The Veterinary Journal 208 (2016) 76–80

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Pharmacokinetics and disposition of flupirtine in the horse

M. Giorgi [a,*], V. De Vito [a], A. Poapolathep [b], R. Rychshanova [c], M. Sgorbini [a], H. Owen [d]

[a] Department of Veterinary Sciences, University of Pisa, Via Livornese (lato monte), San Piero a Grado, Italy
[b] Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, University of Kasetsart, Bangkok, Thailand
[c] Veterinary School, Kostanay State A. Baitursynov University, Kostanay, Kazakhstan
[d] School of Veterinary Science, The University of Queensland, Gatton Campus, Gatton, Queensland 4343, Australia

ARTICLE INFO

Article history:
Accepted 15 August 2015

Keywords:
Flupirtine
Horses
Intravenous
Oral
Pain reliever
Pharmacokinetics

ABSTRACT

Flupirtine (FLU) is a non-opioid analgesic drug, with no antipyretic or anti-inflammatory effects, used in the treatment of a wide range of pain states in human beings. It does not induce the side effects associated with the classical drugs used as pain relievers. The aim of this study was to evaluate the pharmacokinetic profiles of FLU after IV and PO administration in healthy horses. Six mixed breed adult mares were randomly assigned to two treatment groups using an open, single-dose, two-treatment, two-phase, paired, cross-over design (2 × 2 Latin-square). Group 1 ($n = 3$) received a single dose of 1 mg/kg of FLU injected IV into the jugular vein. Group 2 ($n = 3$) received FLU (5 mg/kg) via nasogastric tube. The animals then swapped groups after a 1-week wash-out period and the doses were repeated. Blood samples (5 mL) were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 24, 36 and 48 h and plasma was then analysed by a validated HPLC method.

Some mild and transient adverse effects (that spontaneously resolved within 5 min) were observed in 2/6 animals after IV administration. No adverse effects were noticed in the PO administration group. After IV and PO administrations, FLU was detectable in plasma for up to 36 h. The mean elimination half-life was longer after PO (10.27 h) than after IV (3.02 h) administration. The oral bioavailability was 71.4 ± 33.1%. After compartmental simulation/modelling, an oral dose of 2.6 mg/kg was calculated to give C_{max} and AUC values in horses similar to those reported in humans after a clinical dose administration with a theoretical FLU effective plasma concentration of 187 ng/mL. These findings may form the basis for further studies concerning this active ingredient in equine medicine.

Introduction

Flupirtine (FLU) is an aminopyridine drug (ethyl {2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl}carbamate) approved in Europe in 1984 for the treatment of pain (Kumar et al., 2013). FLU is a centrally acting analgesic with a mechanism of action unlike that of opiates and non-steroidal anti-inflammatory drugs (NSAIDs); it is active with a favourable tolerability, and has no antipyretic or anti-inflammatory effects (Devulder, 2010). FLU was the first drug to be recognised in the class of '*Selective Neuronal Potassium Channel Openers*' (SNEPCOs) (Kornhuber et al., 1999).

FLU interacts with the G-protein-regulated, Inwardly Rectifying K^+ channels (GIRKs), a novel family of K^+ channels (distinct from the voltage-dependent ones) that are regulated by neurotransmitters and expressed in different parts of the brain. FLU activates GIRKs and stabilizes the membrane resting potential by activating KCNQ potassium channels so generating a neuronal hyperpolarizing current (M-current); the increased M-current results in decreased neuronal excitability (Kolosov et al., 2012). In addition, FLU inhibits the NMDA receptor indirectly by acting as an oxidizing agent at the redox site of the NMDA receptor, maintaining the Mg^{2+} block on the NMDA receptor (Devulder, 2010).

FLU can be useful in the treatment of a wide range of pain states in humans. In line with its mechanism of action reducing neuronal hyperexcitability, it has proven useful in conditions involving neuronal hyperexcitability such as chronic pain (non-malignant and malignant), migraine and neurogenic pain (Luben et al., 1994; Wörz et al., 1996; Mueller-Schwefe, 2003; Ringe et al., 2003; Li et al., 2008; Szelenyi, 2013). Furthermore, its effect as a muscle relaxant represents and adds value in painful conditions associated with increased muscle tension, such as musculoskeletal back pain, myofascial pain and tension headaches (Worz, 1991; Wörz et al., 1995, 1996; Banerjee et al., 2012; Kumar et al., 2013). FLU has also been shown to be beneficial in the short-term treatment of acute to moderate pain, such as post-operative pain, trauma and dysmenorrhoea (Heusinger, 1987).

The approved indications for FLU differ between countries but mainly include the clinical management of musculoskeletal pain, post-operative pain, headache, dysmenorrhoea, neuralgia and

* Corresponding author. Tel.: +39 5022 10154.
E-mail address: mgiorgi@vet.unipi.it (M. Giorgi).

http://dx.doi.org/10.1016/j.tvjl.2015.08.019
1090-0233/

neuritis, post-traumatic pain (trauma and chemical burns) and pain associated with cancer (Devulder, 2010; Harish et al., 2012). It has not been used to its full potential as an analgesic in the first decade of the 21st century, but there has recently been a resurgence in FLU use after discovery of its powerful additive effects when used with opioids (Goodchild et al., 2008; Capuano et al., 2011; Kolosov et al., 2012; Lee et al., 2015) in addition to its properties when used alone (Wilhelmi, 2013).

While there is a substantial body of evidence on the efficacy of FLU in humans, only a small number of studies on the analgesic effect of FLU in laboratory animals are to be found in the literature (Gordon et al., 1987; Schwarz et al., 1995; Nielsen et al., 2004) and its pharmacokinetic profiles in cats (De Vito et al., 2014a) and dogs (De Vito et al., 2014b) have been recently described. Advanced studies (phase III) in dogs and horses are ongoing in the USA, and although data are not yet available, FLU is likely to be launched on the veterinary market in the near future.[1] The aim of the present study was to evaluate the pharmacokinetic profiles of FLU after IV and PO administration in healthy horses.

Materials and methods

Chemicals and reagents

Pure FLU maleate salt and the Internal Standard trazodone (IS) powder (both >99.0% purity) were supplied by Sigma-Aldrich. Acetonitrile (ACN; HPLC grade), methanol (MeOH), dichloromethane (CH_2Cl_2) and ethyl acetate (AcOEt) were purchased from Merck. Ammonium acetate ($AcONH_4$) was purchased from Carlo Erba. Deionised water was produced by a Milli-Q Water System (Millipore). All other reagents and materials were of analytical grade and supplied from commercial sources. The LC mobile phase was filtered through 0.2 μm cellulose acetate membrane filters (Sartorius Stedim Biotech) with a solvent filtration apparatus.

Animal and experimental design

The subjects were six racehorse mares (Italian trotters), aged 9–13 years and weighing 480–590 kg. The horses were determined to be clinically healthy based on physical examination, serum chemistry and haematological analyses. Animals were evaluated daily (for 1 week) for visible adverse effects by specialized personnel. Animal care and handling was performed according to the provision of the EC council Directive 86/609 EEC and also according to Institutional Animal Care and Use directives issued by the Animal Welfare Committee of the University of Pisa, which approved the study protocol.

Horses were randomly assigned to two treatment groups (six slips of paper marked with the numbers 1 to 6 in a box), using an open, single-dose, two-treatment, two-phase, paired, cross-over design (2 × 2 Latin-square). All subjects were fasted for 12 h overnight before each experiment. During the first phase each horse in group 1 ($n = 3$) received a single dose of 1 mg/kg of FLU (Katadolon vials containing 164.5 mg/3 mL FLU D-gluconate [corresponding to 100 mg FLU/3 mL]; AWD Pharma) injected IV into an indwelling catheter previously inserted in the right jugular vein (flow rate 3 mL/min). Group 2 ($n = 3$) received a dose of 5 mg/kg orally (Efiret 100 mg hard capsules containing FLU maleate; Meda Pharma). The oral formulation of FLU was given to all animals via nasogastric tube and consisted of capsules in 500 mL of distilled water. After administration, the nasogastric tube was rinsed with 500 mL of distilled water to ensure complete delivery of the drug into the stomach.

A 1-week wash out period was observed between the phases, then the groups were rotated and the experiment was repeated. The left jugular vein was catheterised to facilitate blood sampling. Blood samples (5 mL) were collected at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 24, 36 and 48 h after administration of FLU and placed in collection tubes containing lithium heparin. Samples were immediately centrifuged at 2000 *g* (10 min), and the harvested plasma was stored at –20 °C until use within 30 days from collection.

High performance liquid chromatography

The analytical method was based on a previous method validated in dog plasma (De Vito et al., 2015). In brief, the HPLC system was an LC Jasco consisting of quaternary gradient system (PU 980) and an inline multilambda fluorescence detector (FP 1520). The chromatographic separation assay was performed with a Luna $C18_{(2)}$ analytical column (250 mm × 4.6 mm inner diameter, 5 μm particle size; Phenomenex) preceded by a guard column with the same stationary phase ($C18_{(2)}$; Phenomenex). The system was maintained at 25 °C. The mobile phase consisted of ACN:$AcONH_4$ (20 mM) solution, pH 6.8 (60:40, v/v) at a flow rate of 1 mL/min. Excitation and emission wavelengths were set at 323 and 370 nm, respectively. The elution of the substances was carried out in isocratic mode.

Sample extraction

The procedure was performed in a 15 mL polypropylene vial. A 500 μL aliquot of plasma was added to 100 μL of IS (100 μg/mL) and vortexed for 60 s. Four millilitres of AcOEt:CH_2Cl_2 (7:3 v/v) were added, then the sample was vortexed (30 s), shaken (100 osc/min, 10 min) and centrifuged at 3000 *g* for 10 min at 10 °C. Three millilitres of the supernatant were collected in a separate clean vial. The organic phase was evaporated under a gentle stream of nitrogen at 40 °C and reconstituted with 500 μL of the mobile phase. Twenty microlitres of this latter solution were injected onto the HPLC-FL.

Pharmacokinetic evaluation

FLU plasma concentration vs. time curves were modelled for each subject using a mono- or two-compartment open model (Gibaldi and Perrier, 1982). Comparison between competing models was made using the residual plots, visual inspection of the goodness of fit curves and the Akaike's information criterion. The pharmacokinetic calculations were carried out using WinNonLin v 5.3.1 (Pharsight). The PO bioavailability was calculated from the ratio of the areas under the plasma FLU concentration curve after PO and IV administration, respectively, indexed to their respective dose:

$$F(\%) = (AUC_{PO} \times Dose_{IV}) / (AUC_{IV} \times Dose_{PO}) \times 100$$

Based on the PK analysis of pooled data, computer simulations (WinNonlin 5.3.1) were performed to calculate the oral dose that should be administered to horses in order to achieve the values of C_{max} (773 ng/mL) and AUC (6070 h ng/mL) reported in humans after oral administration of a clinical dose (Abrams et al., 1988). These calculations were based on the assumptions that the plasma protein binding is the same in humans and horses and that the effective plasma concentrations are of the same order of magnitude for the two species. When the theoretical dosage regimen in horses (a PK/PD hybrid variable) was evaluated, the relative effective plasma drug concentration (assumed at the steady state) was calculated according the following formula (Toutain, 2009):

$$EC = (ED \times Bioavailability) / Clearance$$

where EC is the average effective target plasma concentration needed to obtain the desired clinical response, ED is the dose per dosing interval (amount/time), bioavailability is the extent of systemic bioavailability (a factor between 0 and 1), and clearance is the plasma clearance expressed for the given dosing interval.

Statistical analysis

Pharmacokinetic variables were evaluated using Student's t test to determine statistically significant differences between the treatment groups. Both pharmacokinetic parameters and FLU plasma concentrations are presented as means ± standard deviation (normality tested by Shapiro–Wilk test). All analyses were conducted using GraphPad InStat (GraphPad Software). In all experiments, differences were considered significant if $P < 0.05$.

Results

The HPLC method was re-validated using horse plasma. Briefly, FLU was linear ($r^2 > 0.99$) in the range 10–1500 ng/mL. When samples exceeded the upper limit of the range, they were re-analysed after appropriate dilution. The intra-day repeatability was measured as coefficient of variation and was <5.3%, whereas accuracy, measured as closeness to the concentration added on the same replicates, was <6.2%.

Immediately after IV injection of the drug, 2/6 horses showed adverse effects including muscle twitching, head shaking and agitation but they resolved spontaneously within 5 min. No behavioural changes or alterations in health parameters (heart rate, rectal temperature and intestinal sounds) were observed in the remaining animals during or up to 7 days after the study. Health parameters and behaviour were evaluated once a day and were found to be normal.

A bi-compartmental model best fitted the plasma concentrations after IV and PO administrations in all the six horses. A two-compartment model with bolus input and first-order output, with micro-constants as primary parameters was used for the IV administration while a first-order input, first-order output, no lag time

[1] See http://www.kindredbio.com/#!pipeline/c1ktj (accessed 10 August 2015).