the therapeutic and adverse effects of flunixin-meglumin in cats are scarce.

Ketoprofen was used in chapter 2. This drug has been approved for use in cats in Europe, Australasia, Canada, and Japan. However, to our knowledge, no studies investigating the therapeutic and adverse effects of ketoprofen in cats have been reported yet.

The purpose of the present study was to elucidate the differences in therapeutic and adverse effects of flunixin and ketoprofen between young and adult cats.

Chapter 1

Therapeutic and adverse effects of non-steroidal anti-inflammatory drugs in adult and young cats

Introduction

In the analgesic study of cats, the effect of flunixin-meglumine (FNX) has been reported in 40 cats undergoing a variety of surgical procedures [13]. It is also well known that NSAIDs cause some adverse effects such as gastrointestinal side-effects in the veterinary medicine. 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.

Flunixin is well absorbed from the gastrointestinal tract and undergoes enterohepatic circulation, resulting in a bioavailability of >100% in dogs and cats [14, 20]. For many NSAIDs, glucuronidation is an important pathway. For example, the metabolism of ketoprofen is dominated by glucuronidation reactions in dogs [6]. However, many studies have reported that glucuronidation in cats is limited. Therefore, NSAIDs may induce more severely adverse-effects in cats compared with other species.

There are many NSAIDs include FNX in veterinary medicine market. In USA and European countries, FNX is used in the horse and the dog. In a study comparing postoperative analgesia of Phenylbutazone, FNX and carprofen; FNX had the longest duration of analgesic effect (12.8 h) compared to Phenylbutazone (8.4 h) and carprofen (11.7 h) in horses [16]. But there is no clear data about therapeutic and adverse effects in the cat.

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.

Material 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, Japan), sodium pentobarbital (Nembutal®, Dainippon Sumitomo Pharmaceutical Co. Ltd, Osaka, Japan), and lipopolysaccharide (LPS, Lot no.116354OJC, 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 (0.3μg/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). HPLC analysis was performed on 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 Asahipak 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 group 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 LPS injection, and then decreased gradually. Body temperature was significantly (P<0.01) higher at 2, 4 and 6 hrs 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 temperatures in FNX0.5 group were significantly (P<0.05 to 0.01) lower compared to the LPS group at 1, 2 and 4 hr after LPS injection. 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 (cm^2) 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 adlut 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 maximam 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.

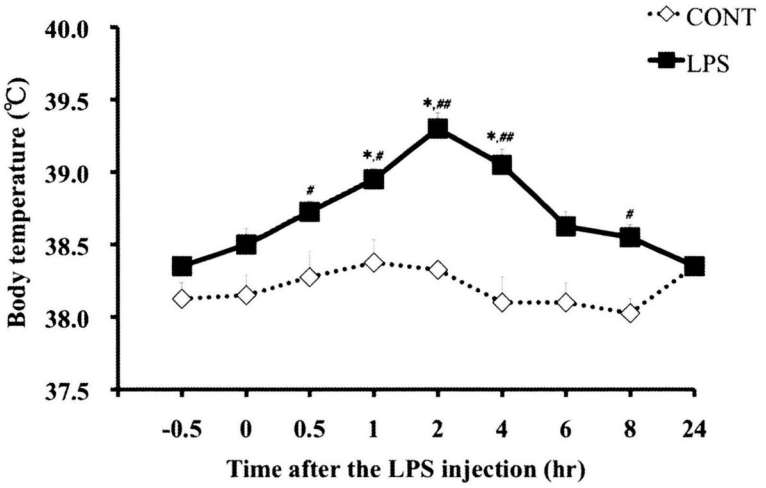

Fig. 1. Hyperthermia induced by LPS in young cats. LPS (0.3 μg/kg body weight) was injected i.v. at 0 hr. The control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was injected PSS before LPS injection. Those groups are hereafter referred to as CONT and LPS. Data show the mean value and S.E. of 4 cats. At 0.5, 1, 2, 4 and 8 hr after LPS injection, body temperature was significant higher than control (CONT). * $P<0.05$, significantly different from body temperature at -0.5 hr value. # $P<0.05$; ## $P<0.01$, significantly different from the CONT group.

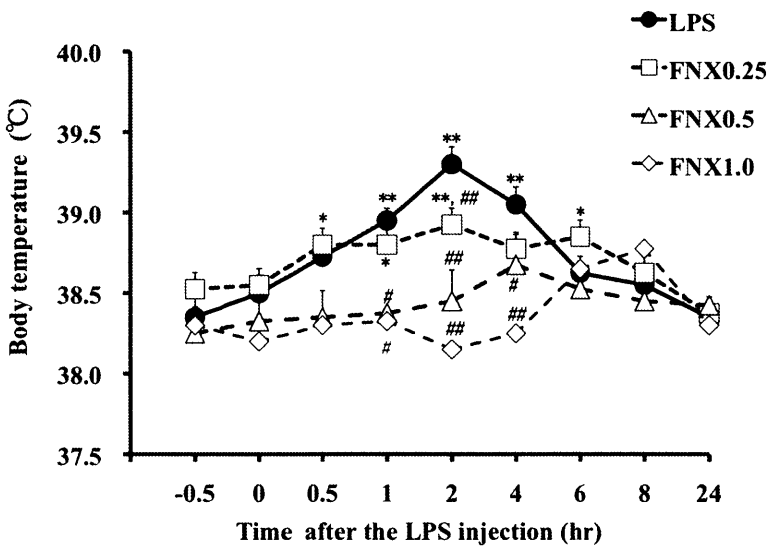

Fig. 2. Effect of flunixin on hyperthermia induced by LPS in young cats. LPS (0.3 μg/kg body weight) was injected i.v. at 0 hr. Flunixin was administered s.c. at 0.5 hr before LPS injection. The control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was 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. Data show the mean value and S.E. of 4 cats. At 1, 2 and 4 hr after LPS injection, both FNX0.5 and FNX1.0 significantly suppressed hyperthermia induced by LPS. FNX 0.25 also significantly suppressed hyperthermia induced by LPS at 2 hr after LPS injection. * $P<0.05$, significantly different from body temperature at -0.5 hr value. # $P<0.05$; ## $P<0.01$, significantly different from the LPS group. The LPS group is same as for Fig. 1.

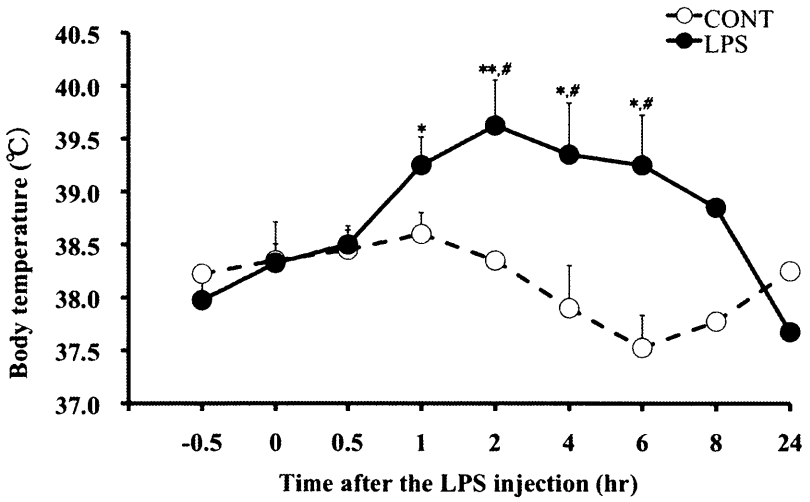

Fig. 3. Effect of flunixin on hyperthermia induced by LPS in adult cats. LPS (0.3 μg/kg) was injected i.v. at 0 hr. The control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was injected PSS before LPS injection. Those groups are hereafter referred to as CONT and LPS. Data show the mean value and S.E. of 4 cats. Body temperature was significant higher at 2, 4 and 6 hr in the LPS group than the control (CONT) group. * $P<0.05$; ** $P<0.01$, significantly different from -0.5 hr value. # $P<0.01$, significantly different from the CONT group.

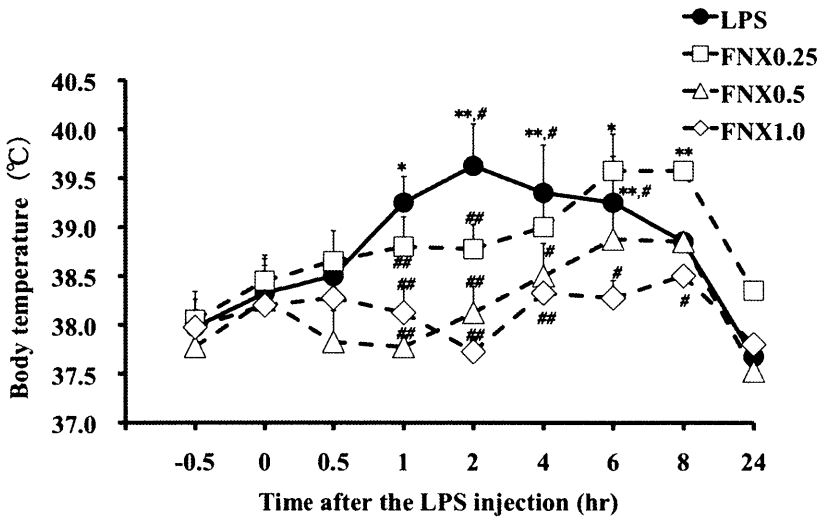

Fig. 4. Effect of flunixin on hyperthermia induced by LPS in adult cats. LPS (0.3 μg/kg) was injected i.v. at 0 hr. The control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was injected PSS before LPS injection. The cats in the other groups received 0.25, 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. Data show the mean value and S.E. of 4 cats. At 1 and 2 hr after LPS injection, FNX0.25 significantly suppressed hyperthermia induced by LPS. FNX0.5 and FNX 1.0 groups were almost-completely suppressed hyperthermia induced by LPS. * $P<0.05$; ** $P<0.01$, significantly different from body temperature at -0.5 hr value. # $P<0.05$; ## $P<0.01$, significantly different from the LPS group. The LPS group is same as for Fig. 3.

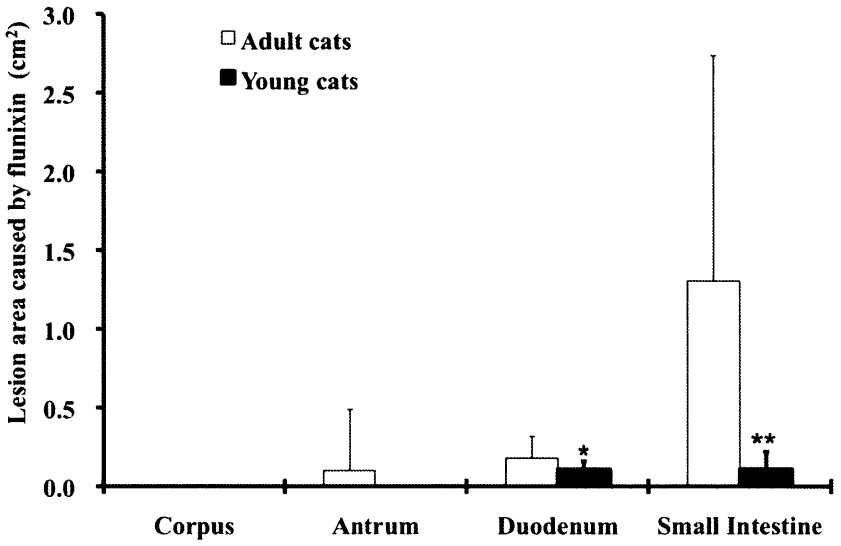

Fig. 5. Gastrointestinal lesions induced by flunixin in adult and young cats. Flunixin (1 mg/kg body weight s.c.) was administered for 3 days; once a day after a morning meal. The animals were sacrificed 24 hr after the final dose of flunixin, and gastrointestinal mucosal lesions were examined. Data show the mean value and S.E. of 5 cats. In young cats, very few gastrointestinal lesions were produced by flunixin. * $P<0.05$; ** $P<0.01$, significantly different from adult cats.

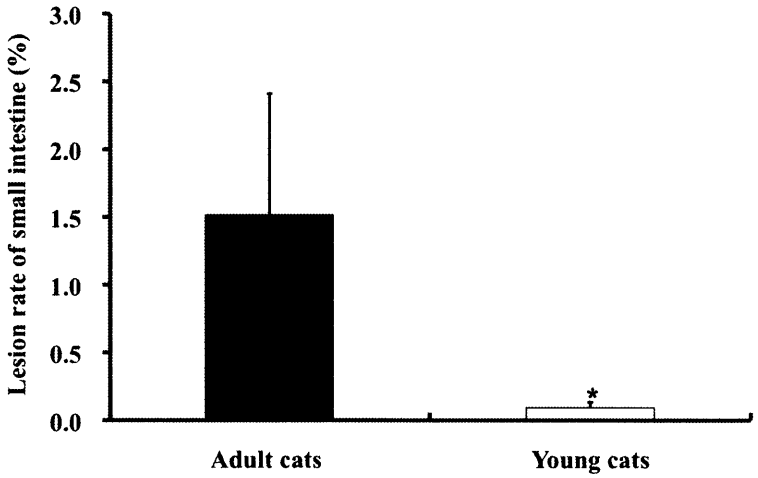

Fig. 6. Percentages of small intestinal lesion area induced by flunixin in adult and young cats. The percentage of the lesion area was calculated as 100% of total small intestine area. Flunixin (1 mg/kg body weight, s.c.) was administered for 3 days; once a day after a morning meal. The animals were sacrificed 24 hr after the final dose of flunixin, and gastrointestinal mucosal lesions were examined. Lesion rate (%) = (lesion area in small intestine / total of small intestine area) × 100. Data show the mean value and S.E. of 5 cats. In young cats, very few small intestinal lesions were produced by flunixin. * $P<0.01$, significant different from control group.

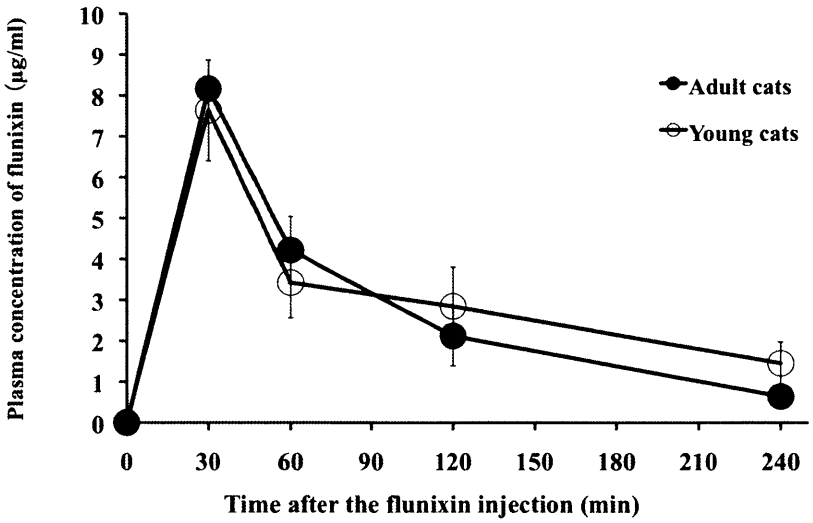

Fig. 7. Time-dependent changes of plasma concentration of flunixin in both adult and young cats. Flunixin (1.0 mg/kg) was injected s.c. at 0 min. Data show the mean value and S.E. of 5 cats. There is not significant difference on plasma concentration of flunixin between adult and young cats.

Discussion

Flunixin is one of the traditional NSAIDs, and this drug has the strong anti-cyclooxygenase(COX) activity [21]. 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 [22, 24]. 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 synthesised by COX-1, and in particular to reduce the gastro-intestinal bleeding caused by COX-1 inhibition [8, 23]. In a vitro study, FNX is lower COX-2 selectivity comparing 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, 5, 19, 29]. These pyrogenic cytokines are transported to the thermoregulatory 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 [19].

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, FNX was

administration 1 mg/kg/day during 7 consecutive days for the cat. In this study, biochemical and haematological variables did not affect in the cat [32]. So, the dose of 0.5 mg/kg may be safe.

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 cm^2) and young cats (234 ± 15.7 cm^2), 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 $1.52 \pm 0.89\%$ of total surface area, while in the young cats it was $0.09 \pm 0.04\%$, 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, T_{max} after oral doses of flunixin is approximately 1.3–2 hr in cats [32]. The elimination half-life has been found to be 1–1.5 hr, using an assay with a limit of 0.25 μg/mL [31, 32]. In our study, T_{max} 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 defence. Alterations of intestinal

glycocalyx 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 [1]. 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 investigation suggesting that bacterial flora may play a role on the pathogenesis of NSAIDs bowel injury. The study have demonstrated that antimicrobials attenuated NSAIDs induced enteropathy in rats [33]. From this observation, it may be possible that gastrointestinal bacteria 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. But we can not make out that why gastrointestinal effect in the young cat milder then that 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 this study, FNX occurred severe gastrointestinal adverse effects in adult cats. So, FNX may be not used for the cat. 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.

Chapter2

Comparison of gastrointestinal adverse effects of ketoprofen between adult and young cats

Introduction

Ketoprofen is a member of the 2-arylpropionic acid subgroup of carboxylic acid NSAIDs. It is a chiral molecule. Similar to other members of this subgroup (e.g., carprofen and vedaprofen), it contains a single asymmetric carbon atom and therefore exists in two, mirror-image, enantiomeric forms: R(−) and S(+) ketoprofen. In most species, NSAIDs are very effective in alleviating acute and chronic pain. For example, NSAIDs are often the first drugs used in treating pain caused by osteoarthritis in veterinary medicine [26]. Ketoprofen is also used for pain treatment. In an analgesic study of cats, the single-observer visual analog score (VAS) for pain and the need for intervention were significantly lower in the ketoprofen group than in the meperidine and buprenorphine groups [30]. However, NSAIDs have been known to cause some adverse effects, including gastrointestinal side effects, in veterinary medicine. However, no studies have been published about gastrointestinal side effects of ketoprofen in cats.

Most NSAIDs are cleared from the body through hepatic metabolism primarily comprising glucuronidation followed by excretion of the resultant polar metabolites via bile and/or urine. Ketoprofen bioavailability in cats has been reported as 112% and 85% for S(+) and R(−) ketoprofen, respectively [18]. However, this value of bioavailability for S(+) ketoprofen may be an overestimate because of inversion of R(−) to S(+) ketoprofen. Nevertheless, the percentage absorption of R(−) and S(+) ketoprofen seems relatively high [18]. For many NSAIDs, glucuronidation is an important pathway. For example, ketoprofen metabolism is dominated by glucuronidation reactions in dogs [6]. However, many studies have reported that glucuronidation in cats is limited. Therefore,

NSAIDs may induce more severe adverse effects in cats than in other species.

The veterinary medicine market utilizes many NSAIDs including ketoprofen. Ketoprofen is approved for use in cats in Europe, Australasia, Canada, and Japan. However, no studies have investigated the therapeutic and adverse effects of ketoprofen in cats. The purpose of the present study was to elucidate differences in therapeutic and adverse effects of ketoprofen between adult and young cats.

Material and methods

Animals

This study compared the effects of ketoprofen administration (ketoprofen, 2.0 mg/kg, subcutaneous administration) between young and adult cats in terms of antipyretic, pharmacokinetic, and gastrointestinal adverse effects. All of cats were obtained by self-breeding in the laboratory animal facilities of Tottori University (pharmacological laboratory, department of veterinary medicine). 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 for more than >7 days before the start of the experiment. Three to four cats were housed together in a cage (PEC-902; IRIS Ohyama Inc., Sendai, Japan). During the experiments, the animals were housed individually at an ambient temperature of 25 ± 1°C with a 12- hr light/dark cycle, with the (lights were being switched on at 07:00). All of cats received commercially available dry cat food. 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

Ketoprofen (1% Ketofen®, Dainippon Sumitomo Pharmaceutical Co. Ltd., Osaka, Japan), xylazine (Ceractal®, 2% solution, Bayer, Japan), sodium pentobarbital (Nembutal®, Dainippon Sumitomo Pharmaceutical Co. Ltd.), and lipopolysaccharide (LPS, Lot no.116354OJC, W. E. Coli, Wako Pure Chemical Industries Ltd., Osaka, Japan) were used in this study.

Prevention of LPS-induced hyperthermia using ketoprofen

The experiment consisted of 3 treatment groups in both young and adult cats. Four cats were assigned to each of the 3 treatment groups (2 males and 2 females in each group). For induction of pyrexia, LPS (0.3 μg/kg) was administered intravenously (i.v.) at 0 hr. Ketoprofen was administered subcutaneously (s.c.) 0.5 hr before LPS injection. In both young and adult cats, the control group was administered 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). PSS was injected before LPS in the LPS group. Cats in the other groups received ketoprofen (2.0 mg/kg, s.c.) and LPS (0.3 μg/kg, i.v.). These groups will hereafter be referred to as CONT, LPS, and KP. Body temperature was measured 0.5 hr before and 0, 0.5, 1, 2, 4, 8, and 24 hr after LPS injection.

Examination of gastrointestinal lesions

This experiment consisted of non-injected (young and adult) and ketoprofen-injected (young and adult) animals. In this design, each group was composed of 2 males and 2 females. To determine gastrointestinal lesions, ketoprofen (2.0 mg/kg,

s.c.) was administered once a day after the morning meal for 3 days. In all groups, 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 ketoprofen. The stomach and small intestine were excised as follows. Stomachs were opened along the greater curvature. Small intestines were opened on the antimesenteric side. These organs were then immersed in 1000 ml saline. Thirty minutes later, visible mucosal lesions in the stomach and small intestine were examined by stereomicroscopy. This method has been used in the study of rats [12]. The percentage of the lesion area was calculated as 100% of total small intestine area. Lesion rate (%) = (lesion area in small intestine / total of small intestine area) × 100.

Analysis of pharmacokinetics of ketoprofen

ketoprofen (2.0 mg/kg, s.c.) was administered to young (2 males, 2 females) and adult cats (2 males, 2 females). The pharmacokinetics of this NSAID were analyzed by high-performance liquid chromatography (HPLC). HPLC analysis was performed using a model L-62000 instrument (Hitachi Co. Ltd., Tokyo, Japan). For pharmacokinetic analysis, blood samples were collected from the jugular vein 0, 0.5, 1, 2, and 4 hr after NSAID injection. Heparin was used as an anticoagulant. Plasma was separated, stored at −30°C for 1 week, and analyzed by HPLC. Chromatographic separations were performed on Bioptic AV (4.6 mm ID × 150 mm L; GL Sciences Inc., Tokyo, Japan). The mobile phase was composed of 50 mM phosphate buffer (pH 7.3)/acetonitrile (5:95, v/v). The solution was filtered through a 0.45-μm membrane prior to use. The flow rate was 1.0 ml/min, and column temperature was maintained at 40°C. The channel on the

UV detector was configured at 254 nm. The volume injection was 20 μl. Each sample was analyzed once.

Statistical analysis

All values were expressed as means and standard errors. In the data for body temperature, one-way analysis of variance for repeated measures was used to examine time effects within each group and the combined effect of all 3 groups at each time point. Least significant difference (LSD) test was used to compare means for those values that showed significant differences. Other data were subjected to analysis of variance. When F values were not significant, differences between groups were analyzed by Student's t-test. When F values were significant, Wilcoxon–Mann–Whitney test was used for statistical evaluation. The level of significance in all tests was set at $P < 0.05$.

Results

Prevention of LPS-induced hyperthermia using ketoprofen

As shown in Fig. 8, in young cats, mean body temperature before LPS injection was 38.43 ± 0.09°C (n = 4). In the LPS group, body temperatures at 1, 2, 4, and 6 hr were significantly higher than those at −0.5 hr ($P < 0.05$). After LPS injection, body temperatures increased and reached a maximum 2 hr after injection (39.40 ± 0.13°C). Body temperature decreased after the peak of LPS-induced hyperthermia at 2 hr. Body temperature was significantly higher in the LPS group than in the CONT group at 0.5, 1, 2, 4, and 8 hr ($P < 0.05$ or 0.01). In the KP group, body temperatures did not change

significantly at each elapsed time than at −0.5 hr. In the KP group, body temperatures 2 and 4 hr after LPS injection were significantly lower than those in the LPS group ($P <$ 0.01). Thus, ketoprofen suppressed LPS-induced hyperthermia in young cats.

As shown in Fig. 9, in adult cats, mean body temperature in the LPS group was significantly ($P < 0.05$ or 0.01) higher at 1, 2, 4, and 8 hr than at −0.5 hr. Body temperature increased to a maximum (39.65 ± 0.16°C) 4 hr after LPS injection and then decreased gradually. Body temperature was significantly ($P < 0.05$ or 0.01) higher at 1, 2, 4, and 6 hr in the LPS group than or 0.01) lower than that in the LPS group 2 and 4 hr after LPS injection. In the KP group, body temperatures did not change significantly at each elapsed time than at −0.5 hr. Thus, ketoprofen suppressed LPS-induced hyperthermia in adult cats.

Gastrointestinal side effects

In both adult and young cats, lesions were observed in the duodenum and lower small intestine following repeated doses of ketoprofen. In the stomach, some lesions were observed in adult cats but not in young cats. As shown in Fig. 10, the area (cm^2) of lesions caused by ketoprofen in the small intestine was smaller in young cats than in adult cats. In the duodenum, the lesion frequency rate in young cats (50%) was lower than that in adult cats (75%). No significant difference was observed in the lesion area in the duodenum between young and adult cats. In addition, the area (%) of small intestine occupied by lesions was lower in young cats (0.39 ± 0.14%) than in adult cats (0.47 ± 0.06%) (Fig. 11). In both adult and young cats in the non-injected groups, no gastrointestinal lesions were observed.

Pharmacokinetics of ketoprofen

Figures 12 and 13 show the pharmacokinetics of R(−) and S(+) ketoprofen, respectively. In both adult and young cats, blood levels of ketoprofen reached a maximum 0.5 hr after injection (Figs. 12 and 13). One hour after injection, ketoprofen concentrations in both adult and young cats decreased to approximately 50% of the maximum and then decreased gradually. The area under the curve (AUC) of the plasma concentration in young cats was similar to that in adult cats. AUC of S(+) ketoprofen in young and adult cats was 12.71 ± 1.53 (μg hr/ml) and 11.67 ± 2.25 (μg h/ml), respectively. AUC of R(−) ketoprofen in young and adult cats was 0.66 ± 0.06 (μg hr/ml) and 0.91 ± 1.63 (μg hr/ml), respectively. No significant difference in AUCs was observed between adult and young cats.

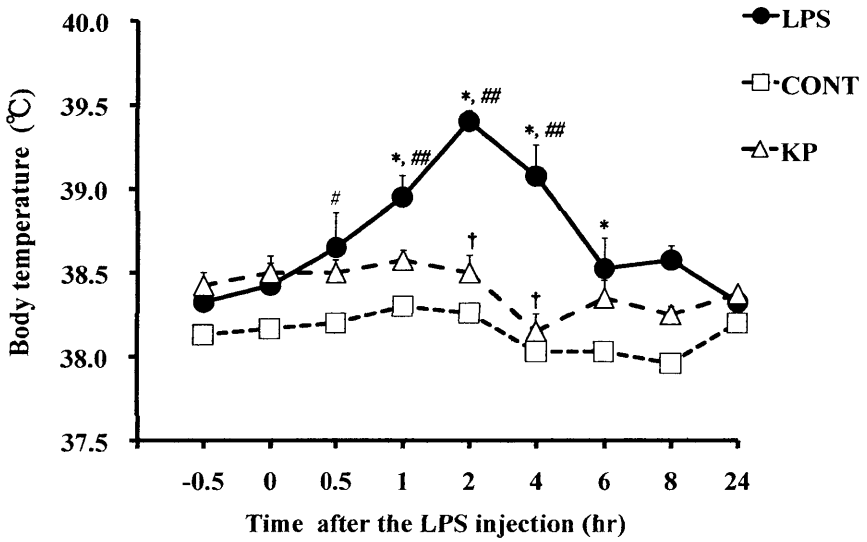

Fig. 8. Effect of ketoprofen on hyperthermia induced by LPS in young cats. LPS (0.3 μg/kg body weight) was injected i.v. at 0 hr. Ketoprofen was administered s.c. at 0.5 hr before LPS injection. The control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was injected PSS before LPS injection. Those groups are referred to as CONT, LPS and KP. Data show the mean value and S.E. of 4 cats. At 0.5, 1, 2 and 4 hr after LPS injection, body temperature was significantly higher than CONT. At 2 and 4 hr after LPS injection, ketoprofen significantly suppressed hyperthermia induced by LPS. Body temperature in the KP group was not significantly different from the CONT group. * $P<0.05$; ** $P<0.01$, significantly different from body temperature at -0.5 hr value. # $P<0.05$; ##$P<0.01$, significantly different from the CONT group. † $P<0.01$, significantly different from the LPS group.

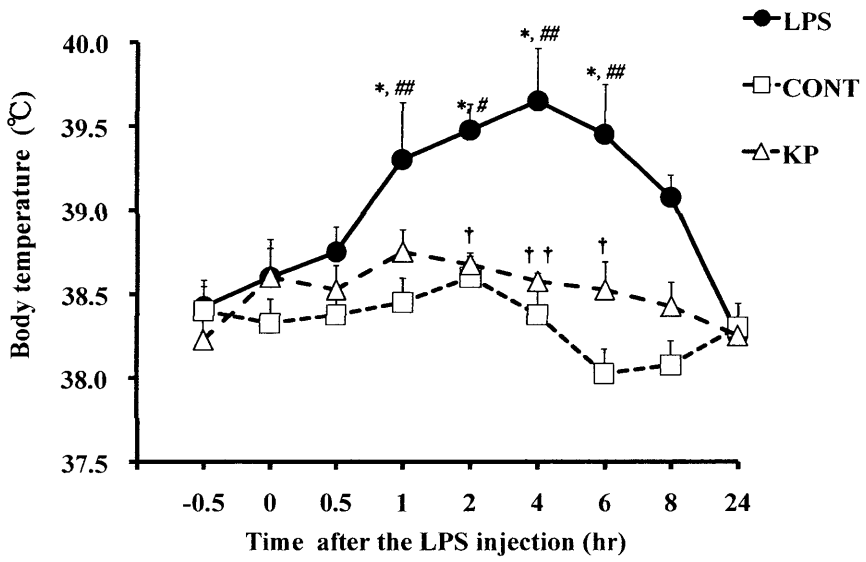

Fig. 9. Effect of ketoprofen on hyperthermia induced by LPS in adult cats. LPS (0.3 μg/kg body weight) was injected i.v. at 0 hr. Ketoprofen was administered s.c. at 0.5 hr before LPS injection. The control group was received 0.5 ml/kg physiological saline solution (PSS, s.c. and i.v.). The LPS group was injected PSS before LPS injection. Those groups are referred to as CONT, LPS and KP. Data show the mean value and S.E. of 4 cats. At 1, 2, 4 and 6 hr after LPS injection, body temperature was significantly higher than CONT. At 2, 4 and 6 hr after LPS injection, ketoprofen significantly suppressed hyperthermia induced by LPS. Body temperature in the KP group was not significantly different from the CONT group. * $P<0.01$, significantly different from body

temperature at -0.5 hr value. # $P<0.05$; ##$P<0.01$, significantly different from the CONT group. † $P<0.05$; ††$P<0.01$, significantly different from the LPS group.

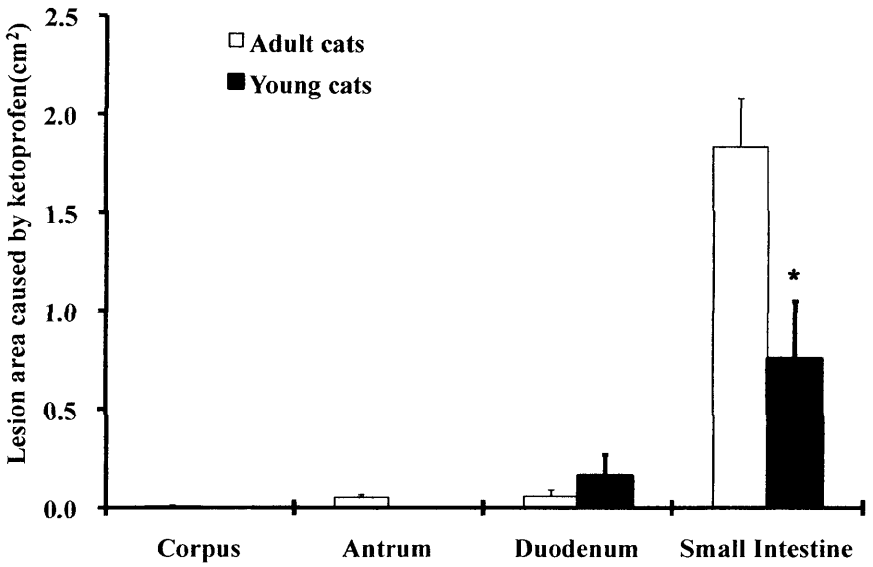

Fig. 10. Gastrointestinal lesions induced by ketoprofen in adult and young cats. Ketoprofen (2.0 mg/kg body weight s.c.) was administered for 3 days; once a day after a morning meal. The animals were sacrificed 24 hr after the final dose of ketoprofen, and gastrointestinal mucosal lesions were examined. Data show the mean value and S.E. of 4 cats. In young cats, very few gastrointestinal lesions were produced by ketoprofen. * $P<0.05$; ** $P<0.01$, significantly different from adult cats.

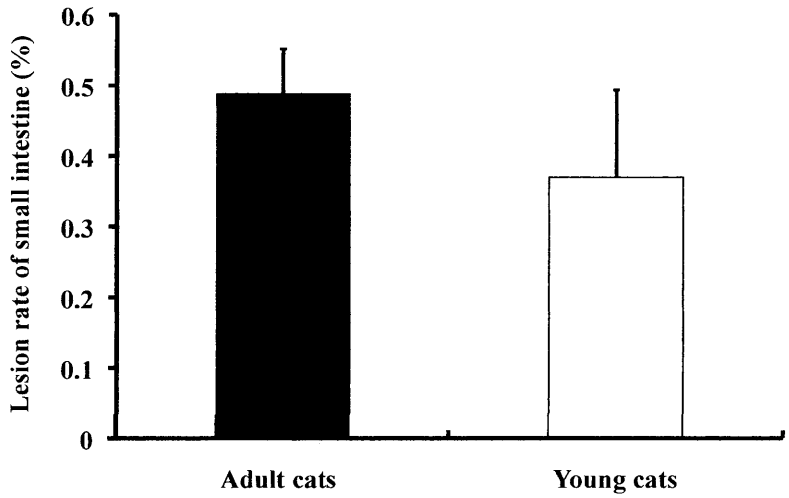

Fig. 11. Percentages of small intestinal lesion area induced by ketoprofen in adult and young cats. The percentage of the lesion area was calculated as 100% of total small intestine area. Ketoprofen (2.0 mg/kg body weight, s.c.) was administered for 3 days; once a day after a morning meal. The animals were sacrificed 24 hr after the final dose of ketoprofen, and gastrointestinal mucosal lesions were examined. Lesion rate (%) = (lesion area in small intestine / total of small intestine area) × 100. Data show the mean value and S.E. of 4 cats. In young cats, few small intestinal lesions were produced by ketoprofen.

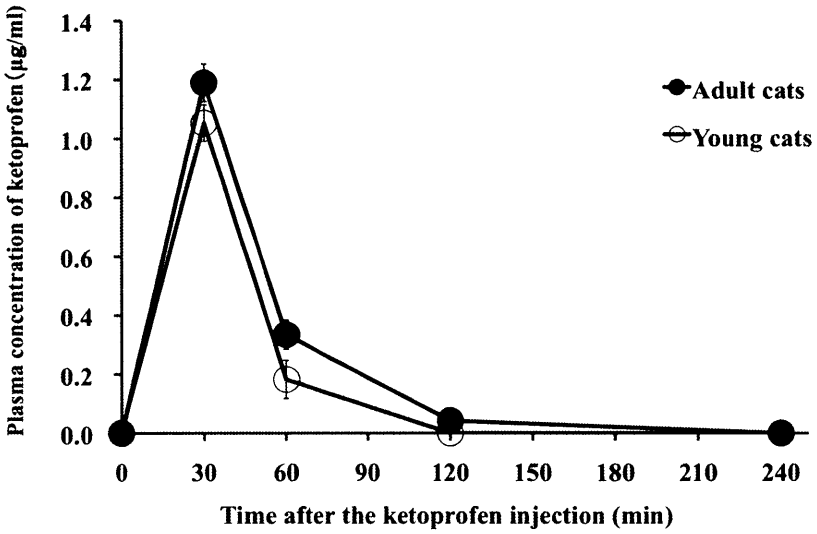

Fig. 12. Time-dependent changes of plasma concentration of R (-) ketoprofen in both adult and young cats. Ketoprofen (2.0 mg/kg) was injected s.c. at 0 min. Data show the mean value and S.E. of 4 cats. There was no significant difference on plasma concentration of ketoprofen between adult and young cats.

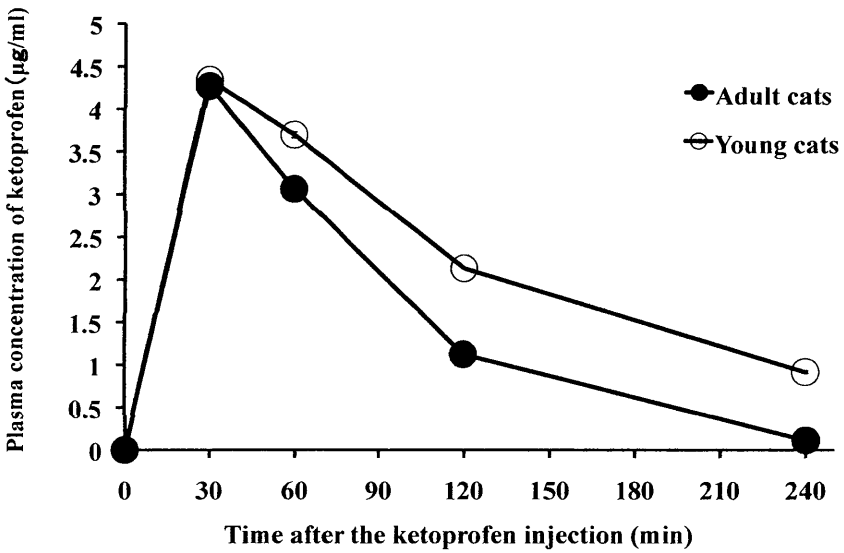

Fig. 13. Time-dependent changes of plasma concentration of S (+) ketoprofen in both adult and young cats. Ketoprofen (2.0 mg/kg) was injected s.c. at 0 min. Data show the mean value and S.E. of 4 cats. There was no significant difference on plasma concentration of ketoprofen between adult and young cats.

Discussion

Cyclooxygenase (COX) has been recognized as the principal catalyst in the synthesis of prostanoids from arachidonic acid. COX has two isoforms, COX-1 and COX-2 [8, 9]. COX-1 is constitutively expressed in most 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 homeostatic functions of prostanoids preferentially synthesized by COX-1, and in particular to reduce gastrointestinal bleeding caused by COX-1 inhibition [8, 9]. In an in vitro study, ketoprofen demonstrated lower COX-2 selectivity in comparison with ibuprofen and piroxicam [9]. LPS from gram-negative bacteria stimulates host defense cells to release several endogenous pyrogens. Many studies show that LPS-induced fever is mediated by a number of endogenous pyrogenic cytokines [4, 5, 19, 29], which are transported to the thermoregulatory center in the preoptic area. They stimulate the production of COX-2-dependent prostaglandin (PG) E2, the putative final mediator of the febrile response [19].

The present study demonstrated that LPS caused hyperthermia in both young and adult cats and that ketoprofen suppressed LPS-induced hyperthermia. This study also indicated that ketoprofen significantly suppresses hyperthermia in cats. Ketoprofen may thus be useful in the treatment of bacterial febrile conditions in veterinary practice, except in cats because of its adverse effects. The analgesic effects of ketoprofen were not monitored in this study. However, ketoprofen significantly suppressed LPS-induced

hyperthermia 2 hr after LPS injection. Therefore, ketoprofen may show analgesic effects from 2 hr after injection. Further study is required to confirm this possibility.

In the present study, in both adult and young cats, lesions were observed in the duodenum and lower small intestine following repeated doses of ketoprofen. In the stomach, some lesions were observed in adult cats but not in young cats. Because this discrepancy could be a result of the difference in the small intestinal area between adult (389 ± 31.7 cm^2) and young cats (179 ± 7.7 cm^2), the percentage of eroded tissue relative to the surface area of the intestinal lumen was calculated. As shown in Fig. 4, in adult cats, the eroded area was $0.47 \pm 0.06\%$ of the total surface area, while in young cats, the eroded area was $0.39 \pm 0.14\%$. These results of lower values for the percentage of lesion-eroded tissue differed from the results using flunixin. However, in our previous study using flunixin, the mucosal lesion area was more severely damaged than that in this study. Therefore, gastrointestinal lesions caused by ketoprofen administration may be milder than those caused by flunixin administration. These differences may be related to the selectivity of COX-1 and COX-2.

Possible reasons for these differences in erosion between adult and young cats are as follows. First, plasma concentrations of both enantiomeric forms of ketoprofen were determined. HPLC analysis showed that pharmacokinetic data of both drugs were similar for young and adult cats. In pharmacokinetic studies of ketoprofen, T_{max} of S(+) and R(−) ketoprofen after oral administration has been reported to be approximately 0.87 and 0.6 hr, respectively, in cats [18]. In our study, T_{max} of both ketoprofen enantiomers after injection was 0.5–1 hr in both adult and young cats. The elimination

half-life of R(−) and S(+) ketoprofen was approximately 1 and 1.5 hr, respectively, in both young and adult cats. Therefore, our study demonstrated no difference in plasma concentrations of ketoprofen between young and adult cats. Significant age-based differences in bile acids, enterobacteria, and mucosal defense may also play a role in progression of gastrointestinal lesions caused by ketoprofen administration, as suggested previously [35]. Alterations in the intestinal glycocalyx have been reported in rats after indomethacin administration, suggesting that changes in epithelial mucin content may contribute to NSAID-induced deleterious effects 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 [1].

Intraluminal factors including bacteria may play a key role in the initiation of damage in NSAID-induced mucosal erosions. On the other hand, indomethacin administration has been reported to induce an increase in bacterial counts in the mucosa [1]. One study suggested that bacterial flora may play a role in the pathogenesis of NSAID-induced bowel injury. That study demonstrated that antimicrobials attenuated NSAID-induced enteropathy in rats [33]. In pigs, one study reported that microbial flora in feces changed with age [17]. From these observations, the possibility arises that changes in gastrointestinal bacterial counts or bacterial flora may be responsible for the gastrointestinal adverse effects. These factors may be among the causes of variation in ketoprofen-induced erosion between young and adult cats.

Another recent study documented that soluble dietary fibers can protect cats from indomethacin-induced small intestinal lesions; dietary fibers act same as endogenous

mucin [28]. Dietary fibers may also influence enterobacteria. The other possibility is that differences in age influence the activity or quantity of COX. In rats, COX-2 protein was reduced in the cortex of old rats than of young rats of both sexes [27]. And then, in this study, gastrointestinal lesion may be not relation to differences in sexes, too.

In conclusion, this study demonstrated that ketoprofen suppressed LPS-induced hyperthermia in both young and adult cats and that ketoprofen-induced small intestinal lesions were fewer and smaller in young cats than in adult cats. This difference between young and adult cats in gastrointestinal adverse effects was unrelated to plasma concentrations of ketoprofen. In the present study, because ketoprofen caused severe gastrointestinal adverse effects in adult cats, it may not be suitable for use in adult cats. Although the essential causes of differences in reactions are unknown, the present results suggest that ketoprofen may be safer for use in young cats than in adult cats. These results are similar to those of our previous study using flunixin in cats. Thus, some NSAIDs may be used more safely in young cats than in adult cats from the viewpoint of intestinal adverse effects.

General Conclusion

In this study, we tried to elucidate the differences in the therapeutic and adverse effects of flunixin-meglumine and ketoprofen between adult and young cats. We observed that the therapeutic effects of these NSAIDs were equivalent in young and adult cats. In Japan, flunixin-meglumine is not approved for use in cats; however, we demonstrated that flunixin-meglumine can suppress the hyperthermia that is induced by LPS in cats (chapter 1). In chapter 1, we confirmed the dose dependency of the effects of flunixin-meglumine. In chapter 2, we confirmed that ketoprofen, which is approved for use in cats in Japan, can suppress hyperthermia equivalently in young and adult cats.

Conversely, the adverse effects of these NSAIDs were not equivalent in young and adult cats. We tried to assess the adverse effects of these drugs on the gastrointestinal tract. In chapter 1, we demonstrated that the area (cm2) of the lesions caused by flunixin in both the duodenum and the small intestine was significantly smaller in young cats compared with adult cats (Fig. 5).

In chapter 2, we assessed the adverse effects of ketoprofen on the gastrointestinal tract. There were no significantly different gastrointestinal adverse effects between young and adult cats. However, we observed a trend toward less adverse effects for ketoprofen in young cats compared with adult cats.

These observations regarding the adverse effects of NSAIDs on the gastrointestinal tract revealed that the occurrence of gastrointestinal lesions was lesser in young cats compared with adult cats. We inspected the blood levels of NSAIDs using high-performance liquid chromatography (HPLC) to confirm the differences observed; however, no significant differences were observed in the blood levels of NSAIDs between the young and adult cats.

There are several potential explanations for these results, which include the presence of significant age-based differences in bile acids, enterobacteria, and mucosal defense mechanisms. The observation that NSAIDs caused severe gastrointestinal adverse effects in adult cats in the present study implies that these drugs may not be suitable for use in adult cats. Although the essential mechanisms underlying these differences are unknown, the present results suggest that with regard to gastrointestinal adverse effects, NSAIDs are safer in young cats compared with adult cats.

Abstract

In this study, we confirmed the presence of differences in nonsteroidal anti-inflammatory drug (NSAID) sensitivities between young and adult cats regarding their therapeutic and adverse effects. Flunixin-meglumin (chapter 1) and ketoprofen (chapter 2) were used here. In both chapters, young (<3 months of age) and adult (>12 months of age) cats of both sexes were given lipopolysaccharides (LPS; 0.3 μ g/kg, i.v.) to test the prevention of LPS-induced hyperthermia by NSAIDs. NSAIDs were administered 30 min before LPS injection, and body temperature was measured 24 hr after injecting LPS. LPS-induced hyperthermia was almost completely inhibited by pretreatment with NSAIDs in both young and adult cats. In addition, NSAIDs exhibited similar antipyretic effects in both young and adult cats. In both the chapters, the animals were administered NSAIDs once a day for 3 days and were sacrificed 24 hr later to examine the gastrointestinal mucosal lesions. In adult cats, NSAIDs caused many severe lesions in the small intestine. In contrast, very few gastrointestinal lesions were produced by NSAIDs in young cats. The results of chapter 1 revealed the presence of significant differences in the lesions caused by flunixin between young and adult cats. However, the results of chapter 2 revealed that the differences in the lesions caused by ketoprofen were not considerably significant; however, the lesions induced in young cats tended to be smaller than those in adult cats. Using HPLC, the plasma concentrations of these drugs were analyzed to evaluate the pharmacokinetics of

NSAIDs. There were no significant differences in the plasma concentrations of NSAIDs between young and adult cats from 0.5 to 4 hr after the injection of the drug (in both chapters). These results suggest that NSAIDs can be used more safely in young cats compared with adult cats considering the gastrointestinal adverse effects. Furthermore, the differences in gastrointestinal lesions observed between young and adult cats showed no significant correlation with the plasma concentration of NSAIDs.

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My gratitude to my family for their moral support and warm encouragements.

REFERENCES

1. Basivireddy, J., Jacob, M., Ramamoorthy, P. and Balasubramanian, K. A. 2005. Alteration in the intestinal glycocalyx and bacterial flora in response to oral indomethacine. *Int. J. Biochem. Cell Biol.* 37: 2321–2332.
2. Beretta, C., Garavaglia, G. and Cavalli, M. 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an in vitro analysis. *Pharmacol. Res.* 52: 302–306.
3. Berde, C.B. and Sethna, N.F. 2002. Analgesics for the treatment of pain in children. N Engl J Med. 347:1094-103.
4. Blatteis, C.M., Li, S., Li, Z., Feleder, C. and Perlik, V. 2005. Cytokines, PGE2 and endotoxic fever: a re-assessment. *Prostaglandins & other Lipid Mediators.* 76:1-18.
5. Bishai, I., Dinarello, C. A. and Coceani, F. 1987. Prostaglandin formation in feline cerebral microvessels: effect of endotoxin and interleukin-1. *Can. J. Physiol. Pharmacol.* 65: 2225–2230.
6. Chakir, S., Marie-Helence, M., Jacques, M., Pierre, L., Nathalie, D., Francoise, L., Zaid, A. and Alain, N. 1994. High-performance liquid chromatographic enantioselective assay for the measurement of ketoprofen glucuronidation by liver microsomes. *J. Chromatography B* 654: 61–68.
7. Court, M. H. and Greenblatt, D. J. 2000. Molecular genetic basis for deficient acetaminophen glucuronidation by cats: UGT1A6 is a pseudogene, and evidence for reduced diversity of expressed hepatic UGT1A isoforms. *Pharmacogenetics* 10:

355–369.

8. Cronstein, B. N. 2002. Cyclooxygenase-2 selective inhibitors: translating pharmacology into clinical utility. *Clev. Clin. J. Med.* 69:SI13–19.

9. Cryer, B. and Feldman, M. 1998. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *Am. J. Med.* 104: 413–421.

10. Davis, L. E. and Westfall, B. A. 1972. Species differences in biotransformation and excretion of salicylate. *Am. J. Vet. Res.* 33: 1253–1262.

11. Frank, E. and Landgraf, R. 2008. The vasopressin system—from antidiuresis to psychopathology. *Eur. J. Pharmacol.* 583: 226–242.

12. Filaretova, L. Bagaeva, T. and Makara, G.B. 2002. Aggravation of nonsteroidal antiinflammatory drug gastropathy by glucocorticoid deficiency or blockade of glucocorticoid receptors in rats. *Life Sci.* 71: 2457–2468.

13. Fonda, D. 1996. Postoperative analgesic actions of flunixin in the cat. *J. Vet. Anaesth.* 23: 52–55.

14. Horii, Y., Ikenaga, M., Shimoda, M. and Kokue, E. 2004. Pharmacokinetics of flunixin in the cat: enterohepatic circulation and active transport mechanism in the liver. *J. Vet. Pharmacol. Therap.* 27: 65–69.

15. Jernigan, A. D. 1988. Idiosyncrasies of feline drug metabolism. *In:* 12th Annual Kal Kan Symposium for the Treatment of Small Animal Diseases. Kal Kan, Vernon, CA, USA. 65–69.

16. Johnson, C. B., Taylor, P. M., Young, S. S. and Brearley, J. C. 1993. Postoperative

analgesia using PBZ, flunixin or carprofen in horses. *Vet. Rec.* 133: 336–338.

17. Kim, H. B., Borewicz, K., White, B. A., Singer, R. S., Sreevatsan, S., Tu, Z. J. and Isaacson, R. E. 2011. Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. *Vet. Microbiol.* 153: 124–133.

18. Lees, P., Taylor, P. M., Landoni, F. M., Arifah, A. K. and Waters, C. 2003. Ketoprofen in the cat: pharmacodynamics and chiral pharmacokinetics. *Vet. J.* 165: 21–35.

19. McCann, M. E., Rickes, E. L., Hora, D. F., Cunningham, P. K., Zhang, D., Brideau, C. and Black, W. C. and Hickey, G. J. 2005. In vitro effects and in vivo efficacy of a novel cyclooxygenase-2 inhibitor in cats with lipopolysaccharide-induced pyrexia, *Am. J. Vet. Res.* 66: 1278–1284.

20. Mckellar, Q. A, Galbraith, E. A., Bogan, J. A., Russell, C. S., Hoke, R. E. and Lees, P. 1989. Flunixin pharmacokinetics and serum thromboxane inhibition in the dog. *Vet. Rec.* 124: 651–654.

21. Menozzi, A., Pozzoli, C., Giovannini, E., Solenghi, E., Grandi, D., Bonardi, S., Bertini, S., Vasina, V. and Coruzzi, G. 2006. Intestinal effect of nonselective and selective cyclooxygenase inhibitor in the rat. *Eur. J. Pharmacol.* 552: 143–150.

22. Morita, I., Schindle, M., Regier, M., Otto, J. C., Hori, T. and De Witt, D. L. 1995. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J. Biol. Chem.* 270: 10902-10908.

23. Raskin, J. B. 1999. Gastrointestinal effects of non steroidal anti-inflammatory therapy. *Am. J. Med.* 106: 3–12.

24. Ren, Y., Walker, C., Loose-Mitchel, D. S., Deng, J., Ruan, K. H. and Kulmacz, R. J. 1995. Topology of prostaglandin H synthase-1 in the endoplasmic reticulum membrane. *Arch. Biochem. Biophys.* 323: 205–214.

25. Robinson, D. and Williams, R. T. 1958. Do cats form glucuronides? *Biochem. J.* 68: 23–24.

26. Rychel, J. K. 2010. Diagnosis and Treatment of Osteoarthritis. *Top. Companion Anim. Med.* 25: 20–25.

27. Sanguino, E., Roglans, N., Alegret, M., Sanchez, R. M., Vazquez, C.M. and Laguna, J. C. 2004. Prevention of age-related changes in rat cortex transcription factor activator protein-1 by hypolipidemic drugs. *Biochem. Pharmacol.* 68: 1411–1421.

28. Satoh, H. Hara, T. Murakawa, D. Matsuura, M. and Takata, K. 2010. Soluble dietary fiber protects against nonsteroidal anti-inflammatory drug-induced damage to the small intestine in cats. *Dig. Dis. Sci.* 55: 1264–1271.

32. Savides, M. C., Oehme, F. and Nash, S. 1984. The toxicity and biotransformation of single doses of acetaminophen in dogs and cats. *Toxicol. Appl. Pharmacol.* 74: 26–34.

29. Sirko, S., Bishai, I. and Coceani, F. 1989. Prostaglandin formation in the hypothalamus in vivo: effect of pyrogens, *Am. J. Physiol.* 256: R616–R624.

30. Slingsby, L. S. and Waterman-Pearson, A. E. 1998. Comparison of pethidine, buprenorphine and ketoprofen for postoperative analgesia after ovariohysterectomy in the cat. *Vet. Rec.* 143: 185–189.

31. Taylor, P. M., Lee, P. and Reynoldson, J. 1991. Pharmacodynamics and pharmacokinetics of flunixin in the cat: a preliminary study. *Vet. Rec.* 128: 258.

32. Taylor, P. M., Winnard J. G. and Jefferies R. R. 1994. Flunixin in the cat; a pharmacodynamic, pharmacokinetic and toxicological study. *Br. Vet. J.* 150: 253–262.

33. Takeuchi, K., Tanaka, A., Kato, S., Amagase, K. and Satoh, H. 2010. Roles of COX inhibition in pathogenesis of NSAID-induced small intestinal damage. *Clin. Chim. Acta. Phamacol.* 411: 459–466.

34. Yeh, S., Chernov, H. and Woods. L. 1971. Metabolism of morphine by cats. *J. Pharm. Sci.* 60: 469–471.

35. Zelena, D. and Ludmila F. 2010. Age-dependent role of vasopressin in susceptibility of gastric mucosa to indomethacin-induced injury. *Regulatory Peptides* 161: 15–21.