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Pharmacokinetics and metabolism study of firocoxib in camels after intravenous administration by using high-resolution bench-top orbitrap mass spectrometry

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ABSTRACT

In this study, we developed a high-resolution liquid chromatography mass spectrometry method for the pharmacokinetic study of firocoxib followed by full method validation. Following a solid-phase extraction, the firocoxib and internal standard (celecoxib) were separated on an Agilent Zorbax ZDB C18 column (50 mm × 2.1 mm i.d., 3.5 μm) with a gradient elution using methanol and 0.1% aqueous formic acid. Data acquisition was performed at 25,000 resolution with the automatic gain set to 1,000,000 and the maximum injection time of 100 ms. Data were acquired in full-scan mode over a mass range of 100–550 Da in positive electrospray mode. Linear calibration curves were obtained over the concentration ranges of 0.5–200 ng/mL and no interfering peaks were detected at the retention time of firocoxib and internal standard in blank camel plasma samples. The mean extraction recoveries of firocoxib at three concentrations of 5, 25 and 75 ng/mL ranged from 92 to 104%. Coefficient of variation of intra-day and inter-day precision were both <10%. The accuracy of the method ranged from 95 to 107%. The validated method was then successfully applied in evaluating the pharmacokinetics and metabolism of firocoxib in camels (*Camelus dromedarus*) ($n = 5$) following intravenous (i.v.) administration of a dose of 0.1 mg kg/body weight. The results obtained (mean ± SD) were as follows: the terminal elimination half-life ($t_{1/2\beta}$) was 5.75 ± 2.26 h, and total body clearance (Cl_T) was 354.1 ± 82.6 mL/kg/h. The volume of distribution at steady state (V_{SS}) was 2344.4 ± 238.7 mL/kg. One metabolite of firocoxib was tentatively identified as desalkyl firocoxib (m/z 283). Firocoxib could be detected in plasma 3–5 days following i.v. administration in camels using a sensitive liquid chromatography high-resolution orbitrap mass spectrometry method.

1. Introduction

Firocoxib [3-(cyclopropylmethoxy)-4-(4-(methylsulfonyl) phenyl-5,5-dimethylfuranone)] is a non-steroidal anti-inflammatory drug (NSAID) that inhibits prostaglandin synthesis via selective inhibition of cyclo-oxygenase-2 (COX-2), imparting analgesic, antipyretic and anti-inflammatory properties [1]. It is marketed for use in horses and dogs. The clinical trials and field studies conducted as part of the development of firocoxib have demonstrated its efficacy in reducing pain and improving the mobility of dogs with osteoarthritis, with a low rate of adverse events [2].

The anti-inflammatory activity of all NSAIDs is mediated by inhibition of COX, a key enzyme in facilitating prostaglandin synthesis. Firocoxib preferentially inhibits COX-2 [1]. This isozyme is induced in response to the actions of external stimuli which induce inflammation. The other isoform, COX-1, has a physiological role, notably in the gastric mucosa and possibly the renal vasculature [3]. COX-2 inhibitors have therefore fewer gastrointestinal side effects and renal side effects.

NSAIDs are among the most commonly prescribed drugs for race camels for various orthopedic injuries. They are also the most common group of drugs reported as prohibited substances. However, most of the current NSAID drugs can be detected for long periods especially with current sensitive analytical techniques. An NSAID with a short detection time would be highly welcomed by practicing veterinarians and trainers working in the field of camel racing.

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The pharmacokinetics of firocoxib has been studied in horses [1] and donkeys [4] but not in camels. The pharmacokinetic parameters varied considerably among these species. For example, total body clearance was 37 and 239 mL/h/kg in horses and donkeys, respectively. The manufacturer of firocoxib (Previcox®, Merial, France) reports a total body clearance value of 400 mL/h/kg in dogs [5]. Although firocoxib is marketed for horses and dogs, it has already found its use in race camels using the dose recommended for horses.

The purpose of the current study was to develop a sensitive high-resolution liquid chromatography mass spectrometry method for the detection of firocoxib and its metabolites in camel fluids and to apply it to characterize the pharmacokinetics and metabolites of firocoxib in camels after a single bolus administration. Another goal of the study was to advice on a withdrawal period before camel racing following a therapeutic dose of firocoxib as an effective way of controlling illegal firocoxib use in camel racing.

2. Materials and methods

2.1. Animals

Five clinically healthy male camels (*Camelus dromedarius*), 6–9 years old and ranging in bodyweight from 300 to 400 kg were used in this study. The camels were out of training and kept in open pens. They were fed good-quality hay and lucerne (alfalfa) once daily, with water allowed ad libitum. No camel had received any drug for at least 1 month. Before the study, blood samples were collected for clinical chemistry (alanine aminotransferase, aspartine aminotransferase, alkaline phosphatase, glutamyltransferase, creatine kinase, total protein, albumin, globulin, urea nitrogen, creatinine, calcium, phosphorus, iron, copper and a complete hemogram). These tests were repeated for 7 consecutive days after firocoxib administration using a clinical chemistry analyzer (Hitachi 704). Animals which were normal on physical examination and their clinical chemistry tests were within the normal ranges were chosen in the study.

2.2. Treatment

Firocoxib (Eqioxx, 20 mg/mL, Meryl) was administered as a bolus i.v. injection (jugular vein) at a dose of 0.1 mg/kg (manufacturer recommended dose for horses). Venous blood samples were drawn from the opposite jugular vein at 0, 5, 10, 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 12 and 24 h timed from the start of injection of firocoxib. Blood samples were also collected 2, 4, 5, 7 and 10 days after drug administration for evaluation of detection time. Blood samples were immediately placed on ice; plasma was separated by centrifugation at 4500 × *g* at room temperature for 10 min. The harvested plasma was frozen at −20 °C until analysis.

2.3. Determination of Firocoxib in plasma

Firocoxib and celecoxib were obtained commercially. All solvents and chemicals were of analytical grade or HPLC grade.

To an aliquot of 1 mL of plasma samples, calibrator or QC was added 25 μL of methanol containing 1 μg/mL of celecoxib as internal standard (IS) solution and 2 mL of sodium phosphate buffer (0.1 mM, pH 6.0). The samples were immediately agitated for 30 s followed by centrifugation at 4500 × *g* for 10 min. The samples were extracted by solid-phase extraction as reported previously [6]. Briefly, Clean Screen extraction cartridges (C8 with a cationic exchanger; Clean Screen, Worldwide Monitoring) were conditioned sequentially with methanol (5 mL), water (5 mL) and 0.1 M phosphate buffer (pH 6.0, 3 mL). The samples were passed through the cartridges and were followed by 0.1 M phosphate buffer (3 mL). The cartridges were then washed with 1 M acetic acid (2 mL) and dried with air at 45 psi for 5 min before being washed with hexane (2 mL). Acid and neutral fractions, containing firocoxib and IS, were eluted with dichloromethane (5 mL).

Extracts of the samples were analyzed on a ThermoFisher Accela UPLC system (Thermo Fisher Scientific, San Jose, USA). The chromatographic separation was performed using an Agilent Zorbax ZDB C18 column (3.5 μm × 2 mm × 50 mm, Santa Clara, CA) linked to a Phenomenex pre-column filter (4 × 2 mm, Torrance, CA) operating in gradient mode at 35 °C. The mobile phases were 0.1% formic acid (solvent A) and methanol (solvent B). A linear gradient was run at 0.3 mL/min, with 40% solvent B at the start (t = 0 min), increasing to 90% solvent B at t = 4 min. The gradient was then returned to 40% solvent B at t = 4.20 min, and stabilized until t = 7.3 min before starting the next injection. The temperature of the autosampler tray and of the column compartment was set at 10 and 35 °C, respectively.

Detection was performed by means of an Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, USA) equipped with a heated electrospray (HESI-II) ion source, operated in a positive mode. Source parameters were optimized by introducing solution of firocoxib at a concentration of 10 μg/mL at a rate of 3 μL/min into a solvent flow of 300 μL/min consisting of 50% organic mobile phase and 50% aqueous mobile. This was achieved using the autotune functionality within the Xcalibur operating software using the exact mass m/z 337.1032 from firocoxib. The optimized source parameters were as follows: sheath gas flow rate, auxiliary gas flow rate and sweep gas flow rate: 45, 10 and 0 arbitrary units, respectively, capillary temperature: 275 °C; heater temperature: 300 °C; spray voltage: 4.50 kV. Nitrogen was used as both the source and the collision cell gas. Data acquisition was performed at 25,000 resolution (enhanced resolution mode), the automatic gain control (AGC) was set to 1,000,000 and the maximum injection time was 100 ms.

Data were acquired in full-scan mode over a mass range of 100–550 Da. The ion m/z 113.23 derived from uracil added to the mobile phase (300 μg/L) was used as a lock mass for the Orbitrap. The monoisotopic accurate mass of firocoxib (m/z 337.1032) and of the internal standard celecoxib (m/z 382.37217) were extracted from the total ion chromatogram, at a mass tolerance of 2 ppm for integration and quantification.

The orbitrap was calibrated every 3 days with MSCAL5-1EA and MSCAL6-1EA for positive ion modes. The calibration was performed when the daily results of the deviation mass accuracy reached ±3 ppm. Data processing was performed with the help of ToxID software (version 2.1.1) and Xcalibur software (version 2.1) ThermoFisher Scientific, San Jose, CA, USA).

2.4. Method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, matrix effects and stability according to the guidelines set by the United States Food and Drug Administration (FDA) [7]. Validation runs were conducted on 4 consecutive days. Each validation run consisted of one set of calibration standards and two replicates of QC samples (n = 8 total values in 4 days). For intra-assay coefficients of variation, a total of 10 replica were used in the same day.

The selectivity of the method was evaluated by analyzing 20 blank camel plasma samples. Calibration curves were constructed by analyzing spiked calibration samples on 4 separate days. Peak area ratios of firocoxib to IS were plotted against firocoxib concentrations, and standard curves were fitted to the equations by linear regression without weighting in concentration of 0.5, 1.0, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0 and 200.0 ng/mL. The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curves, which can be quantified reliably, with an acceptable accuracy (80–120%) and precision (<20%).