Pharmacokinetics Of Flupirtine In Dogs And Cats

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ABSTRACT

Flupirtine (FLU) is a non-opiod analgesic drug belonging to the class of N-methyle-D-aspartate. In human medicine FLU is recommended for the treatment of a wide range of pain states, but no study been performed in Pets.

The aim of this study is to evaluate the pharmacokinetic profiles (PK) of FLU in healthy dogs and cats after different routes of administrations.

Six healthy Labrador breed adult dogs and six healthy mixed breed adult cats were used in two different experiments.

Dogs: single dose, four-group and four-treatment (Intravenous IV, Oral immediate release POIR, per rectum [5 mg /kg]; oral prolonged release POPR [200 mg /subject], cross over design.

Cats: single dose, two-groups, two treatments(IV and POIR[5 mg /kg]), cross over design.

The wash out period between trails was 1 week. Blood samples were collected at assigned times and plasma was analysed by a validated HPLC method.

Some adverse effects, rapidly resolved, were noted only in dogs after IV treatment . The FLU plasma concentration were detectable in all groups up to 36 h. The bioavalability (F%) value after POIR, POPR and RC in dogs were 41.9,36.8 and 29.3%respectively, while oral F% in cat was 39.3±9.7%. Oral bioavalability in dogs and cats was similar, but about two times smaller than that found in the human (90%). This large differences demonstrated that PK values drived in pets shouldn`t be extrapolated to human and vice versa.

This study represents the first step that should pave the way for use of this active ingredient in Pet animals.

INTRODUCTION

Flupirtine (FLU) is an aminopyridine drug (ethyl {2-amino-6-[(4- fluorobenzyl) amino] pyridin- 3-yl}carbamate) approved in Europe in 1984 for treatment of pain *(1)*. It 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 *(2)*. It is the first drug to be recognised in the unique class of 'Selective Neuronal Potassium Channel Openers' (SNEPCO) *(3)*.

FLU 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 *(4)*. Moreover, FLU inhibits the NMDA receptor indirectly by acting as an oxidizing agent at the redox site of the N-Methyle-D-Aspartate receptor, maintaining the Mg2+ block on the NMDA receptor *(2)*.

Nowadays, increasing numbers of animal species, especially those commonly kept as pets, are treated as members of the family. 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 *(5)*.

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 proven useful in conditions involving neuronal hyperexcitability such as chronic pain (non-malignant and malignant), migraines and neurogenic pain *(6-11)*. 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 *(1,7,12-14)*. FLU has also been shown as beneficial in the short-term treatment of acute pain to pain of a moderate duration such as postoperative pain, trauma and dysmenorrhoea *(15)*. The approved indications of FLU differ between countries but mainly include the clinical management of musculoskeletal pain, postoperative pain, headaches, dysmenorrhoea, neuralgia and neuritis, post-traumatic pain (trauma and chemical burns) and pain associated with cancer *(16, 17)*. It has possibly not been used to its full potential as an analgesic in the first decade of the 21th century, but in recent years, there has been a resurgence in FLU use after discovery of its powerful-additive effects when used with opioids *(4,18, 19)* in addition to its properties when used alone *(20)*. There is a substantial body of evidence on the efficacy of FLU in humans however this is inadequate to recommend its off-label use in veterinary clinical practice *(21)*.

The aim of this study was to evaluate the pharmacokinetic profiles of FLU in healthy dogs and cats after different routes of administrations.

MATERIALS AND METHODS

Drugs

The drug used in this study was Flupirtine with four different formulations.

FLU (Katadolon® 100 mg/3 mL vials, FLU D-gluconate AWD Pharma, Radebeul, Germany) for intravenous injection(IV).

FLU (Efiret® 100 mg hard capsules, FLU maleate, 100 mg, Meda Pharma S.p.A. Milano, Italy) for oral administration(POIR).

FLU (Katadolon® PR 400 mg Prolonged-Release tablets, FLU maleate, 400 mg, AWD Pharma, Radebeul, Germany) for oral administration (POPR).

FLU (Katadolon® Zäpfchen, suppositories, FLU maleate, 150 mg, AWD Pharma, Radebeul, Germany) for rectal administration (RC).

Animals

Study in cats

Six mixed breed adult cats, three male and three female aged between 3-6 years, with a bodyweight in a range of 2.9-5.2 kg, were enrolled in this study.

Study in dogs

Six adult, Labradors, one male and five female dogs aged between 3-6 years, with a body weight in the range of 34-40 kg, were enrolled in the study.

Chemicals and reagents

Pure FLU maleate salt and the Internal Standard trazodone (IS) powders (both >99.0% purity) were supplied by Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN), methanol (MeOH),dichloromethane (CH2CL2) and ethyl acetate (AcOEt) were purchased from Merck (Darmstadt, Germany). Ammonium acetate (AcONH) was purchased from Carlo Erba (Milano, Italy). Deionised water was produced by a Milli-Q Milli-pore Water System (Millipore, MA,USA).Formic acid (HCOOH) was purchased from Sigma-Aldrich (Steinheim, Germany).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 S.A., Aubagne Cedex, France) with a solvent filtration apparatus.

Instruments and devices

High performance liquid chromatography (HPLC-FL)

The HPLC-FL system was an LC Jasco (Como, Italy) consisting of quaternary gradient system (PU 980) and an in line multilambda fluorescence detector (FP 1520).

Others: Printing PH Meter, Sample Mixer steromag. (DYNAL)®,Ultra sonic cleaner (VWR),Electronic Analytical and Precision balance, Centrifuge, Magnetic stir/ heater, Vortex.vv3 (VWR),pipettes, magnetic bars, flasks, beakers, polypropylene vials, cylinders, nitrogen gas and filter membrane.

Experimental design

Study in cats

The cats were determined to be clinically healthy on physical examination, serum chemistry and haematological analyses. Cats 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 (2x2 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, Radebeul, Germany) 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 S.p.A. Milano, Italy). 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. 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.

Study in dogs

The dogs were determined to be clinically healthy on physical examination, serum chemistry and haematological analyses. Dogs were randomly assigned to four treatment groups (six slips of paper marked with the numbers 1 to 6 in a box), using an open, single-dose, four-treatment, four-phase, unpaired, cross-over design (4 x4 Latinsquare). All dogs were fasted for 10 h overnight before each experiment. During the first phase, each dog in group 1 (n = 2) received a single dose of 5 mg/kg FLU (Katadolon® 100 mg/3 ml vials, FLU D-gluconate AWD Pharma, Radebeul, Germany) injected IV into the jugular vein with a 1 mL/min injection rate. Group 2 (n = 2) received the same dose via the PO route as an IR formulation (Efiret® 100 mg hard capsules, FLU maleate, 100 mg, Meda Pharma S.p.A. Milano, Italy). All subject doses were prepared by weighing and partitioning the marketed drug. Group 3 (n = 1) received FLU at 200 mg/dog as a PR formulation (Katadolon® PR 400 mg Prolonged-Release tablets, FLU maleate, 400 mg, AWD Pharma, Radebeul, Germany) via the PO route. The PR tablet was manually split according to its division engraving. Group 4 (n = 1) received FLU at 5 mg/kg via the RC route (Katadolon® Zäpfchen, suppositories, FLU maleate, 150 mg, AWD Pharma, Radebeul, Germany). Suppositories were dipped in cold water for 30 min before use, to facilitate their partitioning and insertion. Emptying of bowels was controlled by housing the dogs in individual cages and checking for defecation for at least 2 hours after insertion of the suppositories, to allow the drug to be fully absorbed. Animals were given the suppositories

whilst in a prone position. A 1-week wash out period was observed between the four phases, then the groups were rotated and the experiment was repeated. At the end of the protocol study, each animal had received all the formulations. To facilitate blood sampling, 30 minutes before the start of the study, an 18-gauge soft cannula (Vasofix Braunule, Luer Lock, BBraun Melsungen AG, 34209 Germany) was placed into the saphenous vein, and fixed in place with a cohesive flex wrap bandage (Petflex, Salisbury,MA, USA). Blood samples (1 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 2000g (10 min), and the harvested plasma was stored at -20°C until use, within 30 days from collection. Animals were evaluated daily (up to 1 week after the completion of the study) for visible adverse effects by specialized personnel. Two weeks after the end of the study the dogs underwent a health check for physical and behavioural abnormalities. All dogs used in this study were certified as healthy and thus suitable for rehoming.

Chromatographic conditions

The chromatographic separation assay was performed with a Luna C18 $_{(2)}$ analytical column(250 mm x 4.6 mm inner diameter, 5 µ particle size [Phenomenex, Bologna, Italy]) preceded by a security guard column with the same stationary phase(C18$_{(2)}$ [Phenomenex, Bologna, Italy]). The system was maintained at 25° C. The mobile phase consisted of ACN: AcONH4 (20mM) 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 370nm, 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 wasadded to 100 µL of IS (100 µg/mL) and vortexed for 60 sec. Four mL of AcOEt:CH2Cl2(7:3 v/v)was added, then the sample was vortexed (30 sec), shaken (100 osc/min, 10 min) and centrifuged at3000 g for 10 min at 10°C. Three mL 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 500µL of the mobile phase. Twenty µL of this latter solution was injected onto the HPLC-FL.

Pharmacokinetic evaluation

Study in cats

FLU plasma concentration vs. time curves were modeled for each subject using a mono- or atwo-compartment open model *(22)*. Comparison between competing models was made using the residual plots, visual inspection of the goodness of fit curves and the Akaike'sinformation 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:

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

Study in dogs

The pharmacokinetic calculations were carried out using WinNonLin v 5.3 (Pharsight Corp, Cary NC, USA). The $AUC_{0-\infty}$ was calculated using the log-linear trapezoidal rule. Changes in plasma FLU concentrations were evaluated by use of standard non-compartmental analysis, and the relative pharmacokinetic parameters were determined using standard non-compartmental equations **(23).** Systemic availability (F%) was calculated from the ratio of the areas under the plasma FLU concentration curve, after each single extravascular route and the respective IV administration, indexed to their respective dose:

$$F(\%) = (AUC_{POIR,\ POPR,\ RC} \times Dose_{IV})/(AUC_{IV} \times Dose_{POIR,\ POPR,\ RC}) \times 100$$

Statistical analysis

Study in cats

Pharmacokinetic variables were evaluated using the 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 $P < 0.05$.

Study in dogs

Pharmacokinetic data were evaluated using the ANOVA test to determine statistically significant differences. 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

Results of feline study

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. 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, micro-constants as primary parameters was used 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.

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

After IV administration, FLU plasma concentration varied widely, especially in the initial samples. (concentration range at 15 min, 12,937-25,388 ng/mL). 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 Cmax(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. No statistical differences in pharmacokinetics were found between the genders (P=0.12). The complete pharmacokinetic parameters are reported in Table 1.

The average plasma concentration vs. time curves after both the administrations are reported in Fig.1.

Results of canine study

There were adverse effects in all dogs in the IV group, including salivation, tremors and vomiting. All the adverse effects resolved rapidly (within 10 min) and spontaneously. No observable adverse effects were noted after the extravascular administrations: in these groups physiological signs and parameters were normal.

The average and individual plasma concentrations vs. time curves after the four administrations are reported in Fig. 2 and 3(a-f).

After IV administration, FLU plasma concentration varied widely, especially at the initial time points of collection. FLU was detectable in plasma up to 36 h following administration, then at 48 h, the drug concentrations dropped below the LOQ of the

method. After PO (IR and PR) administrations, the FLU plasma concentrations were lower than after IV administration, but were detectable over the same range of time. After POIR administration, the C max (1549.6 ± 916.3 ng/mL) was shown at a T max of 1.42 ± 0.58 h. The POIR bioavailability (F%) was 41.93 ± 8.47%.

After POPR administration, the pharmacokinetic trend was similar to that reported in the POIR group. After POPR administration, the Cmax (1256.1 ± 353.0 ng/mL) was shown at a Tmax of 2.17 ± 0.93 h. The POPR bioavailability (F%) was 36.78 ± 8.44%.

The lowest Cmax was attained after RC administration (635.3 ± 266.5 ng/mL), achieved at a Tmax of 2.17±0.93. The RC bioavailability (F%) was 29.43 ± 8.84%. The terminal phase of all the mean pharmacokinetic curves showed a similar trend of elimination. The half life values did not differ significantly between the treatment groups. The volume of distribution and clearance values were not statistically different among the treatments, after normalization for their F% values.

The complete pharmacokinetic parameters are reported in Table 2.

Table 1. Pharmacokinetic parameters of flupirtine (5 mg/kg) after IV and PO administrations in healthy cats. Mean± S.D (n = 6).

Parameter	Unit	IV route (Mean±SD)	PO route (Mean±SD)
AUC	hr*ng/ml	77299±14908	27856±9719
C Max	ng/ml	/	2460±453
T Max	hr	/	2.78±0.77
K01	1/hr	/	1.66±1.11
K10	1/hr	0.36±0.11	0.12±0.03
K12	1/hr	1.64±1.09	0.07±0.13
K21	1/hr	0.41±0.15	0.20±0.17
K01_HL	hr	/	1.75±0.38
K10_HL	hr	2.32±0.99	3.42±1.38
Alpha	1/hr	2.13±1.07	0.41±0.19
Beta	1/hr	0.036±0.015	0.044±0.023
Alpha_HL	hr	0.42±0.25	3.09±1.94
Beta_HL	hr	11.31±2.24	13.67±4.43
A	ng/ml	22314±10632	/
B	ng/ml	4292±1447	/
CL	ml/hr/kg	45.09±28.01	195.0±55.04
V1	ml/ kg	0.000201±0.000078	0.00156±0.00047
V2	ml/ kg	467.1±463.5	1798±845
F%	%	/	39.3±9.7

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

Table 2. Pharmacokinetic parameters of flupirtine after IV, POIR and RC (5 mg/kg) and POPR (200 mg/dog) administrations in healthy dogs Mean± S.D (n = 6)

Parameter	Unit	IV	POIR	POPR	RC
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
R^2		0.98 ± 0.03	0.98±0.01	0.99±0.01	0.99±0.01
λz	1/hr	0.11 ± 0.02	0.10±0.03	0.10±0.01	0.09±0.02
T1/2λz	hr	6.20 ± 0.88	7.49±1.97	7.08±0.82	7.78±1.93
Tmax	hr	/	1.42±0.58	2.17±0.93	2.17±0.93
Cmax	ng/ml	/	1549.67±916.3	1256.19±353.04	635.34±266.46
AUC 0-∞	hr.ng/ml	23614±9122	10084±4676	9885±5244	7314±4790
Vz/F*	ml/kg	2089±646	6633±4226	7390±4043	9464±4175
CL/F*	ml/hr/kg	240.46±90.52	604.60±289.98	721.19±388.17	921.25±513.18
AUMC 0-∞	hr.ng/ml	102861±54136	84654±42011	88222±58959	87045±89557
MRT 0-∞	hr	6.18±1.07	8.45±1.69	8.38±1.28	10.28±3.18
MAT	hr	/	2.27±0.31	2.20±0.28	4.10±0.44
F %	%	/	41.93±8.47	36.78±8.44	29.43±8.84

R^2correlation coefficient; λz = terminal phase rate constant; T1/2 λz = terminal half-life; T max= time of peak; Cmax = peak plasma concentration; Vz/F = apparent volume of distribution; CL/F = apparent clearance; AUC 0-∞ = area under the plasma concentration time curve; AUMC 0-∞ = area under the first moment curve; MRT0-∞ = mean resident time; MAT = mean absorption time F% = bioavailability. * In the IV administration these values are VZ and CL

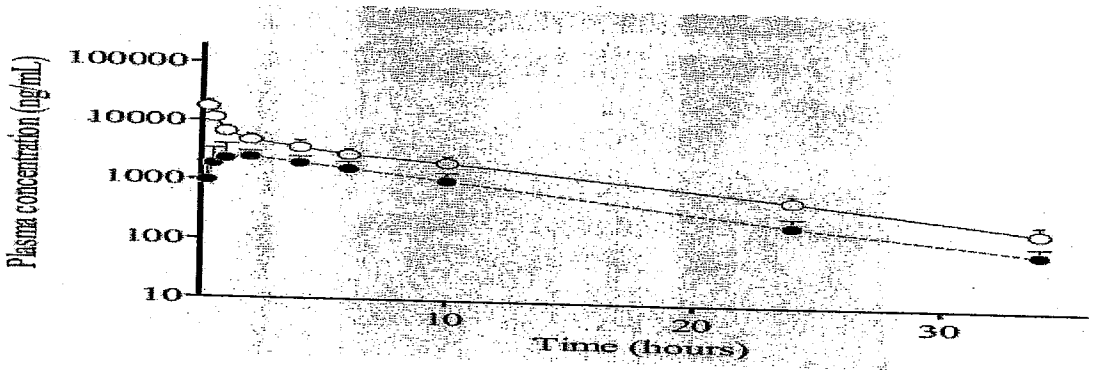

Fig 1.Mean semi logarithm plasma concentrations of FLU vs. time curves following IV(○-)and PO (-●)administrations of FLU (5 mg/kg) in cats (n = 6). Bars represent the standard deviations.

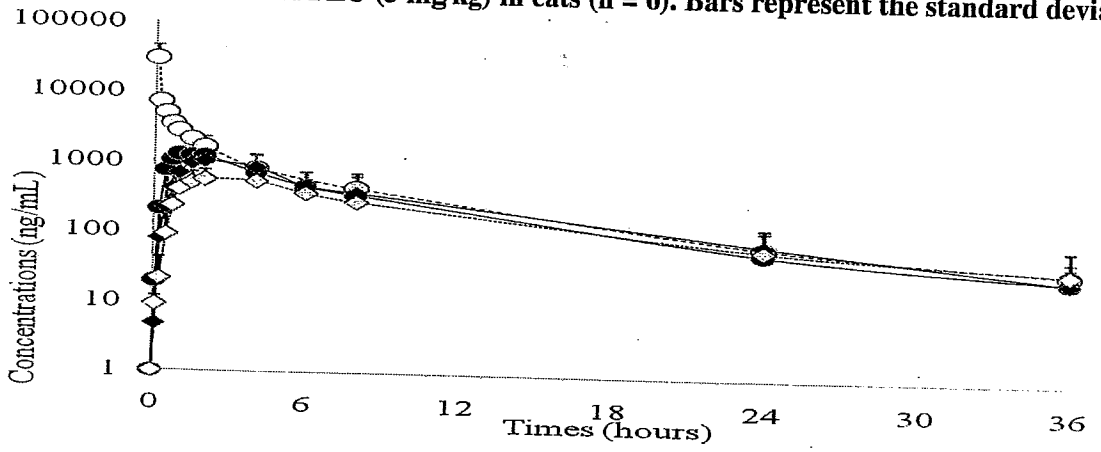

Fig. 2. Mean semi logarithm plasma concentrations of flupirtine vs. time curves following 5 mg/kg IV (-○-), POIR (-●-), and RC (◇) and 200 mg/dog POPR(♦) administrations of flupirtine in dogs (n = 6). Bars represent the standard deviations.

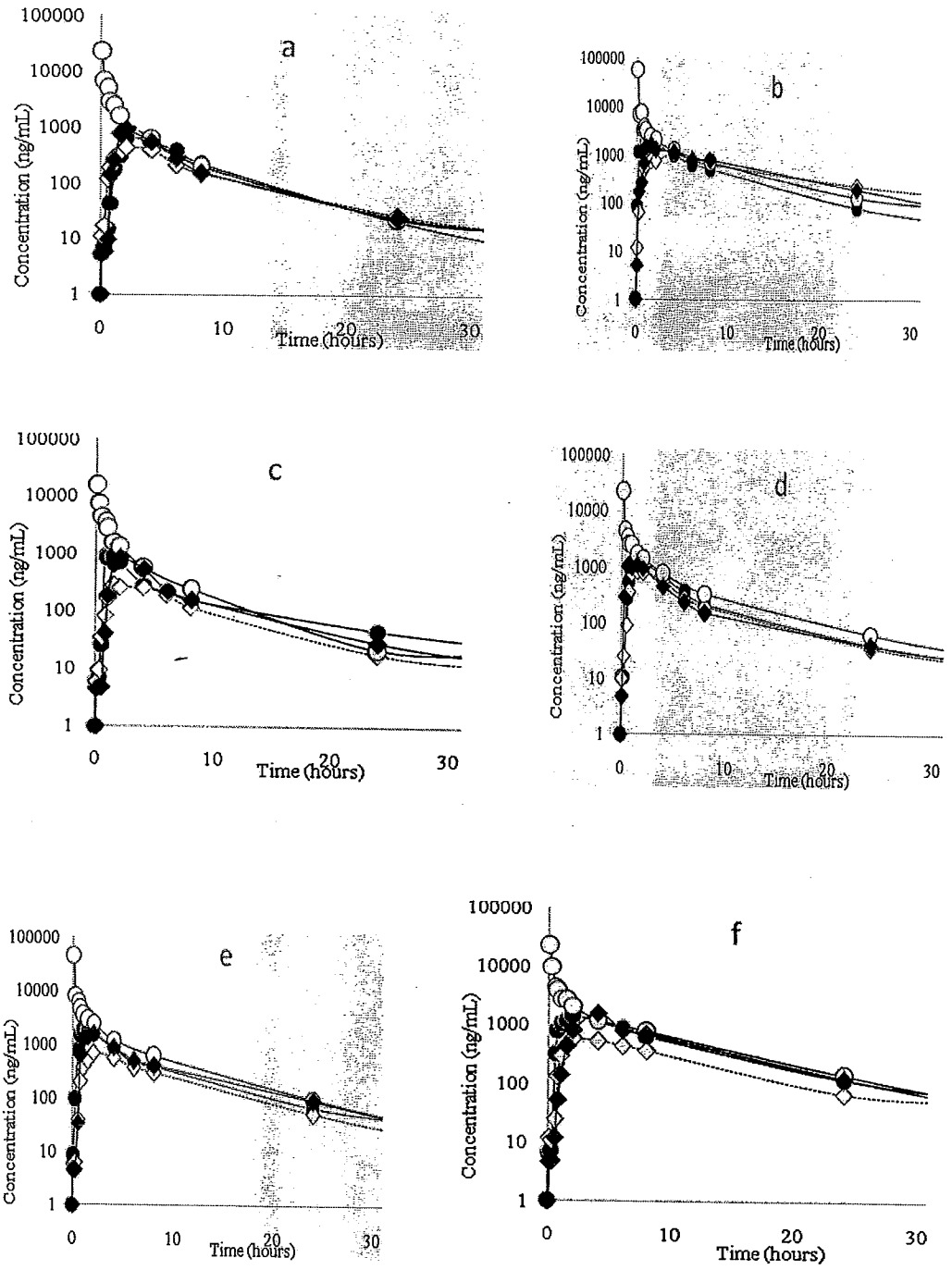

Fig. 3. (a-f). Individual semi logarithm plasma concentrations of flupirtine vs. time curves following 5 mg/kg IV (-○-), POIR (-●-), and RC (◇) and 200 mg/dog POPR(♦) administrations of flupirtine in dogs .

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 *(16)*. The therapeutic benefits seen with FLU relate to its unique pharmacological properties. Recently its potential for use in veterinary medicine has been explored *(21)*. Preclinical studies showed that FLU was more potent than paracetamol and as potent as pentazocine in the electrostimulated pain test in mice *(24)*. FLU significantly prolonged the latency of the tail-flick test in rats *(25)*. FLU produced an efficacy profile superior to that of tramadol for cancer-associated pain *(4,6)*. FLU produced a significant increase in morphine antinociception when the two drugs were administered in combination in different rat models of pain *(18,19)*. If the sparing opioid effect is also evident in cats and dogs, 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 *(26)*. Factly it does not induce the gastrointestinal side effects evoked by classical NSAIDs or the cardio-/cerebrovascularand renal side effects evoked with chronic therapy with COX-2 selective inhibitors *(27)*.

The doses administered in these studies (5 mg/kg IV and PO) for cats and (5 mg/kg IV, POIR and RC or 200 mg/subject POPR) for dogs were about 3 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) *(16)*.

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 *(24)* and 3 mg/kg *(28)*, respectively., and FLU at 5 mg/kg in combinational therapy with morphine increased the antinociceptive activity of morphine 4-fold, without increasing the adverse effects *(18,19)*.

No side effects were reported in these studies. The 5 mg/kg dose in cats did not produce any visible side effect (for 7 days), while Some adverse effects were shown in all the subjects (dogs) receiving IV injection (salivation, tremors and vomiting), while no side effects were reported after the extravascular administrations. It might be inferred that these adverse effects were triggered by the high plasma concentrations detected at the first collection points, as when the drug plasma concentrations fell, the adverse effects were finished. The 5 mg/kg dose did not produce any permanent side effect in any of the dogs or cats in the current study (evaluated up to 1 week after completion of the study), a finding that supports the good safety profile of FLU earlier reported in humans *(29)*. It has been reported that FLU maintains glutathione levels, a property that has prevented cell death in human retinal pigmented epithelial cells *(30)*. This feature could be exploited in animal species that only have small amounts of this enzyme, such as cats.

FLU for IV injection is marketed as a d-glucuronide derivative. The plasma concentrations found in dogs were a slightly higher than that those reported in cats, but the drug was detectable over the same range of time (up to 36 h).

FLU is a water soluble compound in the form of a maleate salt (pKa 5.3) that is rapidly absorbed from the human gastro-intestinal tract *(31)*. The T max after POIR in dogs(1.42 h) in line with that reported for humans (range 1.6-1.8 h) *(32)*, is a slightly shorter than that found in cats (2.78 h). This difference could be triggered by a number of reasons as: the large variation in this parameter in the cat, different absorption or to other species-specific factors.. In contrast, the maximum FLU plasma concentration after POIR administration in dogs was almost half those reported in cats(2460 ng/ml) and humans(770 ng/ml) *(32)*. The maximum FLU plasma concentration 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). The POIR bioavailability was similar to that

reported in cats (39.3%), but about two times smaller than that in humans (90%) *(33)*. Large differences in F% between humans and pets (dogs& cats) have previously been demonstrated, indicating that F% values derived in dogs and cats should not be extrapolated to humans, and vice versa *(34)*.

Values of apparent CL and V2 after PO administration also after their normalization for F% were different from those after IV administration. This suggests 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 and dogs (despite the low oral F%) a dose of 5 mg/kg PO & PO (IR and PR) in cats and dogs respectively produced FLU plasma concentrations higher that the plasma concentrations produced by the PO clinical dose (100 mg/subject) reported in humans **(33)**. Hence, if the clinical plasma concentrations achieved in humans are assumed to also be effective in the dogs and cats, an oral dose of 5 mg/kg might be a valid dose to begin efficacy studies in the canine species.

The half-life values after IV administration (6.2 h) were a slightly shorter than those following the extravascular administrations (range 7.1-7.8 h) in dogs although the difference was not significant (P=0.31). These values were in line with the mean terminal plasma elimination half-life, which was about 6.5 h in healthy humans *(32)*, whereas they were about twice the half-life observed in cats (13.6 h). This is in line with the reduced clearance in cats compared to humans *(32)*. 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 *(35)*, this transformation could be slower or may not occur in cats. Factly, cats lack one of the two N-acetyl-transferases enzymes (the NAT2) normally expressed in humans *(36)* which are responsible for the D13223 metabolite formation.

FLU is predominantly excreted in urine (about 72% in humans, *(33)*. 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 *(32)*. Hence caution should be taken 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 *(32)*. Hence this should be taken into consideration if FLU is to be administered to elderly cats.

Modified release formulations, when used in dogs, might offer a number of potential advantages. Firstly, they could ensure that the titration schedule is simpler and easier to manage because of the reduction in dosing frequency (once daily), with better tolerance and increased compliance from both owner and animal. Secondly, the efficacy of the drug is combined with a low incidence of adverse effects, avoiding the need of additional drugs. Moreover, the likelihood of adverse effects due to abrupt peaks in plasma concentrations and relapses of symptoms (due to rapidly decreasing post-peak plasma concentrations) is reduced because of the uniformity of drug plasma concentrations *(37)*.

Surprisingly, the pharmacokinetic profile after POPR was almost identical to that attained after POIR administration. The two PO treatment groups did not show any statistically different pharmacokinetic parameters. The marketed PR formulation of FLU is a tablet in which the active ingredient is dispersed in a polymeric inert matrix. Hence tablet splitting would not have affected the PR drug release. This is further supported by one indication reported on the package insert is to administer a half tablet (splitting long the engraved line) to patients with liver impairment.

Unfortunately no pharmacokinetic studies concerning FLU PR formulation have been reported in the literature so far and the reason

for this behaviour in dogs remains obscure. However, this is not the first time that a PR formulation marketed for humans has been described as not working as predicted in dogs *(38)*. As the two PO formulations do not differ in pharmacokinetic characteristics, IR is preferable to PR because it is less expensive.

The main rationale for the use of suppositories in human medicine is avoidance of the first pass effect caused by high hepatic clearance. FLU is known to be minimally affected by hepatic clearance, hence the RC formulation has been marketed to allow the drug to be absorbed more rapidly in the systemic circulation (a result of the fatty excipients liquefying at body temperature in the rectum). Another benefit of this formulation is that suppositories can be administered in patients that are difficult to take tablets *(39)*. RC administration of 150 mg of FLU in healthy young volunteers produced a Cmax of 0.89 mg/L after 5.7 h with a F 72.5% *(16)*.

In the present study, RC treatment showed a lower concentration (635.3 ng/mL) and an F% of 29.4 (the lowest among the formulations tested). Venous drainage of the caudal rectum in dogs occurs via caudal and middle rectal veins through the caudal vena cava, thus bypassing the liver, while cranial rectal veins drain into the liver by way of the portal vein *(40)*. A larger systemic availability is likely if the drug was deposited 1 cm into the rectum. However, this latter procedure is quite difficult to attain in dogs, leading to inaccurate drug placement. Another specific concern with RC administration of FLU and a likely explanation for the low systemic availability seen in this route of administration is the possibility for sequestration of drug in faecal matter, a general disadvantage of the RC route for drug administration. These two phenomena might explain the low bioavailability (29.4%) produced by this route of administration. An earlier study reported that suppository formulations (marketed for humans) showed reduced bioavailability in dogs *(41)*.

Conclusion

This is the first study on FLU in a species of veterinary interest. The pharmacokinetic profiles of FLU in cats and dogs were somewhat different compared to the FLU disposition in humans.

Although the oral administrations in dogs gave bioavailability values lower than those produced in humans, a 5 mg/kg dose IR or a 200 mg/subject PR gave plasma concentrations similar to those reported in humans after clinical dosing. However the PO F% of FLU was quite low in cats, a same dose(5 mg/kg) gave plasma concentration exceeding those reported in humans after clinical dosing.

The dose of 5 mg/kg by IV administration gave a high plasma concentration which likely precipitated the observed adverse effects. RC administration gave the lowest bioavailability, while the oral formulations (SR and IR) were similar, both in pharmacokinetic profiles and parameters.

This study could pave the road for the use of this active ingredient in the veterinary field. Further studies need to be undertaken to assess if this drug may be adequate in canine and feline medicine.

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الملخص العربى
المسار الحركى لعقار الفلوبرتين فى الكلاب والقطط

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يعتبر عقار الفلوبرتين مسكن لاينتمى الى المسكنات الافيونيه وينتمى الى طائفه ال ان ميثيل دى اسبرتات ويوصى به كمسكن قوى للألم فى الانسان على نطاق واسع ولكن لم يتم دراسه تأثيره فى الحيوانات الاليفه (القطط والكلاب)

الهدف من هذه الدراسه هو دراسه الحركيه الدوائيه لهذا العقار فى القطط والكلاب الصحيحه من الناحيه الفسيولوجيه بعد اعطائه بعده طرق مختلفه.

استخدم فى هذه الدراسه عدد سته كلاب من فصيله البادور وعدد سته قطط من فصائل مختلفه فى تجربتين مختلفتين حيث تم تقسيم الكلاب الى اربع مجموعات(عن طريق الفم بشكلين مختلفين من الدواء ، عن طريق الوريد ،وعن طريق فتحه الشرج) وتم اعطاء جرعه خمسه ملليجرام بالنسبه للكيلو جرام من وزن الحيوان فى مجموعات الفم والوريد وجرعه مئتان ملليجرام بالنسبه لكل كلب فى مجموعه الكلاب التى تعالج عن طريق الشرج وتم تصميم التجربه عن طريق تبادل المجموعات .

كما استخدم فى التجربه الثانيه عدد سته قطط وتم تقسيمها الى مجموعتين كل منهما تحتوى على ثلاث قطط وتم تجريعهم بنفس الجرعه وهى خمسه ملليجرام بالنسبه للكيلو جرام من وزن الحيوان عن طريف الفم والوريد فقط وتم تصميم التجربه عن طريق تبادل المجموعات

كما روعى ان تكون فتره الغسيل للدواء عباره عن اسبوع واحد بين كل جرعه من اشكال التجريع المختلفه وجمعت عينات الدم من الوريد فى الاوقات المحدده للجدول الزمنى لتجميع العينات وتم تحليلها عن طريق جهاز الكروماتوجرافيا السائله العاليه الاداء بطريقه اثبت صحتها مسبقا

واظهرت النتائج بعض الاثار السلبيه التى سرعان ماحلت تلقائيا فى الكلاب التى تم تجريعها عن طريق الوريد ولكن لم تظهر اى اثار اخرى على بقيه المجموعات واوضحت النتائج ان معدل تركيز الدواء فى البلازما ظل مستمرا حتى الساعه السته وثلاثون من التجميع بعد التجريع وان قيمه اتاحيه الدواء الفميه بعد التجريع عن طريق الفم بشكلين مختلفين(الشكل سريع الانتشار والاخر ممتد الانتشار) وكذلك بعد التجريع عن طريق الشرج بالنسبه للكلاب كانت ٤١,٩ فى المائه و٣٦,٨ فى المائه و٢٩,٣ فى المائه على التوالى بينما فى القطط كانت النسبه حوالى ٤٠ فى المائه مما يوضح ان الاتاحيه الفميه للدواء فى الفصيلتين غالبا متشابهه ولكن تقل بمقدار مرتين عن التى وجدت فى الانسان والتى تبلغ حوالى ٩٠ فى المائه. وهذا الاختلاف الكبير يوضح ان النتائج المتحصل عليها من الحركيه الدوائيه لهذا العقار لايمكن ان تنطبق على الانسان والعكس صحيح.

تمثل هذه الدراسه الخطوه الاولى والتى من استطاعتها ان تمهد الطريق لاستخدام هذاه الماده الفعاله فى المجال البيطرى وخاصه كمسكن قوى للألام فى القطط والكلاب.