The pharmacokinetics and in vitro/ex vivo cyclooxygenase selectivity of parecoxib and its active metabolite valdecoxib in cats

This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/149511 since 2016-07-15T12:42:14Z

Published version:
DOI:10.1016/j.tvjl.2014.07.025

Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.
This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in

Veterinary Journal 202(1) - 2014: 37-42.

http://dx.doi.org/10.1016/j.tvjl.2014.07.025

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

(1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.

(2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.

(3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en),

[http://dx.doi.org/10.1016/j.tvjl.2014.07.025]
The pharmacokinetics and in vitro/ex vivo cyclooxygenase selectivity of parecoxib and its active metabolite valdecoxib in cats

T.W. Kim\textsuperscript{a}, C. Vercelli\textsuperscript{b}, A. Briganti\textsuperscript{c}, G. Re\textsuperscript{b}, M. Giorgi\textsuperscript{c,*}

\textsuperscript{a} College of Veterinary Medicine, Chungnam National University, Daejeon, South Korea

\textsuperscript{b} Department of Veterinary Sciences, Division of Pharmacology & Toxicology, University of Turin, Via L. da Vinci 44, 10095 Grugliasco, Torino, Italy

\textsuperscript{c} Department of Veterinary Sciences, University of Pisa, Via Livornese (lato monte) 1, 56122 San Piero a Grado, Pisa, Italy

Abstract

Parecoxib (PX) is an injectable prodrug of valdecoxib (VX, which is a selective cyclo-oxygenase-2 (COX-2)) inhibitor licensed for humans. The aim of the present study was to evaluate pharmacokinetics and in vitro/ex vivo cyclooxygenase selectivity of PX and VX in cats. In a whole blood in vitro study, PX did not affect either COX enzymes whereas VX revealed a COX-2 selective inhibitory effect in feline whole blood. The IC\textsubscript{50} values of VX for COX-2 and COX-1 were 0.45 and 38.6 μM, respectively. Six male cats were treated with 2.5 mg/kg of PX by intramuscular injection. PX was rapidly converted to VX with a relatively short half-life of 0.4 h. VX achieved peak plasma concentration (2.79 ± 1.59 μg/mL) at 7 h following PX injection. The mean residence times for PX and VX were 0.43 ± 0.15 and 5.94 ± 0.88 h, respectively. In the ex vivo study, PX showed a COX-2 inhibition rate of about 70% in samples taken at 1, 2, 4 and 10 h after injection, with a significant difference compared to the control. In contrast, COX-1 was slightly inhibited, ranging from 0.7% to 9.7% of the control inhibition rate without any significant difference for 24 h after PX administration. The preliminary findings of the present research appear promising and encourage further studies to investigate whether PX can be successfully used in feline medicine.

Keywords: Parecoxib, Valdecoxib, Cat, COX-1 and COX-2, Selectivity
**Introduction**

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain, fever and inflammation in human and veterinary medicine. Despite the well-known anti-inflammatory and analgesic effects, non-specific inhibition of cyclo-oxygenase (COX) derived side effects may limit the long-term clinical use of NSAIDs (Emery et al., 1999; Papich, 2008). The COX-2 selective inhibitors represent another therapeutic option to reduce pain and inflammation. This class of drugs is known to have fewer gastrointestinal adverse effects due to COX-1 sparing effect (Silverstein et al., 2000). In addition, COX-2 selective inhibitor drugs have other benefits over conventional NSAIDs including preservation of platelet function and the maintenance of normal clotting during acute peri-operative pain management (Langford and Mehta, 2006). However, despite the increased margin of safety for COX-2 selective inhibitor drugs, selective COX-2 inhibition alone has been shown to play a key role in some adverse effects. Renal adverse effects due to COX-2 inhibition are still a concern in veterinary medicine especially if there is renal compromise (due, for example, to dehydration, tubular dysfunction, electrolyte depletion, or anaesthesia) (Papich, 2008). Moreover, recent reports have shown that chronic use of these drugs may increase the possibility of adverse cardiovascular events in humans (Rao and Knaus, 2008). Due to relative lack of information about NSAIDs in cats and the increased risks of NSAID associated toxicity in this species, relatively few NSAIDs have been approved for cats compared with dogs (Lascelles et al., 2007). Notably, cats are more sensitive to the gastrointestinal side effects of NSAIDs than other species (Robertson and Taylor, 2004; Carroll and Simonson, 2005). In the last decade, several COX-2 selective inhibitor drugs have been marketed in the veterinary field, including deracoxib, firocoxib, mavacoxib, robenacoxib and most recently cimicoxib (Autefage et al., 2011; Bienhoff et al., 2012; Reymond et al., 2012; Kim and Giorgi, 2013; Kim et al., 2014). Other COX-2 selective inhibitors licensed for humans have also been tested in veterinary species (Kongara et al., 2009, 2010; Giorgi et al., 2012). Currently, robenacoxib is the only COX-2 selective inhibitor drug licensed for cats. Parecoxib (PX) is an injectable prodrug of valdecoxib (VX) belonging to the sulfonamide sub-class. VX is a weak acidic sparingly water soluble compound with a pKa of 9.8. It was formerly marketed as an oral formulation for the management of acute and chronic pain, osteoarthritis and rheumatoid arthritis in humans. Due to serious cutaneous adverse reactions (SCARs) reported in humans following chronic administration, VX was withdrawn from the market in 2004. However during this time, a selective COX-2 inhibiting ability was demonstrated with VX exhibiting anti-inflammatory and analgesic activity comparable to other nonselective NSAIDs (Padi et al., 2004; Gierse et al., 2005). Parecoxib, a weak acidic compound (pKa = 4.9), was developed as a COX-2 inhibitor for acute pain treatment and was designed to be a water soluble and parenterally safe pro-drug form of VX (Teagarden and Nema, 2007). Parenteral administrations of PX have not been associated with SCARs and the drug continues to be available on the market in this form after being reassessed by the regulatory authorities at the same time as VX was under scrutiny (Dalpiaz and Peterson, 2004). Currently, PX is approved for use in humans in much of Europe for short term perioperative pain control, but it is still not approved in the US. A number of experimental studies have assessed the clinical efficacy, pharmacokinetics and safety profile of PX in dogs (Kongara et al., 2009, 2010; Giorgi et al., 2012). However, the use of PX in cats has not yet been reported. The aims of the present study were to evaluate (1) the effect of PX and VX on COX-1 and COX-2 using whole blood in vitro assays, and (2) the pharmacokinetic and ex vivo pharmacodynamics (PD) features of PX and VX after IM administration (2.5 mg/kg) of PX in healthy cats.
Materials and methods

Chemicals and reagents

The standard compounds of PX and VX in powder form (>99.0%) were obtained from Pfizer; 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone (DFU) (>98.9%) pure substance, used as internal standard, was obtained from Sigma-Aldrich. High performance liquid chromatography (HPLC) grade acetonitrile (ACN), methanol (MeOH), n-hexane (C6H14), and diethyl ether (Et2O) were purchased from Merck. Analytical grade trifluoroacetic acid (CF3COOH) was obtained from BDH; sodium chloride (NaCl), acetic acid (AcOH) and ammonium acetate (AcONH4) were purchased from Carlo Erba, and distilled water was produced by a Milli-Q Millipore Water System. All other reagents and materials were of analytical grade and supplied from commercial sources. Lipopolysaccharide (LPS, E. coli serotype O111:B4) was obtained from Sigma-Aldrich. The liquid chromatography (LC) mobile phase was filtered through 0.2 μm cellulose acetate membrane filters (Sartorius Stedim Biotech) with a solvent filtration apparatus. Enzyme-linked immunosorbsent assay (ELISA) kits for prostaglandin (PG) E2 and thromboxane (TX)B2 were obtained from Amersham Biosciences.

Animal studies

The study protocol was approved by the Ethics Committee of the University of Pisa (authorisation 0002473) and transmitted to the Italian Ministry of Health. Six healthy neutered male cats (2.3–5.5 years, 3.1–5.9 kg) were used in the present study. The animals were previously determined to be clinically healthy on the basis of a physical examination and full haematological analyses. Cats received 2.5 mg/kg of PX (Dynastat, Pfizer) via intramuscular (IM) injection over 1 min in the morning after fasting for 12 h overnight. A catheter was placed into the left cephalic vein to facilitate blood collection. Blood samples (1 mL) were collected at 0, 0.08, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12 and 24 h after administration of PX. To reduce stress for the cats while blood was collected, 0.005 mg/kg of medetomidine was administered when necessary. The cats were closely monitored over the initial 24 h and for up to 7 days after administration of the drug. Blood samples for pharmacokinetic analysis were collected in lithium heparinised tubes. Samples were then centrifuged at 3000 g for 30 min of collection and plasma was stored at −80 °C until analysis. Blood samples for ex vivo PD tests were also collected (0, 1, 2, 4, 10 and 24 h) separately in sodium citrate (1 mL) and anticoagulant free (1 mL) tubes, for PGE2 and TXB2 measurements, respectively (Brideau et al., 2001).

In vitro assay

For the COX-1 assay, 5 mL of blood was collected from each cat into anticoagulant free tubes 1 week prior to the beginning of the study. Immediately after collection, aliquots of blood (250 μL) were mixed with 20 μL dimethylsulfoxide (DMSO; Sigma-Aldrich) containing VX or PX to reach a final concentration of 0.0508–1000 μM. Blood aliquots containing only DMSO were used as controls. The tubes were vortexed and then incubated for 1 h at 37 °C. After centrifuging at 16,000 g and 4 °C for 10 min, the supernatant serum was collected and stored at −80 °C before being analysed for TXB2 using commercial ELISA kits (Giraudel et al., 2005a, 2005b, 2009; Donalisio et al., 2012). For the COX-2 assay, 5 mL blood was collected from each cat into sodium citrate tubes 1 week prior to the beginning of the study. Aliquots (245 μL) of blood were added to microtubes containing a
range of concentrations of VX and PX dissolved in 20 μL DMSO as described for the COX-1 assay earlier. Five microlitres of lipopolysaccharide (LPS) (5000 μg/mL in sterile phosphate buffered saline – PBS) was added and vortexed. For each cat, two aliquots were incubated with or without LPS to obtain a positive and negative control, respectively. All samples were incubated for 24 h at 37 °C. After centrifugation, 100 μL of plasma was collected and mixed with 400 μL of MeOH to facilitate protein precipitation. The supernatant was collected and stored at −80 °C prior to analysis for PGE2 using an ELISA assay kit (Giraudel et al., 2005a, 2005b, 2009; Donalisio et al., 2012). All samples were assayed according to the manufacturer’s protocols. Samples were diluted 25- and 15-fold for PGE2 and TXB2, respectively, using the assay buffer supplied with the kit.

**Ex vivo assay**

Blood samples for the ex vivo pharmacodynamic study were collected at 0, 1, 2, 4, 10 and 24 h after PX injection. For the PGE2 and TXB2 assays, aliquots of 245 and 250 μL were used, respectively (Schmid et al., 2010). The blood samples from each tube were treated as per the in vitro study. After preparation, all samples were assayed according to the manufacturer’s protocol using the ELISA kits. Samples were diluted 25- and 15-fold just prior to analysis for PGE2 and TXB2, respectively.

**Inhibition rate comparison study**

Plasma concentrations of VX at 1, 2, 4, 10 and 24 h after PX injection were converted to an inhibition rate based on the PD results from the in vitro assay. Simulated PD data were determined using SigmaPlot 10.0 (SPS). Simulated inhibition rates for each time point were compared with real inhibition rate data from the study.

**Pharmacokinetic study**

The HPLC analysis was carried out according to Saccomanni et al. (2011) with some modifications. Briefly, plasma samples (100 μL) were added to 48 μL of DFU (0.5 μg/mL), then mixed with 10% CF3COOH (120 μL). After briefly vortexing, NaCl (4 mg) was added. After further vortexing, 800 μL of C6H14:Et2O (3:2, v/v) was added, then the samples were shaken (60 osc/min, 10 min) and centrifuged at 21,913 g (rotor radius 10 cm) for 10 min. The supernatant (600 μL) was collected and evaporated under a gentle stream of nitrogen and reconstituted with 200 μL of mobile phase. Twenty microliters of the sample was injected onto the HPLC-FL (Jasco). The plasma concentrations of PX and VX were determined by a multi lambda fluorescence detector (FL 2020 plus). The chromatographic separation assay was performed with a Luna C18 analytical column (150 × 4.6 mm, 3 μm particle size, Phenomenex). The system was maintained at 25 °C. The mobile phase consisted of acetonitrile:buffer (10mMAcONH4, adjusted to pH 4.5 with AcOH) (55:45, v/v) at a flow rate of 0.3 mL/min with isocratic mode. Excitation and emission wavelengths for analysis were set at 265 and 375 nm, respectively. The analytical method was briefly validated as follows. The standard curve was evaluated in addition to nine quality control samples with three different concentrations for each series of analyses. The intra- and inter-day repeatabilities were measured as coefficients of variation and were lower than 3.9%, whereas accuracy, measured as closeness to the concentration of replicate samples, was lower than 2.7%. The limits of detection (LOD, 1 ng/mL) and quantification (LOQ, 5 ng/mL) were determined using signal-to-noise ratios of 3 and 10, respectively. Pharmacokinetic variables were obtained from the individual plasma PX and VX
profiles after a single IM injection of PX. The pharmacokinetic parameters for PX and VX were described with a non-compartment model using WinNonLin v 5.3.1 (Pharsight). The following variables were calculated: terminal half-life (HL_Lambda_z), maximum concentration (Cmax), the time required to reach Cmax (Tmax), area under the concentration/time curve extrapolated to infinity (AUC0–∞), area under the first moment curve extrapolated to infinity (AUMC0–∞) and mean residence time extrapolated to infinity (MRT0–∞). Mean blood concentration profiles and mean values and standard deviations of pharmacokinetic parameters were calculated using the software Microsoft Excel.

**Statistical analysis**

Results were analysed for normal distribution according to the Shapiro–Wilk test and presented as standard deviation of the mean values (±SD). Significant differences among the time points in the ex vivo study were determined using one-way analysis of variance (ANOVA) test. Where significant effects were found, post-hoc analyses using Tukey’s test was performed and P < 0.05 was considered statistically significant. All the analyses were performed using SigmaPlot10.0 (SPSS) and GraphPad InStat v.5.0.

**Results**

**Pharmacokinetics**

The pharmacokinetic parameters of PX and VX were analysed after a single IM injection of PX (2.5 mg/kg) in cats. No adverse effects at the point of injection and no behavioural or health alterations including SCARs were observed during or up to 7 days after the study. The average PX and VX concentration–time profile in blood is presented in Fig. 1. After IM injection, PX was rapidly adsorbed showing a Tmax of 0.10 ± 0.06 h, after which its mean plasma concentration dropped rapidly. In all cats, PX plasma concentrations were lower than the LOQ from 2 h following administration. In contrast, VX showed a slower increase to a maximum peak at about 7 h after PX administration and plasma concentrations had dropped very close to the LOQ by 24 h. Average pharmacokinetic variables for PX and VX are reported in Table 1. Mean residence time and terminal half-life of PX were similar (about 0.4 h). Cmax and AUC0–∞ were 7.86 ± 3.81 μg/mL and 2.91 ± 1.83 h μg/mL, respectively. After PX injection, VX exhibited a Cmax of 2.79 ± 1.59 μg/mL and a half-life of 8.52 ± 6.34 h. The MRT value was 5.94 ± 0.88 h and AUC0–∞ was 15.7 ± 5.27 h*μg/mL, which was relatively longer than that of PX.

**In vitro assay**

Coefficients of variance of intra- and inter-assay were 1.5% and 6.1% for TXB2 and 7.6% and 3.9% for PGE2, respectively (R2 > 0.90). Parecoxib did not affect either TXB2 or PGE2 at the tested concentrations (0.0508–1000 μM data not shown). The PX plasma concentrations achieved in the in vivo pharmacokinetic study were within this range. The VX concentration–response curves for inhibition of plasma PGE2 (COX-2) and serum TXB2 (COX-1) are shown in Figs. 2 and 3, respectively. The Hill slope was slightly steeper in the PGE2 inhibition curve compared to the TXB2 curve (Table 2). The inhibitory concentration 50% (IC50) value was markedly higher for TXB2 compared to that of PGE2 (38.6 ± 18.1 vs. 0.45 ± 0.19 μM), suggesting that VX was
selective for COX-2 inhibition. Mean IC50 values for COX-1 and COX-2 are presented as COX-1:COX-2 ratios in Table 3. The IC ratios increased in proportion to the inhibitory percentage.

**Ex vivo assay**

The ex vivo time vs. PGE2 and TXB2 inhibition curves are presented in Figs. 4 and 5, respectively. Prior to PX injection, variable concentrations of PGE2 and TXB2 were observed in control samples with relatively wide SD values. After PX injection, LPS-induced PGE2 production was significantly decreased to about 70% of the control value at the 1, 2, 4 and 10 h time points. The differences between values for the latter time points were not significant. Twenty-four hours after PX administration, PGE2 production increased. Although it was still 20% lower than the control value, this difference was not significant (P < 0.09). In contrast, the mean value of TXB2 concentrations varied only slightly without any significant differences among all analysed time points.

**Inhibition rate comparison between ex vivo and simulated data**

Inhibition rates were assessed in both the ex vivo and the simulated studies (Table 4). The PGE2 inhibition rate in the ex vivo study was approximately 40% higher than that of the simulated data at 1, 2, 4 and 10 h. In contrast, TXB2 inhibition rate from the ex vivo study was lower than that obtained from simulated data, and with a large SD value.

**Discussion**

Parecoxib was the first COX-2 selective inhibitor to be administered parenterally in humans. It is a pro-drug that is rapidly hydrolysed in vivo in the liver to its active form, VX (Karim et al., 2001). Clinical trials have indicated that PX is effective in treating postoperative pain resulting from oral surgery, orthopaedic surgery and abdominal hysterectomy. Other studies in humans have demonstrated no significant effects on platelet function or the upper gastrointestinal mucosa (Noveck et al., 2001; Stoltz et al., 2002; Harris et al., 2004; Graff et al., 2007). As a result, PX has been approved in European countries for the treatment of postoperative pain. Concerns were raised about the potential for serious adverse effects from PX because of SCARs experienced by some patients as a result of VX. However, successive randomised, double-blind, placebocontrolled clinical human trials concluded that PX is an effective and safe analgesic drug in the postoperative setting (Lloyd et al., 2009). Although PX has not been studied as extensively in veterinary medicine as it has been in humans, some preliminary studies reported that this drug might also be effective and safe (Pozzobon et al., 2008; Kongara et al., 2009, 2010; Giorgi et al., 2012) in animal species. In humans, PX is rapidly and almost completely converted to VX with a plasma half-life in the range of 0.3–0.7 h (Stichtenoth and Frölich, 2003). In the present study, cats showed a half-life value of 0.43 h, which falls into the range established for humans. Conversely, a recent study (Giorgi et al., 2012) reported that PX administered IM in dogs had a terminal half-life that was twice as long (0.91 ± 0.27 h). Such differences in half-life values between species could be explained by multiple factors such as metabolism, clearance, absorption, random variability and differences in protein binding. The apparent disconnect from decreasing PX to increasing VX plasma concentrations is quite remarkable (Fig. 1). The concentration of the parent compound significantly decayed before the concentration of its metabolite began to rise, which is an unusual finding. However these pharmacokinetic profiles were in line with those of PX and VX in humans.
(Karim et al., 2001; Teagarden and Nema, 2007). Tepoxalin and its metabolite RWJ-20142 have also shown a similar trend in horses (Giorgi et al., 2011). On the other hand in dogs, VX elimination followed a similar trend to the parental drug PX, showing a Cmax of 3.23 ± 0.46 μg/mL at 0.40 ± 0.14 h (Giorgi et al., 2012). In cats, the plasma concentration of VX increased slowly with a Cmax of 2.79 ± 1.59 μg/mL at 7 ± 1.67 h, then decreased at a constant rate up to 24 h. These findings are consistent with previously generated human data (Karim et al., 2001; Teagarden and Nema, 2007) which reported a half-life of 8 h for VX. The differences in pharmacokinetic data for VX between cats and dogs might result from several factors alone or in combination. For example, there may be species-derived CYP enzyme differences (VX is metabolised by CYP enzymes differently to PX). Phase II enzymes may also have a role as it is well known that cats have less capacity for glucuronidation than other animal species (Toutain et al., 2010) and this step is responsible for about 20% of the metabolic processing of VX (Talley et al., 2000). Plasma concentrations of VX above 0.2 μg/mL have been reported to be effective for relief of pain triggered by hot plate and formalin tests in mice (Guo et al., 2007). The plasma concentrations of VX in the present study were below this threshold after about 21 h following PX administration. However, caution should be taken in data extrapolation and specific studies are required to evaluate the analgesic action of this drug in cats. In vitro studies provide essential information in helping to understand the pharmacological actions of the tested drug but their results can differ widely from the in vivo conditions (Warner et al., 1999). Therefore, more information can often be provided by ex vivo investigation. In addition, ex vivo studies better reflect the drug’s action under physiological and pathological conditions compared to in vivo (Lees et al., 2004). In the present study, the inhibitory effect of PX on COX-1 and COX-2 pathways was determined using in vitro and ex vivo PD studies. To evaluate the intensity of the inhibition of two isoforms of cyclooxygenase, TXB2 and PGE2 were used as predictive markers for the COX-1 and COX-2 effects, respectively. In vitro assays for TXB2 (COX-1) and PGE2 (COX-2) were performed for both PX and VX. Parecoxib demonstrated no effect on either COX-1 or COX-2 markers, a finding that concurs with findings from a previous study by Teagarden and Nema (2007). Based on these results, PX should not be considered as a COX inhibitor in cats. Efficacy is defined as the maximum effect (Emax) that can be produced in a system. In this study, Emax was referred to Min (% of control) which indicates maximal inhibitory effect. The Min (% of control) value was smaller for PGE2 than for TXB2. Potency is defined as the molarity required to produce a given response (Kenakin, 2004) and in the present study was expressed as the half maximal inhibitory concentration, or IC50 (Toutain and Lees, 2004). IC50 values for both PGE2 and TXB2 were higher than those for human whole blood tests (IC50 for TXB2: 21.9 μM and PGE2: 0.24 μM) (Gierse et al., 2005). The slope of the curve reflects the reactivity of the drug–receptor interaction, that is, one ligand binding to one target yields a slope for the curve of unity (Kenakin, 2004). In our study, the slope was steeper in the PGE2 than in the TXB2 curve, indicating a larger inhibitory response for the COX-2 pathway. To better define the selectivity, PGE2:TXB2 ratios were calculated at three different percentages of inhibition, namely, 20%, 50% and 80%. The IC20 TXB2:IC80 PGE2 ratio was 6.65:1 which was relatively high compared to that for other drugs tested in cats such as robenacoxib (4.23), diclofenac (0.48) and meloxicam (0.19) (Giraudel et al., 2005a, 2005b; Schmid et al., 2010). For a more accurate evaluation, an ex vivo study was performed using blood collected at 0, 1, 2, 4, 10 and 24 h after PX injection during the pharmacokinetic study. Although the maximum active compound level was obtained at 7 h, the ex vivo PGE2 assay demonstrated about 70% inhibition during collection points including 1, 2, 4 and 10 h after PX injection. Although there was wide variation of the values (especially the TXB2 values), it was possible to demonstrate the presence of statistically significant differences among the experimental time-points and to evaluate a trend for PGE2 and TXB2 inhibition.
Conclusions

This study suggests that a single IM injection of PX in cats (2.5 mg/kg) will significantly inhibit PGE2 blood concentrations while not affecting TXB2. The findings of the present research appear promising and should encourage further studies in order to understand whether this drug could be beneficially used clinically in feline medicine. Conflict of interest statement None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors wish to thank Pfizer (Groton, CT, USA) for supplying pure analytical standards of PX and VX. Funds from University of Pisa (Athenaeum ex 60%) supported the study. Cooperlink fund (CII11A2FAV) supported the international cooperation (Ita-Kor). Our external funding did not support or influence the preparation of manuscript. We wish to thank Professor B. KuKanich (Kansas University, USA) for critical revision of the manuscript and Dr H. Owen (University of Queensland, Australia) for the English editing.

References


Fig. 1. Mean plasma concentrations of parecoxib (-○-) and valdecoxib (-●-) vs. time curves in cats (n = 6) following intramuscular injection of parecoxib (2.5 mg/kg). Bars represent the standard deviations.
**Fig. 2.** Mean concentration vs. effect curve for valdecoxib in inhibition of LPS-induced feline plasma PGE2 in vitro (n = 6). Bars represent the standard deviations.
Fig. 3. Mean concentration vs. effect curve for valdecoxib in inhibition of feline serum TXB2 in vitro (n = 6). Bars represent the standard deviations.
Fig. 4. Ex vivo analysis of LPS induced PGE2 contents in cats (n = 6) following IM injection of parecoxib (2.5 mg/kg). Bars represent the standard deviations. *P < 0.05, significant difference in comparison with the control point (T0).
Fig. 5. Ex vivo analysis of TXB2 contents in cats (n = 6) following intramuscular injection of parecoxib (2.5 mg/kg). Bars represent the standard deviations.
Table 1 Mean pharmacokinetic parameters of parecoxib (PX) and its metabolite, valdecoxib (VX), in cats (n = 6) following intramuscular injection of parecoxib (2.5 mg/kg). (\(\text{Lambda}_z\), terminal phase rate constant; \(\text{HL}_z\), terminal half-life; \(T_{\max}\), time of peak; \(C_{\text{max}}\), peak plasma concentration; \(\text{AUC}_{0-\infty}\), area under the plasma concentration–time curve extrapolated to infinity; \(V_z\), apparent volume of distribution; \(\text{Cl}_F\), apparent plasma clearance; \(\text{AUMC}_{0-\infty}\), area under the moment extrapolated to infinity; \(\text{MRT}_{0-\infty}\), mean residence time extrapolated to infinity. NA, not assessable.)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>PX Mean ± SD</th>
<th>VX Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Lambda}_z)</td>
<td>1/h</td>
<td>1.68 ± 0.27</td>
<td>0.13 ± 0.10</td>
</tr>
<tr>
<td>(\text{HL}_z)</td>
<td>h</td>
<td>0.42 ± 0.06</td>
<td>8.52 ± 6.34</td>
</tr>
<tr>
<td>(T_{\max})</td>
<td>h</td>
<td>0.10 ± 0.06</td>
<td>7 ± 1.67</td>
</tr>
<tr>
<td>(C_{\text{max}})</td>
<td>(\mu)g/mL</td>
<td>7.86 ± 3.81</td>
<td>2.79 ± 1.59</td>
</tr>
<tr>
<td>(\text{AUC}_{0-\infty})</td>
<td>(\mu)g/mL</td>
<td>2.91 ± 1.83</td>
<td>15.7 ± 5.27</td>
</tr>
<tr>
<td>(V_z)</td>
<td>mL/kg</td>
<td>654.5 ± 332.4</td>
<td>NA</td>
</tr>
<tr>
<td>(\text{Cl}_F)</td>
<td>mL/h/kg</td>
<td>1142 ± 647.4</td>
<td>NA</td>
</tr>
<tr>
<td>(\text{AUMC}_{0-\infty})</td>
<td>(\mu)g/mL</td>
<td>1.30 ± 0.95</td>
<td>96.9 ± 42.9</td>
</tr>
<tr>
<td>(\text{MRT}_{0-\infty})</td>
<td>h</td>
<td>0.43 ± 0.15</td>
<td>5.94 ± 0.88</td>
</tr>
</tbody>
</table>
Table 2 Mean pharmacodynamic data from in vitro sigmoidal concentration–effect curve from six healthy cats. (PGE₂, prostaglandin E₂; TXB₂, thromboxane B₂; IC₅₀, half maximal inhibitory concentration.)

<table>
<thead>
<tr>
<th></th>
<th>PGE₂</th>
<th>TXB₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min (% of control)</td>
<td>39.1 ± 3.4</td>
<td>45.2 ± 6.1</td>
</tr>
<tr>
<td>Max (% of control)</td>
<td>93.8 ± 6.4</td>
<td>90.6 ± 2.4</td>
</tr>
<tr>
<td>IC₅₀ (µM) of valdecoxib</td>
<td>0.45 ± 0.1</td>
<td>38.6 ± 18</td>
</tr>
<tr>
<td>Hill slope</td>
<td>1.22 ± 0.5</td>
<td>1.00 ± 0.3</td>
</tr>
</tbody>
</table>

Table 3 Potency ratios for inhibition of TXB₂:PGE₂, as an index of selectivity for COX-2, for three levels of inhibition (20%, 50% and 80%).

<table>
<thead>
<tr>
<th></th>
<th>IC₂₀TXB₂:</th>
<th>IC₅₀TXB₂:</th>
<th>IC₈₀TXB₂:</th>
<th>IC₂₀TXB₂:</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₂₀PGE₂</td>
<td>IC₅₀PGE₂</td>
<td>IC₈₀PGE₂</td>
<td>IC₂₀PGE₂</td>
<td>IC₅₀PGE₂</td>
</tr>
<tr>
<td>Mean value ratio</td>
<td>67.5 ± 7.3</td>
<td>88.1 ± 6.8</td>
<td>110 ± 10.2</td>
<td>6.65 ± 2.5</td>
</tr>
</tbody>
</table>

Table 4 Comparison between actual inhibition (%) data from ex vivo study and simulated inhibition (%) data from in vitro study based on VX concentration at each time point (mean ± SD).