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Highlights

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- Flupirtine IV (1 mg/kg) produced mild and transient side effects in some horses.
 - Flupirtine oral bioavailability was calculated to be $71.4 \pm 33.1\%$.
 - The PO dose simulated to give C_{\max} and AUC equivalent to the human dose was 2.6 mg/kg.
 - The theoretical effective plasma concentration was calculated to be 187 ng/mL.
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Pharmacokinetics and disposition of flupirtine in the horse

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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.

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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 Mg²⁺ 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

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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. 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., 2014b). In brief, the HPLC system was an LC Jasco consisting of quaternary gradient system (PU 980) and an inline multimodal fluorescence detector (FP 1520). The chromatographic separation assay was performed with a Luna C18₍₂₎ analytical column (250 mm \times 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:AcONH₄ (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 $\mu\text{g}/\text{mL}$) and vortexed for 60 s. Four millilitres of AcOEt:CH₂Cl₂ (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:

$$F(\%) = (\text{AUC}_{\text{PO}} \times \text{Dose}_{\text{IV}}) / (\text{AUC}_{\text{IV}} \times \text{Dose}_{\text{PO}}) \times 100$$

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 formulae (Toutain, 2009):

$$\text{EC} = (\text{ED} \times \text{Bioavailability}) / \text{Clearance}$$

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 \pm 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).

with micro-constants as primary parameters was used for the PO administration. The average plasma concentration vs. time curves after both the administrations are reported in Fig. 1.

FLU was detectable in plasma up to 36 h, and at 48 h the drug concentrations dropped down the LOQ of the method. After oral administration (5 mg/kg), the FLU plasma concentrations were quite variable, but were detectable over the same range of time. The average C_{max} (1639 ng/mL) was shown at a T_{max} of 2.16 h. The oral bioavailability (F%) was $71.4 \pm 33.1\%$. The half-life of elimination (Beta_HL) value was 3 × higher in the PO compared to the IV group. The mean values of both clearance and volume of distribution were significantly different between the groups including when normalized for dose and F%. The complete pharmacokinetic parameters are reported in Table 1.

After pharmacokinetic simulation of PO multiple dosing, it was found that if the drug is administered once every 24 h the steady

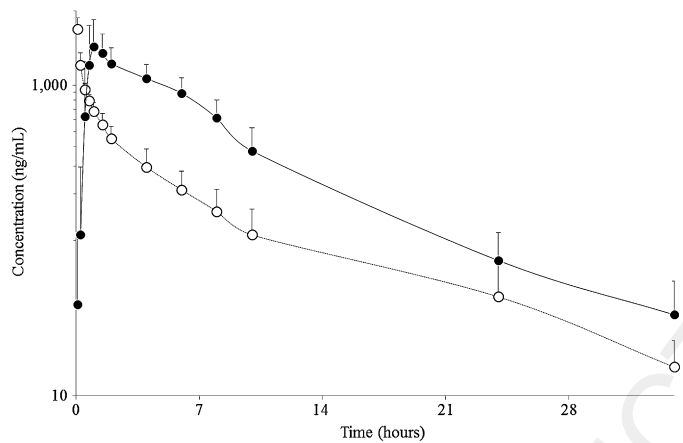


Fig. 1. Mean flupirtine plasma concentration (semi-logarithmic scale) vs. time curves following PO (5 mg/kg) (—●—) and IV (—○—) (1 mg/kg) administrations in healthy horses ($n = 6$). Bars represent the standard deviation of the mean.

Table 1

Relevant pharmacokinetic parameters of flupirtine after IV (1 mg/kg) and PO (5 mg/kg) administrations in healthy horses ($n = 6$).

| Parameters | Units | IV | | PO | |
|-----------------|---------|-------|-------|--------|-------|
| | | Mean | SD | Mean | SD |
| AUC | h*ng/mL | 4003 | 1193 | 13,211 | 4914 |
| K01_HL | h | / | / | 1.38 | 0.62 |
| K10_HL | h | 0.84 | 0.44 | 2.26 | 0.26 |
| Alpha | 1/h | 8.19 | 6.44 | 0.60 | 0.31 |
| Beta | 1/h | 0.27 | 0.12 | 0.07 | 0.03 |
| Alpha_HL | h | 0.12 | 0.06 | 1.41 | 0.65 |
| Beta_HL | h | 3.02 | 1.30 | 10.27 | 3.27 |
| C_{max} | ng/mL | 3706 | 1119 | 1,512 | 643 |
| T_{max} | h | / | / | 2.16 | 0.85 |
| CL ^a | mL/h/kg | 269.7 | 83.58 | 411 | 107.9 |
| V2 | mL/kg | 656.8 | 121.9 | / | / |
| V1 | mL/kg | 289.3 | 80.87 | / | / |
| K01 | 1/h | / | / | 0.61 | 0.30 |
| K10 | 1/h | 1.06 | 0.66 | 0.31 | 0.04 |
| K12 | 1/h | 5.26 | 4.68 | 0.21 | 0.24 |
| K21 | 1/h | 2.13 | 1.40 | 0.15 | 0.10 |
| F% | % | | | 71.4 | 33.1 |

AUC, area under the plasma concentration – time curve; K01_HL, half-life of the absorption phase; K10_HL, half-life of the elimination phase; Alpha, rate constant associated with distribution; Beta, rate constant associated with elimination; Alpha_HL, distribution half-life; Beta_HL, elimination half-life; C_{max} , peak plasma concentration; T_{max} , time of peak; CL, clearance; V2, volume of compartment 2; V1, volume of compartment 1; 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; F%, bioavailability.

^a For the oral dosing this value is divided for its bioavailability.

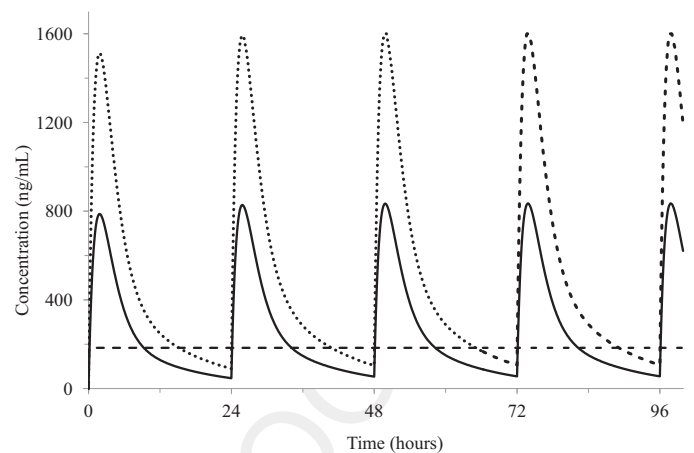


Fig. 2. Mean plasma concentration vs. time curves for flupirtine following a simulated PO multiple dose rate at 5 mg/kg/day (dotted line) and a simulated PO multiple dose rate at 2.6 mg/kg/day (solid line). The dashed line represents the theoretical effective concentration (to be confirmed with experimental data).

state would be achieved after the second administration. The oral dose that theoretically should be administered to horses in order to achieve similar C_{max} and AUC values to those reported with clinical doses in humans is 2.6 mg/kg (Fig. 2). When the theoretical effective drug plasma concentration (EC) was calculated from the relevant parameters ED, clearance and bioavailability, it was shown that the theoretical analgesic effect should be achieved at drug plasma concentrations > 187 ng/mL. The average pharmacokinetic profile indicated that this value is exceeded for over 9 h and 15 h following administration (Fig. 2) of 2.6 and 5 mg/kg of FLU, respectively.

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, 2012). Preclinical studies showed that FLU was more potent than paracetamol and as potent as pentazocine in the electro-stimulated pain test in mice (Nickel, 1987) and significantly prolonged the latency of the tail-flick test in rats (Szelenyi et al., 1989). Furthermore, it produced an efficacy profile superior to that of tramadol for cancer-associated pain (Luben et al., 1994; Kolosov et al., 2012). FLU also 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 et al., 2011). If the sparing opioid effect is also evident in horses, this active ingredient could play an important role in multimodal analgesic therapy in order to avoid moderately high regimens of opioids.

Allometric scaling is an approach for dosage selection that can be used in the absence of either species-specific pharmacokinetic data or prior drug experience in the target species (Hunter, 2010). In the present study, an approach based on data extrapolated from humans rather than an allometric calculation of the dose was preferred. Both the approaches share the assumption that species differences in pharmacodynamics are clinically negligible. The oral dose administered in the present study (5 mg/kg) was about 3 × higher than the minimum dose reported in human clinical practice (100 mg/subject/day). 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 based on earlier preclinical studies in dogs and cats. The ED₅₀ 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. Additionally, recent pharmacokinetic studies carried out with this dose regimen did not show any adverse effects after oral administration (De Vito et al., 2014a and b).

On the other hand as an IV dose, administration of 5 mg/kg FLU produced some adverse effects such as tremors, vomiting and agitation in dogs (De Vito et al., 2014b). In the present study the IV dose was reduced to 1 mg/kg to minimise potential adverse effects. Although the dose was reduced, some mild and transient adverse effects were visible in two subjects. If the IV dose was increased, more severe adverse effects might be expected. This is in line with the unexpected sensitivity of horses to certain drugs when they are injected IV (Jones, 1972). However, it is not clear if the adverse effects are due to the excipients or to the active compound itself. As several adverse effects have been reported in humans after long-term FLU administration (Devulder, 2010), further studies involving multiple drug administrations over a longer period of time are warranted to clarify the safety of this active ingredient in the horse.

FLU is a water soluble compound in the form of maleate salt (pKa 5.3) that is rapidly absorbed from the human gastrointestinal tract (Klawe and Maschke, 2009). The T_{max} found in our study (2.16 h) is between the T_{max} reported for dogs (1.42 h) and that for humans (range 1.6–1.8 h), and cats (2.78 h). A number of factors may be responsible for this difference including the large variation in this parameter in the horse, different absorption due to variable dissolution of the drug in the stomach, or other species-specific factors. In contrast, while the maximal plasma concentrations of FLU after PO administration in humans (100 mg/subject; Abrams et al., 1988) and in cats (De Vito et al., 2014a) were comparable (when normalized for the administered dose and F%), in horses they showed a lower average value compared to that reported for dogs (De Vito et al., 2014b). A large difference (about 40%) has been shown in oral F% between humans (90%) and animals (cats and dogs). In horses, the oral F% was 71%. Wide differences in F% between humans and animals and between animals (carnivorous vs. herbivorous) have previously been demonstrated, indicating that F% values derived in one animal species cannot always be extrapolated to humans or other animal species (Chiou et al., 2000; Kim et al., 2014, 2015).

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 yet been reported. However, it is noteworthy that in horses (despite the lower oral F% than in humans) a dose of 5 mg/kg PO produced FLU plasma concentrations higher than the plasma concentrations produced by the PO clinical dose (100 mg/subject/day) reported in humans (Hlavica and Niebch, 1985). After compartmental modelling/simulation, the calculated dose that produces C_{max} and AUC values (critical parameters for the evaluation of bioequivalence) in horses similar to those reported to be effective in humans is 2.6 mg/kg/day. Further support for this calculation can be obtained using the FLU concentration at a steady state condition for the daily clinical dose in humans. According to Abrams et al. (1988), an AUC value of 6070 µg h/L is obtained with a dose of 100 mg in volunteers having a bodyweight of 63 kg (equivalent to 1.58 mg/kg). The corresponding average plasma concentration in steady state condition is 6070/24 h = 253 ng/mL. To achieve the same average plasma concentration in the horse, this value is multiplied to account for the apparent plasma clearance in the horse (253 ng/mL × 411 mL/kg/h × 24 h = 2.5 mg/kg). This dose (the equivalent analgesic dose to the human clinical dose) is in line with the ED₅₀ values experimentally calculated earlier in cats and dogs (Gordon et al., 1987; Nickel, 1987). The drug plasma EC calculated after the simulation is exceeded for over 9 h and 15 h, after 2.6 and 5 mg/kg FLU oral administration, respectively, suggesting a long lasting therapeutic effect of the drug. It is emphasised here that both the dose and EC

are still theoretical and unconfirmed and further PK/PD studies are required.

Following PO administration of FLU, horses showed mean terminal plasma elimination half-lives in between those reported in cats (13.6 h) and dogs (7.1 h) (De Vito et al., 2014a and b). This is consistent with the clearance value of FLU in horses which is smaller than that reported in dogs (604 mL/h/kg) and larger than that reported in cats (195 mL/h/kg) (De Vito et al., 2014a and b). A likely explanation for the difference in half-life values could be that in cats, FLU is bio-transformed to the N-acetylated analogue D13223, as is the case in humans (Methling et al., 2009); whereas this transformation could be slower or may occur to a lesser extent in horses. Indeed, horses are well known as being poor acetylators (Toutain et al., 2010).

Conclusions

This is the first study on FLU in horses. The pharmacokinetic profiles of FLU in the horse were somewhat different compared to FLU disposition in humans, cats and dogs. Intravenous administration is not advisable in horses because it is likely to produce adverse effects. Although the oral F% of FLU was lower than that in humans, a 5 mg/kg administration produced plasma concentrations exceeding those reported in humans after clinical dosing. An oral dose of 2.6 mg/kg in horses has been calculated to give C_{max} and AUC values similar to those after clinical dose administration in humans. This latter finding is however theoretical and needs to be supported with sound experimental data. The data generated in this research could pave the road for further studies of this active ingredient in equine medicine in order to assess whether this drug may be suitable for horses.

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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References

- Abrams, S.M., Baker, L.R., Crome, P., White, A.S., Johnston, A., Ankier, S.I., Warrington, S.J., Turner, P., Niebch, G., 1988. Pharmacokinetics of flupirtine in elderly volunteers and in patients with moderate renal impairment. *Postgraduate Medical Journal* 64, 361–363.
- Banerjee, M., Bhattacharyya, K., Sarkar, R.N., Ghosh, B., 2012. Comparative study of efficacy and tolerability of flupirtine versus tramadol in non-steroidal anti-inflammatory drug intolerant mechanical low back pain. *Indian Journal of Rheumatology* 7, 135–140.
- Capuano, A., De Corato, A., Treglia, M., Tringali, G., Navarra, P., 2011. Flupirtine antinociception in the rat orofacial formalin test: An analysis of combination therapies with morphine and tramadol. *Pharmacology, Biochemistry, and Behavior* 97, 544–550.
- Chiou, W.L., Jeong, H.Y., Chung, S.M., Wu, T.C., 2000. Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. *Pharmaceutical Research* 17, 135–140.
- De Vito, V., Łebkowska-Wieruszewska, B., Owen, H., Kowalski, C.J., Giorgi, M., 2014a. Pharmacokinetic profiles of the analgesic drug flupirtine in cats. *The Veterinary Journal* 202, 309–313.

- 456 De Vito, V., Lebkowska-Wieruszewska, B., Shaban, A., Lisowski, A., Kowaski, C.J., Giorgi,
457 M., 2014b. Pharmacokinetic profiles of the analgesic flupirtine in dogs after the
458 administration of four pharmaceutical formulations. *Veterinary Anaesthesia and*
459 *Analgesia* doi:10.1111/vaa.12235.
- 460 Devulder, J., 2010. Flupirtine in pain management. *CNS Drugs* 24, 867–881.
- 461 Gibaldi, M., Perrier, D., 1982. *Pharmacokinetics*, Second Ed. Dekker, New York, USA.
- 462 Giorgi, M., Owen, H., 2012. Flupirtine: A human drug with potential for use in the
463 veterinary field. *American Journal of Animal and Veterinary Sciences* 7, 213–
464 217.
- 465 Goodchild, C.S., Kolosov, A., Tucker, A.P., Cooke, I., 2008. Combination therapy with
466 flupirtine and opioid: Studies in rat pain models. *Pain Medicine* 9, 928–938.
- 467 Gordon, R., Sofia, R.D., Diamantis, W., 1987. Effect of flupirtine maleate on the
468 nociceptive pathway, EEG, evoked potentials and polysynaptic reflexes in
469 laboratory animals. *Postgraduate Medical Journal* 63, 49–55.
- 470 Harish, S., Bhuvana, K., Girish, M., Bengalorkar-Kumar, T.N., 2012. Flupirtine: Clinical
471 pharmacology. *Journal of Anaesthesiology Clinical Pharmacology* 28, 172–177.
- 472 Heusinger, J.H., 1987. Efficacy and tolerance of flupirtine and pentazocine in two
473 multicentre trials. *Postgraduate Medical Journal* 63, 71–79.
- 474 Hlavica, P., Niebch, G., 1985. Pharmacokinetics and biotransformation of the analgesic
475 flupirtine in humans. *Arzneimittel-Forschung/Drug Research* 35, 67–74.
- 476 Hunter, R.P., 2010. Interspecies allometric scaling. In: Cunningham, F., Elliott, J., Lees,
477 P. (Eds.), *Comparative and Veterinary Pharmacology*. Springer, London UK, pp.
478 140–155.
- 479 Jones, R.S., 1972. A review of tranquillisation and sedation in large animals. *The*
480 *Veterinary Journal* 90, 613–617.
- 481 Kim, T.W., della Rocca, G., Di Salvo, A., Owen, H., Sgorbini, M., Giorgi, M., 2014.
482 Pharmacokinetics of the novel cyclooxygenase 2 inhibitor cimicoxib in donkeys.
483 *Journal of Equine Veterinary Science* 34, 923–925.
- 484 Kim, T.W., della Rocca, G., Di Salvo, A., Ryschanova, R., Micaela, S., Giorgi, M., 2015.
485 Evaluation of pharmacokinetic and pharmacodynamic properties of cimicoxib
486 in fasted and fed horses. *New Zealand Veterinary Journal* 63, 92–97.
- 487 Klawe, C., Maschke, M., 2009. Flupirtine: Pharmacology and clinical applications of
488 a nonopioid analgesic and potentially neuroprotective compound. *Expert Opinion*
489 *on Pharmacotherapy* 10, 1495–1500.
- 490 Kolosov, A., Goodchild, C.S., Williams, E.D., Cooke, I., 2012. Flupirtine enhances the
491 anti-hyperalgesic effects of morphine in a rat model of prostate bone metastasis.
492 *Pain Medicine* 13, 1444–1456.
- 493 Kornhuber, J., Maler, M., Wiltfang, J., Bleich, S., Degner, D., Rütger, E., 1999. Neuronal
494 potassium channel opening with flupirtine. *Fortschritte Der Neurologie-Psychiatrie*
495 67, 466–475.
- 496 Kumar, R., Keshri, U.P., Sharma, J., 2013. Flupirtine: A mini review. *Journal of Drug*
497 *Delivery and Therapeutics* 3, 113–116.
- 498 Lee, H.K., De Vito, V., Yun, H.Y., Giorgi, M., 2015. Synergistic interaction between
499 tapentadol and flupirtine in the rat orofacial formalin test. *European Journal of*
500 *Pharmacology* *in press*.
- 501 Li, C., Ni, J., Wang, Z., Li, M., Gasparic, M., Terhaag, B., Uberall, M.A., 2008. Analgesic
502 efficacy and tolerability of flupirtine vs. tramadol in patients with subacute low
503 back pain: A double-blind multicentre trial. *Current Medical Research and Opinion*
504 24, 3523–3530.
- 505 Luben, V., Muller, H., Lobisch, M., Wörz, R., 1994. Treatment of tumor pain with
506 flupirtine: Results of a double-blind study versus tramadol. *Fortschritte Der*
507 *Medizin* 112, 282–286.
- 508 Methling, K., Reszka, P., Lalk, M., Vrana, O., Scheuch, E., Siegmund, W., Terhaag, B.,
509 Bednarski, P.J., 2009. Investigation of the in vitro metabolism of the analgesic
510 flupirtine. *Drug Metabolism and Disposition* 37, 479–493.
- 511 Mueller-Schwefe, G., 2003. Flupirtine in acute and chronic pain associated with
512 muscle tenseness: Results of a postmarket surveillance study. *Fortschritte Der*
513 *Medizin. Originalien* 121, 11–18.
- 514 Nickel, B., 1987. The antinociceptive activity of flupirtine: A structurally new analgesic.
515 *Postgraduate Medical Journal* 63, 19–28.
- 516 Nielsen, A.N., Mathiesen, C., Blackburn-Munro, G., 2004. Pharmacological
517 characterisation of acid-induced muscle allodynia in rats. *European Journal of*
518 *Pharmacology* 487, 93–103.
- 519 Ringe, J.D., Miethe, D., Pittrow, D., Wegscheider, K., 2003. Analgesic efficacy of
520 flupirtine in primary care of patients with osteoporosis related pain: A
521 multivariate analysis. *Arzneimittel-Forschung* 53, 496–502.
- 522 Schwarz, M., Schmitt, T., Pergande, G., Block, F., 1995. N-methyl-D-aspartate and alpha
523 2-adrenergic mechanisms are involved in the depressant action of flupirtine on
524 spinal reflexes in rats. *European Journal of Pharmacology* 276, 247–255.
- 525 Szelenyi, I., 2013. Flupirtine, a re-discovered drug, revisited. *Inflammation Research*
526 62, 251–258.
- 527 Szelenyi, I., Nickel, B., Borbe, H.O., Brune, K., 1989. Mode of antinociceptive action
528 of flupirtine in the rat. *British Journal of Pharmacology* 97, 835–842.
- 529 Toutain, P.L., 2009. Mechanisms of drug action and pharmacokinetics/
530 pharmacodynamics integration in dosage regimen optimization for veterinary
531 medicine. In: Riviere, J.E., Papich, M.G. (Eds.), *Veterinary Pharmacology and*
532 *Therapeutics*. Wiley-Blackwell, Iowa, USA, pp. 75–97.
- 533 Toutain, P.L., Ferran, A., Bousquet-Melou, A., 2010. Species differences in
534 pharmacokinetics and pharmacodynamics. In: Cunningham, F., Elliott, J., Lees,
535 P. (Eds.), *Comparative and Veterinary Pharmacology*. Springer, London UK, pp.
536 19–48.
- 537 Wilhelmi, E., 2013. Flupirtine retard – Recommended by the German Society for Pain
538 Management practice guideline on low back pain. *Journal fur Pharmakologie und*
539 *Therapie* 22, 72–73.
- 540 Wörz, R., 1991. Flupirtine in chronic myofascial pain conditions. *Fortschritte Der*
541 *Medizin* 109, 158–160.
- 542 Wörz, R., Lobisch, M., Schwittmann, B., Gessler, M., Grottemeyer, K.H., Langohr, H.D.,
543 Lüben, V., May, A., Nehrlich, D., Schabet, M., 1995. Effectiveness of flupirtine in
544 chronic tension headache: Results of a double-blind study versus placebo.
545 *Fortschritte Der Medizin* 113, 463–468.
- 546 Wörz, R., Bolten, W., Heller, B., Krainick, J.U., Pergande, G., 1996. Flupirtine in
547 comparison with chlormezanone in chronic musculoskeletal back pain: Results
548 of a multicenter randomized double-blind study. *Fortschritte Der Medizin* 114,
549 500–504.