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Pharmacokinetic profiles of the novel COX-2 selective inhibitor cimicoxib in dogs

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ABSTRACT

Cimicoxib (CX) is a novel imidazole derivative that is a cyclo-oxygenase (COX)-2 selective non-steroidal anti-inflammatory drug and the latest COX-2 selective inhibitor to be released for veterinary use. Currently there is limited information available on the pharmacokinetic (PK) properties of CX. The aim of the current study was to evaluate the PK features of CX after administration of the recommended dose and after administration of a more variable dose rate in the form of the commercially available tablet. In addition, the effects of food intake on the PK properties were also evaluated. In the first study, five healthy Beagle dogs received 2 mg/kg CX via the oral route following a period of fasting. The second study was conducted using six healthy Labrador retriever dogs which each received an 80 mg tablet (approximate dose 1.95–2.5 mg/kg) using a crossover design, both in the fasted and fed condition. The plasma concentrations of CX were detected by a validated HPLC method.

No adverse effects were observed in any dogs during the experiment. The results from the PK analysis were similar between the studies, regardless of precision of dose and fasted and fed conditions. The mean peak concentration of CX was 0.49 and 0.43 μg/mL under fasted and fed conditions, respectively. The mean half-life was about 3 h after all treatments. In addition, simulated multiple dosing data revealed that time over minimal effective concentration was similar after 1.95, 2.0 and 2.5 mg/kg dose administrations. These findings suggest that slight variation from the recommended dose should not alter the therapeutic outcome. In addition, CX can be administered to fed dogs without significantly affecting blood levels.

Introduction

For over a decade, osteoarthritis (OA) has been increasingly diagnosed in dogs (Moreau et al., 2005), mainly due to a more humanized life-style (Hoffman and Perkins, 2008). Domesticated dogs are now living longer and, as a result, are more susceptible to age-related diseases such as cancer, arthritis, metabolic disorders, and obesity. Fortunately, the veterinary drug market continues to grow (Hoffman and Perkins, 2008; Giorgi, 2012) although available drugs for the veterinarian remains quite small.

Chronic pain management during inflammatory disease has become a priority in pets. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX) enzymes, preventing the conversion of arachidonic acid to various prostaglandins and thromboxanes. COX-1 is considered to be a housekeeping enzyme and COX-2 is best known for its local up-regulation by pro-inflammatory stimuli, by production of prostaglandins involved in inflammatory responses and in the spinal cord facilitating transduction of painful stimuli (Vardeh et al., 2009). The analgesic and anti-inflammatory activity of NSAIDs is thought to be predominantly mediated through COX-2 inhibition (Seibert et al., 1994; Zhang et al., 1997).

Due to the severe side effects often associated with chronic NSAID use, there has been a boost in research in COX-2 selective inhibitor (coxib) drugs in human and veterinary medicine. In the last decade, five different COX-2 selective drugs have been launched on the veterinary market, namely, deracoxib (2002), firocoxib (2007), mavacoxib (2008), robenacoxib (2009) and the most recent, cimicoxib (CX) (2011). Several studies are available in the literature for most of these drugs (Hanson et al., 2006; Autefage et al., 2011; Bienhoff et al., 2012; Reymond et al., 2012; Kim and Giorgi, 2013), and also for COX-2 inhibitor drugs labelled for human use and applied to veterinary medicine (Giorgi et al., 2012). However, there has been no peer-reviewed study on the pharmacokinetic (PK) profiles of CX in the dog. Recently, an

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analytical method for CX detection in dog plasma has been developed and used to determine PK profile for a single dog (Giorgi et al., 2013).

The objectives of this study were to determine the PK features of CX in healthy dogs after a single oral administration of (1) 2 mg/kg and (2) 80 mg to dogs in the fasting and fed state.

Materials and methods

Animal studies

Beagle and Labrador retriever dogs were used. Each dog was housed in a single 3 m × 2 m box and acclimatized for a minimum of 1 week. The experiments, approved by the Lublin local Animal Research Ethical Board (54/2012), were performed in accordance with the European Regulation on Animal Welfare (Directive 2010/63/EU).

Study 1. CX 2 mg/kg administration (precise dose)

Five healthy male adult Beagles with a weight and age range of 9–12 kg and 4–5 years, respectively, were used. The animals were considered clinically healthy on the basis of a physical examination and complete haematological analyses. The dogs were given CX (Cimalgex, Vetoquinol) at a dose of 2 mg/kg orally, after 12 h of fasting overnight. Water was provided ad libitum. The tailored doses were prepared by carefully partitioning and weighing the marketed chewable tablet.

A catheter was placed into the right cephalic vein to facilitate blood sampling. Blood samples for PK analysis (2–3 mL) were collected at intervals of 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, and 24 h after CX administration, and placed in collection tubes containing lithium heparin. The blood samples were centrifuged at 400 *g* for 5 min within 30 min of collection. The harvested plasma was stored at −20 °C and used within 14 days of collection.

Study 2. CX 80 mg/dog administration (variation on recommended dose)

Six healthy male Labrador retriever dogs weighing 32–41 kg and ranging in age between 2 and 4 years were used in this study. The dogs were considered clinically healthy on the basis of a physical examination and complete haematological analyses. Animals were randomly (drawn from a box) assigned to two treatment groups, using an open, paired, single-dose, two treatment, two-period, crossover design. Each dog received a single dose of 80 mg/subject CX (Cimalgex, Vetoquinol). The first group ($n = 3$) received the treatment in the morning, after fasting for 12 h overnight. The second group ($n = 3$) was treated with a single dose of 80 mg/subject CX in the morning, 15 min after the dogs had been fed canned meat dog food (250 g) with oatmeal (200 g) in a 20 cm squared container. After a wash-out period of 2 weeks the groups were reversed and the treatments repeated. A catheter was placed into the right cephalic vein to facilitate blood sampling. Both the collection times and the sample handling were as reported for Study 1.

Chemical and reagents

Pure CX and parecoxib (internal standard) powders (both >99.0% purity) were donated by Vetoquinol and Pfizer, respectively. High performance liquid chromatography (HPLC) grade acetonitrile (ACN), methanol (MeOH), dichloromethane (CH_2Cl_2), and diethyl ether (Et_2O) were purchased from Merck. Analytical grade trifluoroacetic acid (CF_3COOH) was obtained from BDH. Sodium chloride (NaCl) and ammonium acetate ($AcONH_4$) were purchased from Carlo Erba. Distilled water was produced by a Milli-Q Milli-pore Water System (Millipore). All other reagents and materials were of analytical grade and supplied from commercial sources. The LC mobile phases were filtered through 0.2 μm cellulose acetate membrane filters (Sartorius Stedim Biotech) with a solvent filtration apparatus.

Chromatographic conditions

The analysis procedure was performed according to Giorgi et al. (2013). Briefly, plasma samples (500 μL) were added to internal standard (100 μL, 5 μg/mL) and 10% CF_3COOH (100 μL). After vortexing for 30 s, NaCl (4 mg) was added and the samples were vortexed again. After vortexing, 600 μL of CH_2Cl_2:Et_2O (3:7, v/v) were added, then the samples were vortexed (30 s), shaken (60 osc/min, 10 min) and centrifuged at 21,913 *g* (rotor radius 10 cm) for 10 min at 10 °C. The supernatant (400 μL) was collected in a separate snap cap vial. The organic phase was evaporated under a gentle stream of nitrogen and reconstituted with 200 μL of mobile phase. Twenty microlitres of this latter solution were injected onto the HPLC system which was coupled to a fluorescence detector (HPLC-FL).

The plasma concentration of CX was determined by liquid chromatography (LC Jasco) consisting of a quaternary gradient system (PU 980 plus) and an in line multi lambda fluorescence detector (FL 2020 plus). The chromatographic separation assay was performed with a Kinetex C18 analytical column (100 mm × 4.6 mm inner diameter, 2.6 μm particle size; Phenomenex) preceded by a security guard column with the same stationary phase (Kinetex XB-C18; Phenomenex). The system was maintained at 25 °C. The mobile phase consisted of acetonitrile:buffer (10 mM $AcONH_4$, adjusted to pH 4.5 with AcOH) (35:65, v/v) at a flow rate of 1 mL/min with isocratic mode. Excitation and emission wavelengths for analysis were set at 268 and 430 nm, respectively.

Quantification

The calibration curve of peak area vs. concentration (0.01, 0.05, 0.1, 0.5 and 1 μg/mL) of CX was plotted. Least squares regression parameters for the calibration curve were calculated, and the concentrations of the test samples were interpolated from the regression parameters. Sample concentrations were determined by linear regression, using the formula $Y = aX + b$, where Y = peak area, X = concentration of the standard in μg/mL, a = the slope of the curve and b = the intercept with Y axis. The correlation coefficient of the calibration curve was >0.99. The CX recovery was 76 ± 5.6% with a coefficient of variation (CV) < 6.3%. The limit of quantification was 25 ng/mL. The full method validation can be found elsewhere (Giorgi et al., 2013).

PK evaluation

The PK calculations were carried out using WinNonLin v 5.3.1 (Pharsight). Maximum concentration (C_{max}) of CX in plasma, and the time required to reach C_{max} (T_{max}) were predicted from the data. The area under the concentration/time curve ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule. According to Akaike value and to goodness of fit criteria, changes in plasma concentrations of CX were best described using the mono-compartmental analysis. The adequacy of the curve fitting was assessed by the correlation coefficient and standard error of the estimated parameters.

Determination of CX dosage regimens

Based on the PK analysis of pooled data, computer simulations (WinNonlin 5.3) were performed to compare the time above minimal effective concentration (MEC) after different oral doses (1.95, 2, and 2.5 mg/kg). The MEC was set 0.2 μg/mL according to the previous report (EMA, 2011).

Statistical analysis

The PK data between groups were assessed using ANOVA with Bonferroni's post hoc test. The results are reported as standard deviations of the mean values (±SD). All the analyses were conducted using GraphPad InStat v.5.0. Differences were considered significant if the associated P value was <0.05.

Results

No visible adverse effects were observed in any dogs in either of the studies over the 24 h following drug administration.

Study 1. CX 2 mg/kg administration (precise dose)

The mean time concentration curve for the five Beagle dogs given 2 mg/kg CX is shown in Fig. 1. After oral administration, CX concentrations were detectable in plasma for up to 24 h in 2/5 dogs. A mono-compartmental model best fitted the plasma

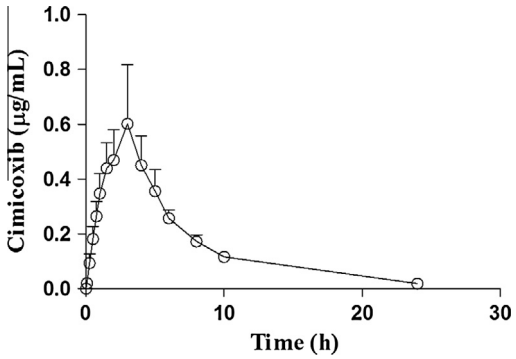

Fig. 1. Mean plasma concentrations of cimicoxib vs. time curve in Beagle dogs ($n = 5$) following PO administration (2 mg/kg). Bars represent the standard deviations.