The Veterinary Journal 202 (2014) 309–313

Contents lists available at ScienceDirect

The Veterinary Journal

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

Pharmacokinetic profiles of the analgesic drug flupirtine in cats

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ARTICLE INFO

Article history:
Accepted 13 June 2014

Keywords:
Cats
Flupirtine
Intravenous
Oral
Pain reliever
Pharmacokinetics

ABSTRACT

Flupirtine (FLU) is a non-opioid analgesic drug with no antipyretic or antiphlogistic effects, used in the treatment of a wide range of pain states in human beings. There is a substantial body of evidence on the efficacy of FLU in humans but this is inadequate to recommend its off-label use in veterinary clinical practice. The aim of this study was to evaluate the pharmacokinetic profiles of FLU after IV and PO administration in healthy cats.

Six mixed breed adult cats 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 5 mg/kg of FLU injected IV into the jugular vein. Group 2 (n = 3) received the same dose via PO route. The wash out period was 1 week. Blood samples (1 mL) were collected at assigned times and plasma was then analysed by a validated HPLC method.

No adverse effects at the point of injection and no behavioural changes or alterations in health parameters were observed in the animals during or after the study (up to 7 days after the full study). After IV administration, FLU was detectable in plasma up to 36 h. After PO administration, FLU plasma concentrations were lower than those following IV administration, but they were detectable over the same time range. The terminal part of both mean pharmacokinetic curves showed a similar trend of elimination. The oral bioavailability was approximately 40%. This is the first study of FLU in an animal species of veterinary interest and it could pave the way for the use of this active ingredient in the veterinary field.

Introduction

Increasing numbers of animal species, especially those commonly kept as pets, are treated as members of the family and pet owners demand the same level of care they expect for themselves. This change in attitude has resulted in the increased development of more effective and innovative veterinary therapies (Giorgi, 2012; Giorgi and Yun, 2012).

Pain management is a steadily emerging concept in veterinary medicine (Lamont, 2008) that has resulted in increased interest in the development of new techniques for pain management (Giorgi and Owen, 2012b; Giorgi et al., 2012). There is a limited number of analgesics licensed for cats, and off-label drug use is commonly practiced (Pypendop and Ilkiw, 2008; Lee et al., 2013). Recent investigations have shown that analgesic drugs are still under-used in feline medicine (Taylor, 2003) for fear of their associated side effects (Robertson and Taylor, 2004). It is therefore critical to investigate new active compounds to increase the drug armamentarium for use in cats.

Flupirtine (FLU) is an aminopyridine drug (ethyl {2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl}carbamate) that was approved in Europe in 1984 for the treatment of pain (Kumar et al., 2013) (Fig. 1). FLU is a centrally acting analgesic with a mechanism of action unlike that of opiates. It is active with a favourable tolerability and with no antipyretic or antiphlogistic effects (Singal et al., 2012). FLU is the first drug to be recognised in the unique class of 'selective neuronal potassium channel openers' (SNEPCOs) (Kornhuber et al., 1999). It interacts with the G-protein-regulated, inwardly rectifying K^+ channels (GIRKs), a novel family of K^+ channels distinct from the voltage-dependent ones. They are regulated by neurotransmitters and are expressed in different parts of the brain. FLU activates GIRKs and stabilizes the membrane resting potential by activating potassium channels KCNQ and thus generating a neuronal hyperpolarizing current (M-current). The increased M-current due to the action of FLU translates to decreased neuronal excitability (Kolosov et al., 2012). Moreover, 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 (Singal et al., 2012).

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http://dx.doi.org/10.1016/j.tvjl.2014.06.011
1090-0233/

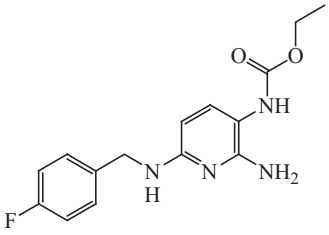

Fig. 1. Molecular structure of flupirtine.

FLU can be useful in the treatment of a wide range of pain states in human beings. In line with its mechanism of action promoting neuronal rest, it has proved 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 added 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 as beneficial in the short-term treatment of acute to moderate pain such as postoperative pain, trauma and dysmenorrhoea (Heusinger, 1987).

The approved indications of FLU differ between countries but mainly include the clinical management of musculoskeletal pain, postoperative pain, headache, dysmenorrhoea, neuralgia and neuritis, post-traumatic pain (trauma and chemical burns) and pain associated with cancer (Devulder, 2010; Harish et al., 2012). It was probably not used to its full potential as an analgesic in the first decade of the 21st century, but in recent years, there has 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) 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, the only study on the analgesic effect of FLU in animals in the literature looked at laboratory species (Gordon et al., 1987). However it is inadequate to recommend its off-label use in veterinary clinical practice (Giorgi and Owen, 2012a). The aim of this study was to evaluate the pharmacokinetic profiles of FLU after IV and PO administration in healthy cats.

Materials and methods

Chemical and reagents

Pure FLU maleate salt and the internal standard trazodone (IS) powders (both >99.0% purity) were supplied by Sigma-Aldrich. HPLC grade acetonitrile (ACN), 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 Milli-pore Water System, and all other reagents and materials were of analytical grade and supplied from commercial sources. The liquid chromatography (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

Six mixed breed adult intact cats, three males and three females, aged between 3 and 6 years, with a bodyweight in the range 2.9–5.2 kg, were enrolled in the study. The cats were determined to be clinically healthy 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 Lublin, which approved the study protocol.

Cats were randomly assigned to two treatment groups (six slips of paper marked with the numbers 1–6 in a box), using an open, single-dose, two-treatment, two-phase, paired, cross-over design (2 × 2 Latin-square). All cats were fasted for 12 h overnight before each experiment. During the first phase each cat in group 1 (n = 3) received a single dose of 5 mg/kg of FLU (Katadolon 100 mg/3 mL vials, FLU D-gluconate AWD Pharma) injected IV into the jugular vein. Group 2 (n = 3) received the same dose via the PO route (Efiret 100 mg hard capsules, FLU maleate, Meda Pharma). A 1 week wash out period was observed between the phases, then the groups were rotated and the experiment was repeated.

The right cephalic vein was catheterised to facilitate blood sampling. Blood samples (1 mL) were collected at 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., 2014). In brief, the high performance liquid chromatography (HPLC) system was an LC Jasco consisting of quaternary gradient system (PU 980) and an in line 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 security 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 milliliters of AcOEt:CH_2Cl_2 (7:3 v/v) was 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 milliliters of the supernatant was collected in a separate 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 microliters of this latter solution was injected onto the HPLC–FL.

Pharmacokinetic evaluation

FLU plasma concentration vs. time curves were modelled for each subject using a mono- or a 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 Akaike's information criterion. A weighting (1/[actual plasma concentration]^2) was used. The pharmacokinetic calculations were carried out using WinNonLin v 5.3 (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:

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

Statistical analysis

Pharmacokinetic variables were evaluated using Student's t test to determine statistically significant differences between the treatment groups and the gender. 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 cat plasma. Briefly, FLU was linear ($r^2 > 0.99$) in the range 10–2000 ng/mL. When samples exceeded the upper limit of the range, they were re-analysed after appropriate dilution. The intraday repeatability was measured as coefficient of variation and was <6.1%, whereas accuracy, measured as closeness to the concentration added on the same replicates, was <5.9%.

No adverse effects were noted at the point of injection and no behavioural changes or alterations in health parameters were observed in the animals during or (up to 7 days) after the study. Physiological signs and parameters were normal.