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Pharmacokinetic profiles of the analgesic drug flupirtine in cats

V. De Vito [a], B. Łebkowska-Wieruszewska [b], H. Owen [c], C.J. Kowaski [b], M. Giorgi [a,*]

[a] *Department of Veterinary Sciences, University of Pisa, Via Livornese (lato monte), San Piero a Grado, Italy*

[b] *Department of Pharmacology, University of Life Sciences, Akademicka 13 20-950 Lublin, Poland*

[c] *School of Veterinary Science, The University of Queensland, Gatton Campus, Gatton, Queensland 4343, Australia*

* Corresponding author: Tel.: +39 5022 10154.

E-mail address: mgiorgi@vet.unipi.it (M. Giorgi).

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 x 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.

Keywords: Cats; Flupirtine; Intravenous; Oral; Pain reliever; Pharmacokinetics

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).

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; Worz 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; Worz et al., 1995; Worz et al., 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 this 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 3-6 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.

115

116 Cats were randomly assigned to two treatment groups (six slips of paper marked with the
117 numbers 1 to 6 in a box), using an open, single-dose, two-treatment, two-phase, paired, cross-over
118 design (2x2 Latin-square). All cats were fasted for 12 h overnight before each experiment. During
119 the first phase each cat in group 1 (n = 3) received a single dose of 5 mg/kg of FLU (Katadolon 100
120 mg/3 mL vials, FLU D-gluconate AWD Pharma) injected IV into the jugular vein. Group 2 (n = 3)
121 received the same dose via the PO route (Efiret 100 mg hard capsules, FLU maleate, Meda
122 Pharma). A 1 week wash out period was observed between the phases, then the groups were rotated
123 and the experiment was repeated.

124

125 The right cephalic vein was catheterised to facilitate blood sampling. Blood samples (1 mL)
126 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
127 and placed in collection tubes containing lithium heparin. Samples were immediately centrifuged at
128 2000 *g* (10 min), and the harvested plasma was stored at -20 °C until use within 30 days from
129 collection.

130

131 *High performance liquid chromatography*

132 The analytical method was based on a previous method validated in dog plasma (De Vito et
133 al., 2014). In brief, the high performance liquid chromatography (HPLC) system was an LC Jasco
134 consisting of quaternary gradient system (PU 980) and an in line multilambda fluorescence detector
135 (FP 1520). The chromatographic separation assay was performed with a Luna $C18_{(2)}$ analytical
136 column (250 mm × 4.6 mm inner diameter, 5 µ particle size [Phenomenex]) preceded by a security
137 guard column with the same stationary phase ($C18_{(2)}$ [Phenomenex]). The system was maintained at
138 25 ºC. 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 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 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 the 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.

A bi-compartmental model best fitted the plasma concentrations after IV and PO administrations in all the six cats. Two-compartment with bolus input and first-order output, were the micro-constants used as primary parameters for the IV administration while a first-order input, first-order output, no lag time and micro-constants as primary parameters was used for the PO administration. The average plasma concentration vs. time curves after both the administrations are shown in Fig. 2.

After IV administration, the FLU plasma concentration varied widely, especially in the initial samples. FLU was detectable in plasma up to 36 h, then at 48 h, the drug concentrations dropped down the LOQ of the method. After oral administration, the FLU plasma concentrations were lower than after IV administration, but were detectable over the same range of time. The C_{max} (2460 ng/mL) was shown at a T_{max} of 2.78 h. The oral bioavailability (F%) was 39.3 ± 9.7%. The half-life of elimination (Beta_HL) values were similar for both routes. The terminal phase of both mean pharmacokinetic curves showed a similar trend of elimination. The mean values of both clearance (CL) and volume of distribution (V2) were significantly different between the groups. The complete pharmacokinetic parameters are reported in Table 1. No statistical differences in pharmacokinetics were found between the genders ($P = 0.12$).

Discussion

FLU is a centrally acting, non-opioid analgesic that is available in a number of European countries for the treatment of a variety of pain states (Devulder, 2010). The therapeutic benefits seen with FLU relate to its unique pharmacological properties. Recently its potential for use in veterinary medicine has been explored (Giorgi and Owen, 2012a). Preclinical studies showed that FLU was more potent than paracetamol and as potent as pentazocine in an electrostimulated pain test in mice (Nickel, 1987). FLU significantly prolonged the latency of the tail-flick test in rats (Szelenyi et al., 1989) and produced an efficacy profile superior to that of tramadol for cancer-associated pain (Luben et al., 1994; Kolosov, 2012). FLU produced a significant increase in morphine antinociception when the two drugs were administered in combination in different rat models of pain (Goodchild et al., 2008; Capuano 2011). If the sparing opioid effect is also evident in cats, this active ingredient could play an important role in combinatorial analgesic therapy in order to avoid moderately high regimens of opioids. FLU might be also an attractive alternative for

patients with a history of adverse drug reaction to NSAIDs (Papich, 2008). Indeed it does not induce the gastrointestinal side effects evoked by classical NSAIDs or the cardio-/cerebrovascular and renal side effects evoked with chronic therapy with COX-2 selective inhibitors (Treudler et al., 2011).

The dose administered in the present study (5 mg/kg) was about three times higher than the minimum reported in human clinical practice (100 mg/subject). However, it was still within the recommended human clinical range (100-400 mg/subject/day) (Devulder, 2010). The rationale for dose selection of 5 mg/kg was that the ED_{50} of FLU after oral administration in the electrical tooth pulp stimulation test in dogs and cats was 3.5 mg/kg (Nickel, 1987) and 3 mg/kg (Gordon et al., 1987), respectively. Moreover, FLU at 5 mg/kg in combinational therapy with morphine, increased the antinociceptive activity of morphine 4-fold without increasing the adverse effects (Goodchild et al., 2008; Capuano et al., 2011). No side effects were reported in these studies. The 5 mg/kg dose did not produce any visible side effect in the cats in the current study (for 7 days), a finding that supports the good safety profile of FLU in humans (Friedel and Fitton, 1993). It has been reported that FLU maintains glutathione levels, a property that has prevented cell death in human retinal pigmented epithelial cells (Wood et al., 1998). This feature could be exploited in animal species that only have small amounts of this enzyme, such as cats.

FLU is a water soluble compound in the form of maleate salt (pKa 5.3) that is rapidly absorbed from the human gastro intestinal tract (Klawe and Maschke, 2009). The T_{max} reported for humans (range 1.6-1.8 h) is a bit shorter than that found in this study (2.78 h). This difference could be attributed to a number of potential reasons including the large variation in this parameter in the cat, different efficacy of absorption or other species-specific factors. In contrast, the FLU plasma

maximal concentrations after PO route in humans (100 mg/subject) and in cats (5 mg/kg) were comparable if normalized for the administered dose (770 ng/mL vs. 2460 ng/mL) (Abrams et al., 1988). A large difference between humans and cats has been shown in oral F%. This value was more than two times lower in cats than in humans (39.3% vs. 90%) (Hlavica and Niebch, 1985).

Large differences in F% between humans and pets have previously been demonstrated, indicating that F% values derived in pets may be inapplicable to human and vice versa (Chiou et al., 2000). Values of apparent CL and V2 after PO administration even after their normalization for F%, were different from those after IV administration suggesting that other phenomena such as the different pharmaceutical composition used in the IV and PO routes (D-gluconate vs. maleate, respectively) or a saturation of the metabolic enzymes (triggered by the high drug concentrations in the IV group), might have generated these differences.

Although FLU has been used in the treatment of acute and chronic states in humans for 25 years, no minimal effective concentration for pain relief has been reported yet. However, it is noteworthy that in cats (despite the low oral F%) a dose of 5 mg/kg PO produced FLU plasma concentrations higher that the plasma concentrations produced by the PO clinical dose (100 mg/subject) reported in humans (Hlavica and Niebch, 1985).

Following PO administration of FLU 100 mg, the mean terminal plasma elimination half-life was about 6.5 h in healthy humans (Abram et al., 1988), whereas it was about twice this time in cats (13.6 h). This is in line with the reduced clearance in cats compared to humans (Abram et al., 1988). A likely explanation for the long half-life shown in cats, is that while FLU is bio-transformed in the N-acetylated analogue D13223 in humans (Methling et al., 2009) the transformation could be

slower or may not occur in cats. Indeed, cats lack one of the two N-acetyl-transferases enzymes (the NAT2) normally expressed in humans (Trepanier et al., 1998 responsible for the D13223 metabolite formation.

FLU is predominantly excreted in urine (about 72% in humans; Hlavica and Niebch, 1985). Although the CL value of FLU did not significantly change in patients with mild renal impairment compared to healthy patients, the half-life almost doubled (Abrams et al., 1988). Hence caution should be used in cats with presumed renal impairment. It has also been proven that old age is associated with increased half-life of the drug in humans (Abrams et al., 1988) and this should be taken into consideration if FLU is to be administered to elderly cats.

Conclusion

This is the first study on FLU in a species of veterinary interest. The pharmacokinetic profiles of FLU in the cat were somewhat different compared to the FLU disposition in humans. Although the PO F% of FLU was quite low, a 5 mg/kg administration gave plasma concentrations exceeding those reported in humans after clinical dosing. This study could pave the way for the use of this active drug in the veterinary field.

Conflict of interest statement

None of the authors of this paper does have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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434 Table 1 Pharmacokinetic parameters of flupirtine (5 mg/kg) after IV and PO administrations in

435 healthy cats (n = 6)

Parameters	Units	Route	
		IV	PO
AUC	h*ng/mL	77299 ± 14908	27856 ± 9719
C_{max}	ng/mL	/	2460 ± 453
T_{max}	h	/	2.78 ± 0.77
K01	1/h	/	1.66 ± 1.11
K10	1/h	0.36 ± 0.11	0.12 ± 0.03
K12	1/h	1.64 ± 1.09	0.07 ± 0.13
K21	1/h	0.41 ± 0.15	0.20 ± 0.17
K01_HL	h	/	1.57 ± 0.38
K10_HL	h	2.32 ± 0.99	3.42 ± 1.38
Alpha	1/h	2.13 ± 1.07	0.41 ± 0.19
Beta	1/h	0.063 ± 0.015	0.044 ± 0.023
Alpha_HL	h	0.42 ± 0.25	3.09 ± 1.94
Beta_HL	h	11.31 ± 2.24	13.67 ± 4.43
A	ng/mL	22314 ± 10632	/
B	ng/mL	4292 ± 1447	/
CL	mL/h/kg	45.09 ± 28.01	195.0 ± 55.04
V2	mL/kg	467.1 ± 463.5	1798 ± 845
F%		/	39.3 ± 9.7

436

437 AUC, area under the plasma concentration–time curve; C_{max}, peak plasma concentration; T_{max}, time of peak; K01,
438 absorption rate; K10, elimination rate from compartment 1; K12, rate of movement from compartment 1 to 2; K21, rate
439 of movement from compartment 2 to 1; K01_HL, half-life of the absorption phase; K10_HL, half-life of the elimination
440 phase; Alpha_HL, distribution half-life; Beta_HL, elimination half-life; Alpha, rate constant associated with
441 distribution; Beta, rate constant associated with elimination; A, intercept for the distribution phase; B, intercept for the
442 elimination phase; CL, clearance; V1, volume of compartment 1; V2, volume of compartment 2; F%, bioavailability.

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446 **Legends to figures**

447 Fig. 1. Molecular structure of flupirtine

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449 Fig. 2. Mean semi logarithm plasma concentrations of flupirtine vs. time curves following PO
450 (—●—) and IV (--○--) administrations of flupirtine (5 mg⁄kg) in healthy cats (n = 6). Bars represent
451 the standard deviations.