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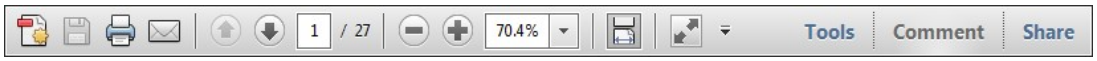

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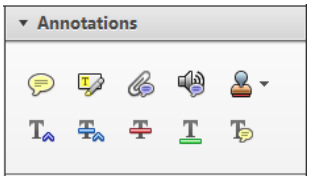

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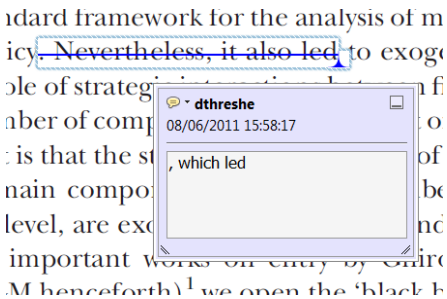

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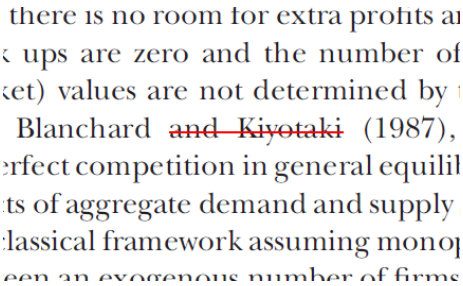

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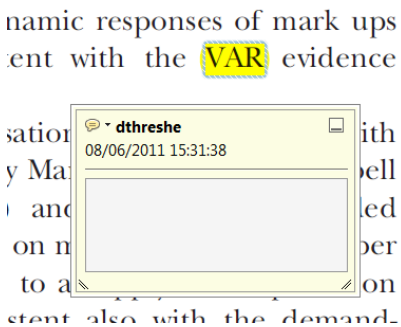

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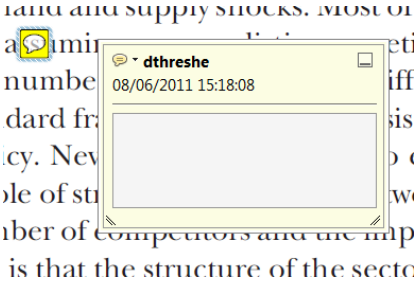

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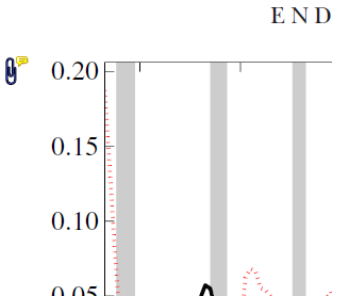

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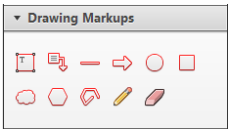

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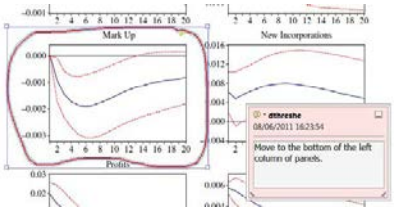

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Pharmacokinetic/pharmacodynamic evaluation of grapiprant in a carrageenan-induced inflammatory pain model in the rabbit

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De Vito, V., Salvadori, M., Poapolathep, A., Owen, H., Rychshanova, R., Giorgi, M. Pharmacokinetic/pharmacodynamic evaluation of grapiprant in a carrageenan-induced inflammatory pain model in the rabbit. *J. vet. Pharmacol. Therap.* doi: 10.1111/jvp.12380.

Grapiprant is the novel selective EP4 receptor inhibitor recently issued on the veterinary market for dogs affected by osteoarthritis. The aim of this study was twofold: to evaluate the pharmacokinetics and the pharmacodynamics of grapiprant in the induced inflammatory pain model in the rabbit after a single IV injection of 2 mg/kg; to compare the thermal antinociception effect after 2 mg/kg IV grapiprant, with that generated by 0.5 mg/kg meloxicam SC injected. Rabbits (n = 12) were randomly assigned to two crossover studies (single-dose, two-period crossover). The first study group A (n = 3) received a single IV dose of grapiprant at 2 mg/kg dissolved in ethanol. Group B (n = 3) received a single IV injection of ethanol (equivalent volume to grapiprant volume) at the same site. The second study group C (n = 3) received a single SC dose of meloxicam at 0.5 mg/kg. Group D (n = 3) received a single SC injection of 15% ethanol (equivalent volume to grapiprant volume) at the same site. After a 2-week washout period, the groups were rotated and the experiments repeated. Blood samples (0.7 mL) were collected from the right ear artery at assigned times and grapiprant plasma concentrations determined by a validated HPLC-FL method. Three hours prior to administration of the drugs, inflammation was induced by SC injection of lambda carrageenan (200 μL, 3% in physiological saline) under the plantar surface of the right hind paw. At a similar time to the blood collection, an infrared thermal stimuli (40 °C) was applied to the plantar surface of the rabbits' hindlimbs to evaluate the thermal withdrawal latency (TWL). The thermal antinociceptive effect was expressed as maximum possible response (% MPR). Grapiprant plasma concentrations were detectable up to the 10-h time point (concentration range 17–7495 ng/mL). The grapiprant-treated group showed a significant increase in TWL from 1 h and up to 10 h after drug administration compared to the control. In contrast, the meloxicam group showed a significant increase in TWL from 4 up to 10 h after drug administration, compared to control. The maximal MPR% was not statistically different between the grapiprant and meloxicam group from 4 to 8 h, while significant differences were shown at 1, 1.5, 2, 10 and 24 h. Given these findings, grapiprant appears to be an attractive option for antinociception in rabbits, due to its rapid onset and extended duration of effect.

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INTRODUCTION

Veterinary medicine faces the unique challenge of having to treat many animal species, including mammals, birds, reptiles and fish. The main challenge for veterinarians is not just to select a drug but to determine, for the selected agent, a rational dosage regimen. Determining this is a long and complicated endeavour because of differences in the expression of enzymes, receptors and signal transduction molecules between species (Giorgi, 2012). Both inter- and intraspecies differences in drug response can be accounted for as either being due to variations in drug pharmacokinetics (PK) or drug pharmacodynamics (PD), the magnitude of which varies from drug to drug (Riviere *et al.*, 1997). Hence, PK/PD studies are critical when a drug is applied to a new animal species.

Nowadays we are far more cognizant of pain in animals (Fajt *et al.*, 2011; Thomsen *et al.*, 2012; Giorgi *et al.*, 2016). Animal species that years ago were considered wild or farm animals are now pets and owners expect an adequate level of care to be provided. This change in attitude has resulted in a push for the development of more effective and innovative veterinary therapies (Moore, 2016). Companion rabbit medicine is a relatively new field quite distinct from laboratory and commercial rabbit medicine and given the differences, there is a requirement for increased information that is specific to this area (Lichtenberger & Lennox, 2012).

Grapiprant is an active ingredient that was discovered in 2007. It was identified as a competitive antagonist of prostanoid EP4 receptors with similar potency in humans, rats (Nakao *et al.*, 2007) and recently in dogs (Nagahisa & Okumura, 2016). It is highly selective for the EP4 receptor compared with other prostanoid receptors (i.e. EP1, EP2, EP3, prostaglandin D, F and I receptors and thromboxane A receptor). The EP4 receptor is the primary mediator of the PGE2-elicited sensitization of sensory neurons and PGE2-elicited inflammation (Lin *et al.*, 2006; Nakao *et al.*, 2007; Chen *et al.*, 2010; Boyd *et al.*, 2011). The EP4 receptor is not the only receptor involved in inflammation and pain, but its inhibition may mediate central sensitization and play a role in pain in humans and animals (Lin *et al.*, 2006; Nakao *et al.*, 2007). Grapiprant has been recently approved by FDA for use in canine medicine (Giorgi, 2015). Studies have already determined its good safety and efficacy profiles in dogs (Rausch-Derra *et al.*, 2016a), and its pharmacokinetics at high doses have been investigated in dogs and cats (Rausch-Derra & Rhodes, 2016; Rausch-Derra *et al.*, 2016b). To the best of the authors' knowledge, no information exists on the pharmacokinetics and pharmacodynamics (PK/PD) of this drug in rabbits.

The objectives of this study were to perform initial investigations on this promising molecule by assessing the PK/PD in rabbits after a single IV injection of grapiprant and to compare its thermal antinociceptive effect with that generated, in the same experimental model, by the current gold standard clinical option meloxicam.

MATERIALS AND METHODS

Animals and experimental design

Twelve adult female New Zealand White rabbits (Pampaloni, Fauglia, Pisa, Italy), with body weights ranging from 2.7 to 3.1 kg (mean 2.88 kg), were used for the study. Animal care and handling was performed according to the provision of the EC council Directive 2010/63/EU and also according to Institutional Animal Care and Use directives issued by the Animal Welfare Committee of the University of Pisa. Rabbits were housed three per cage on a 12-h/12-h light–dark schedule with food and water freely available. The period between arrival at the housing facility and the PK/PD testing was 2 weeks. Rabbits were randomly assigned to two crossover study groups ($n = 6$), using slips of paper marked with the numbers 1–12, selected blinded from a box. Each trial was designed according to a single-dose, two-period crossover study.

Pharmacokinetic experimental design. In the first study, six rabbits were divided into two equal groups. During the first phase of the study, animals in group A ($n = 3$) received a single IV dose of grapiprant at 2 mg/kg via the marginal vein of the left ear. This dose was selected based on previous information describing the effectiveness of grapiprant in dogs (Nagahisa & Okumura, 2016). The injectable grapiprant solutions were freshly prepared by dissolving the pure grapiprant powder in ethanol to produce a 30 mg/mL solution, which was then passed through a 0.45 μm filter, maintaining sterile conditions. Group B ($n = 3$) received a single IV injection of ethanol (equivalent volume to grapiprant volumes) into the same left marginal vein. An indwelling catheter was inserted in the right artery of the ear of each rabbit to facilitate the blood collections. A 2-week washout period was observed. This period was assumed to ensure complete metabolism and excretion of grapiprant as well as the resolution of the induced inflammation (*vide infra*). After the washout period, the groups were rotated and the experiment was repeated (second period). A new grapiprant solution was freshly prepared for the second phase.

The second crossover study was identical in study design and procedure. Six rabbits were divided randomly into two equal groups. Animals in group C ($n = 3$) received a single SC dose of meloxicam (5 mg/mL 15% ethanol solution, Metacam, Boehringer Ingelheim, Milan, Italy) at 0.5 mg/kg. This dose was selected based on the leaflet information. Group D ($n = 3$) received a single SC injection of ethanol 15% in distilled water (equivalent volume to meloxicam volumes). After a 2-week period, the groups were rotated and the experiment was repeated (second period).

By the end of each crossover study, each rabbit ($n = 6$/study) had received both the drug and control treatment. Blood samples (0.7 mL) were collected from the right catheter site at 0, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10 and 24, h after drug (grapiprant or meloxicam) or control (pure or 15% ethanol) administration and placed in collection tubes

containing lithium heparin (MiniCollect, Greiner Bio-One). After each blood collection, 1 mL of saline (0.9% NaCl) supplemented with 10 UI/mL heparin was injected in the right catheter. Specimens were centrifuged at 1000 *g* within 30 min of collection, and the harvested plasma was stored at −70 °C and used within 15 days of collection.

Pharmacodynamic experimental design. Measuring baseline thermal thresholds (prior to inflammation)—Each rabbit was weighed, and the plantar surface of its right hind paw was shaved. A small dot was drawn near the centre of the plantar surface of the rabbit's hindlimb using a marker. Each rabbit was then placed into an individual Plexiglas enclosure without the floor and allowed to acclimatize to the enclosure for 30 min. After the 30-min acclimation period, baseline thermal withdrawal thresholds were determined for each animal. Experiments were conducted by applying infrared thermal stimuli to the plantar surface of the rabbit's hindlimb with a plantar antinociception device (Hargreaves's instrument, model 37370, Ugo Basile) according to previously described methods (Ren & Dubner, 1999) with slight modifications.

Three withdrawal readings were taken from the right hind paw of each rabbit, and the mean of the three readings was used as the rabbit's baseline withdrawal threshold. A minimum interval of 1 min was observed between each of the three withdrawal trials (Dong *et al.*, 2008).

Induction of inflammation-associated hyperalgesia—The next phase of experimentation involved induction of inflammation in a hind paw. Each rabbit received an injection of lambda carrageenan (3% in physiological saline, 200 μL injection volume) SC under the plantar surface of the right hind paw (Dong *et al.*, 2008). The injection was performed such that the point of entry of the needle was remote from the marker dot but the bolus was centred under the dot. Immediately after the carrageenan injection, the rabbit was placed in a holding cage. The determination of thermal withdrawal latency (TWL) after inflammation induction occurred at the three-h time point postcarrageenan injection (Dong *et al.*, 2008). As such, 2.5 h after carrageenan injection, each rabbit was returned to its Plexiglas enclosure. At 3 h postcarrageenan injection, three more thermal withdrawal readings were determined from rabbits. This was also the time zero for the drug or ethanol administrations (pharmacokinetic study). Each reading was separated by 1 min, and the mean of the three readings was used as the rabbit's postcarrageenan TWL.

An infrared radiation source was activated (40 °C) directly below the surface upon which the rabbit rested the plantar surface of their right hindlimb. Hindlimb TWLs were measured by a motion-sensitive timer, which stopped automatically when the hindlimb was removed from the noxious stimulus. The increasing temperature caused the rabbit to withdraw the limb, and the time to withdrawal was automatically measured. A maximum exposure duration of 22.5 sec (cut-off time) was allowed to prevent severe tissue damage. The observer (V D) in the analgesia experiments was blinded to treatments received. TWL was measured before drug administration (baseline) and at 0.5, 1, 1.5, 2, 4, 6, 8, 10 and 24 h after treatment. In the second phase of the crossover study, the whole pharmacodynamics procedure was repeated on the contralateral paw.

The thermal antinociceptive effect was expressed as percentage of maximum possible response (% MPR) (Harris & Pierson 1964), which was calculated as follows:

$$\%\,\mathrm{MPR} = \frac{T_{\mathrm{test}} - T_{\mathrm{con}}}{T_{\mathrm{cut}} - T_{\mathrm{con}}} \times 100$$

where T_{test} represents TWL value after injection of grapiprant or meloxicam, T_{con} is TWL value after injection of pure or 15% ethanol (control) and T_{cut} is the cut-off time (22.5 sec).

Materials

Pure grapiprant analytical standard (> 99.0% purity) was purchased from ChemBo Pharma (Nanjing, China). The Internal Standard (IS) metoclopramide powder (> 99.0% purity) was supplied by Sigma-Aldrich (St. Louis, MO, USA). Meloxicam (Metacam injectable 10 mL 2 mg/mL, Boehringer Ingelheim, Milan, Italy) was supplied by a commercial pharmacy. Lambda carrageenan (Sigma–Aldrich Co.) was dissolved in 0.9% physiological saline after sonication at 40 °C. HPLC grade acetonitrile, methanol, chloroform and ethanol were purchased from Merck (Darmstadt, Germany). Ammonium acetate and acetic acid were purchased from Carlo Erba (Milano, Italy). Deionized water was produced by a Milli-Q Millipore Water System (Millipore, MA, USA). All the other reagents and materials were of analytical grade and supplied from commercial sources. The aqueous and organic components of the mobile phase, degassed under pressure, were mixed by the pumps of the HPLC machine. The LC mobile phases were filtered through 0.2 μm cellulose acetate membrane filters (Sartorius Stedim Biotech S.A., France) with a solvent filtration apparatus.

High-performance liquid chromatography (HPLC)

A previously published validated HPLC technique (De Vito *et al.*, 2015) was revalidated for rabbit plasma samples. The intra- and interday repeatability was measured as a coefficient of variation and was lower than 7.2%, whereas accuracy, measured as closeness to the concentration added on the same replicates, was lower than 5.6%. Within- and between-run precision was lower that 6.7%. The extraction efficiency was 91.1 ± 5.3%. The limits of detection (LOD) and quantification (LLOQ) were 1 ng/mL and 10 ng/mL, respectively. The HPLC system was a LC Jasco (Como, Italy) consisting of quaternary gradient system (PU 2080 plus) and an in-line multilambda fluorescence detector (FP 2020). The chromatographic separation assay was performed with a Synergi Polar-RP 80A analytical column (150 mm × 4.6 mm inner diameter, 4 μm particle size [Phenomenex, Italy]) preceded by a security guard column with the same stationary phase (Phenomenex, Italy).

The system was maintained at 25 °C. The mobile phase consisted of ammonium acetate:acetonitrile (20 mM) solution, pH 4 (70:30, v/v) at a flow rate of 1 mL/min in isocratic mode. The wave lengths were 240 and 400 nm for excitation and emission, respectively.

Preparation of plasma samples

The sample preparation was carried out according to the validated method developed in dog plasma by De Vito *et al.* (2015). Briefly, the procedure was performed in a 15-mL snap cap polypropylene tube. A 0.5-mL aliquot of plasma sample was added to 100 µL of IS (Metoclopramide 25 µg/mL in methanol). After vortexing for 30 sec, 4 mL of chloroform was added, and the sample was vortexed (30 sec), shaken (60 osc/m, 10 min) and centrifuged at 21 913 ***g*** for 10 min at 25 °C. Three millilitres of the supernatant was collected in a separate clean snap cap polypropylene tube. The organic phase was evaporated under a gentle stream of nitrogen and reconstituted with 500 µL of mobile phase. Fifty microlitres of this latter solution was injected onto the HPLC.

Pharmacokinetic evaluation

The concentration vs. time curves of grapiprant in rabbits were described by a noncompartmental model using WinNonlin software (version 5.3.1) (Pharsight, NC, USA). The terminal rate constant (λ) was determined from the slope of the terminal phase of the plasma concentration curve that included a minimum of three points. The half-life of the terminal phase ($T_{1/2}\ \lambda z$) was calculated using $T_{1/2} = 0.693/\lambda$. The area under the concentration vs. time curve ($AUC_{0\text{-}\infty}$) was calculated using the linear trapezoidal rule. Changes in plasma concentration of grapiprant were evaluated using the standard noncompartmental analysis, and the relative pharmacokinetic parameters were determined using standard noncompartmental equations (Gabrielsson & Weiner, 2002). The % of the AUC last to infinity was lower than 9%.

Statistical analysis

For each rabbit, the TWLs measured at a given time point were averaged. These mean TWLs were then averaged for all rabbits given the same treatment. Kolmogorov–Smirnov test was applied to verify data distribution. Pharmacodynamic data were evaluated using the two-way ANOVA (repeated-measures) to determine statistically significant differences between treatment and control values (crossover design). Post hoc comparisons were made by use of Student–Newman–Keuls test. As the two control values were not statistically different, they were merged to determine a single control group of 12 animals. The grapiprant plasma concentrations and the pharmacokinetic parameters are presented as means ± standard error (SD). All analyses were conducted using GraphPad InStat (GraphPad Software). In all experiments, differences were considered significant if $P < 0.05$.

RESULTS

Pharmacokinetics (PK)

Average grapiprant plasma concentration vs. time curve after IV administration of 2 mg/kg in rabbits is presented in Fig. 1. The quantifiable plasma concentrations of grapiprant were in the range 17–7,495 ng/mL and detectable up to 10 h, in all the subjects. The corresponding pharmacokinetic parameters are shown in Table 1. Grapiprant was eliminated quite rapidly with a terminal half-life value of 2.18 h. Clearance was 739.48 mL/h/kg with an extraction ratio in the range 7.7–8.9%, and volume of distribution was wide (2434.4 mL/kg).

Pharmacodynamics (PD)

Differences in TWL in each control group animal ($n = 6$) were not statistically significant at any point tested (first vs second phase). In addition, no significant difference was found between control data determined from the control groups of the two studies ($P > 0.15$). Hence, to establish the TWL baseline, all the pure and 15% ethanol treatment data were grouped for each time point. Hereafter the control group consisted of data from 12 animals. No side effects both systemic and at the injection site were observed from the ethanol injection in both studies. The baseline thermal thresholds prior to inflammation were not statistically different among the groups (14.7–16.3 sec) (Fig 2).

The TWL 3 h after the carrageenan injection (T0) was drastically reduced (5.2–6.5 sec) and did not show any significant differences among the groups (Fig 2).

In the control group, TWL values were constant up to 10 h following the placebo injection. Twenty-seven hours after the carrageenan injection, TWL average returned to a value similar to that observed precarrageenan injection (TWL 15.1 ± 1.3 sec).

Animals given grapiprant showed a significant increase in TWL 1 h after drug administration (7.2 ± 2.0 sec) compared

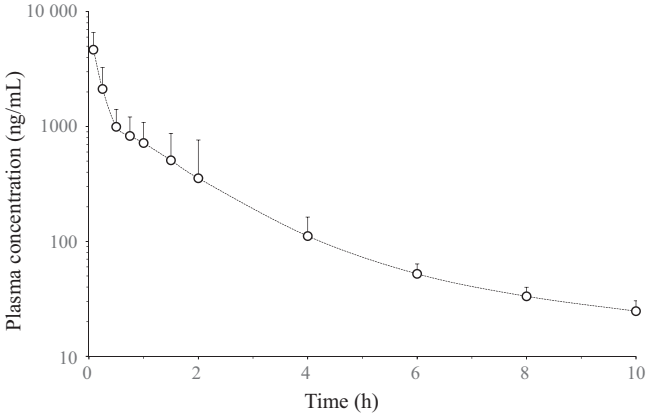


Fig. 1. Mean plasma concentrations (± SD) vs. time curve of grapiprant after IV administration in rabbits ($n = 6$). LLOQ = 10 ng/mL.

Table 1. Mean and SD value of the pharmacokinetic parameters of 2 mg/kg grapiprant following IV administration in rabbits (n = 6)

	IV	
Parameter	Mean	SD
λz (1/h)	0.32	0.05
$T_{1/2}$ λz (h)*	2.18	0.31
C_0 (ng/mL)	7086.90	2812.09
Vz (mL/kg)	2434.40	1405.72
Vss (mL/kg)	1258.26	756.02
CL (mL/h/kg)	739.48	328.77
AUC_{last} (h ng/mL)	3160.62	1590.74
$AUC_{0-\infty}$ (h ng/mL)	3213.49	1590.55
$AUMC_{last}$ (h^2 ng/mL)	4271.49	1886.63
MRT_{last} (h)	1.62	0.26

Λz, terminal phase rate constant; $T_{1/2}\lambda z$, terminal half-life; C_0, drug plasma concentration estimated at time zero; Vz, volume of distribution; Vss, volume of distribution at the steady state; CL, clearance of the terminal phase; AUC_{last}, area under the plasma concentration–time curve; $AUC_{0-\infty}$, area under the plasma concentration–time curve extrapolated to infinity; $AUMC_{last}$, area under the first moment curve; MRT, mean resident time.
*Harmonic mean.

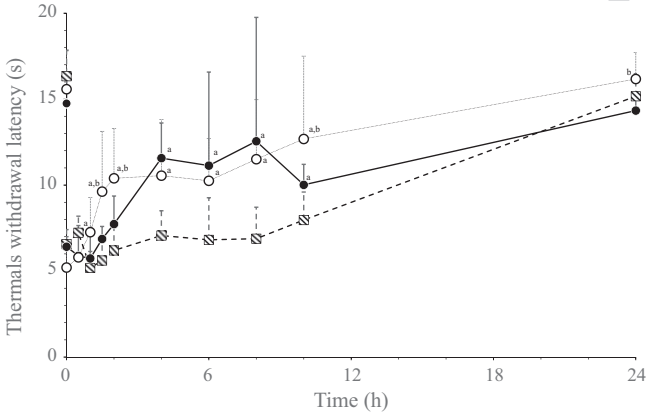


Fig. 2. Mean (± SD) TWL vs. time curve in rabbits (n = 12) after control (zebra square) and IV grapiprant (2 mg/kg) (white circle), and SC meloxicam (0.5 mg/kg) (black circle) administration. [a]Significantly different ($P < 0.05$) from the control group, [b]significantly different ($P < 0.05$) from the meloxicam group. The markers in the upper part of the Y axis represent the mean baseline values (± SD) of the thermal thresholds prior to inflammation.

to the control value. Subsequently, TWL increased in proportion to time with significant differences from the control group still apparent up to 10 h. The average TWL value in the grapiprant group after 24 h was 16.1 ± 1.5 sec which is not significantly different to that of baseline thermal threshold (15.5 ± 2.2 sec) or to the control group at 24 h.

Animals given meloxicam showed a significant increase in TWL 4 h after drug administration (11.5 ± 2.0 sec) compared to control value. TWL achieved steady values (11.3–12.5 sec) up to 8 h, values then decreased to 10.1 ± 1.1 sec at 10 h, these values were still significantly different from the control.

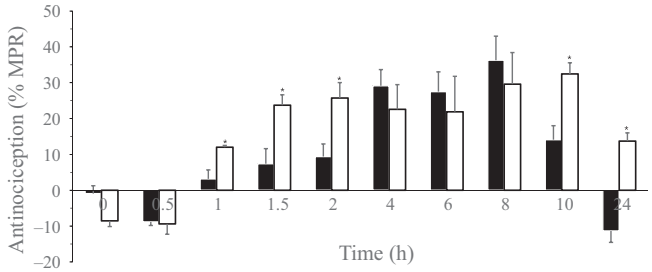


Fig. 3. Mean (±SD) % MPR after IV administration of grapiprant (2 mg/kg) (white bar) and SC meloxicam (0.5 mg/kg) (black bar). *Significantly different ($P < 0.05$) between the groups.

The average TWL value in the meloxicam group after 24 h was 14.3 ± 0.8 sec which is not significantly different from that of the baseline thermal threshold (14.7 ± 1.7 sec) or that of the control group at 24 h.

Mean MPR after grapiprant administration showed thermal antinociception values of around 20–30% over the time period 1.5–10 h. Similarly, meloxicam produced a similar effect (27–36%) but over a shorter range of time (4–8 h). Grapiprant showed significantly higher antinociception effects than meloxicam at 1, 1.5, 2, 10 and 24 h after drug treatments (Fig. 3).

PK/PD evaluation

The pharmacokinetic/pharmacodynamic correlations are reported in Fig. 4. While the mean grapiprant plasma concentration vs. time curve declined, the % MPR vs. time curve rose (Fig. 4). The effect of the drug, albeit small, was also reported at 24 h when the plasma concentration of grapiprant was below the limit of detection of the method. The lag time between grapiprant effect and grapiprant plasma concentration appeared to be generating a large counterclockwise hysteresis loop over an extended period (Fig. 5).

DISCUSSION

If it is difficult to define and recognize whether an animal feels pain, it is even more challenging to objectively determine whether pain medication is effective in exotic animals. In general, to determine the efficacy of drugs in any species, it is important to determine the pharmacokinetic and pharmacodynamic properties of the drug in that species (Toutain & Lees, 2004). Knowing the pharmacokinetic values for a particular analgesic is often insufficient to determine appropriate doses and dosing frequencies, because plasma levels of drugs do not always correlate with analgesia. Plasma concentrations can provide guidance for dosing frequencies, but that does not always hold true because the duration of effect of analgesics (e.g. NSAID) may be much longer than what would be expected from plasma levels. The pharmacokinetics of analgesics also vary considerably across all species that have been studied, so extrapolating clinical doses and dosing intervals

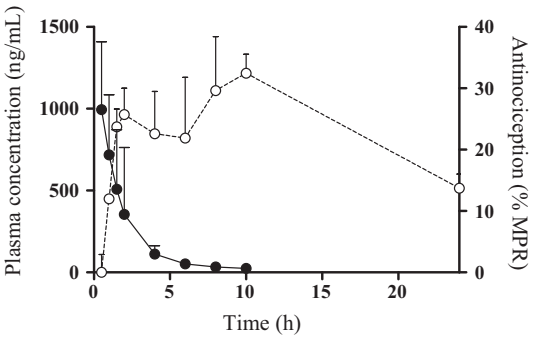
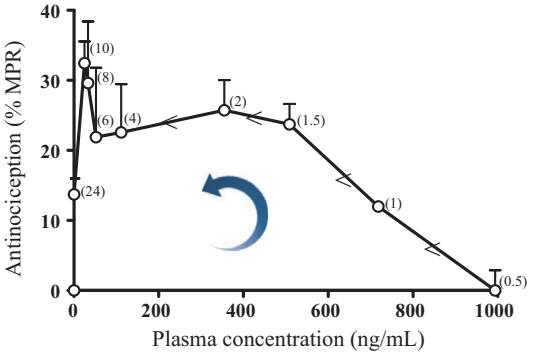


Fig. 4. Mean (±SD) experimental plasma concentrations (open circles) of grapiprant (left Y axis) and mean (±SD) % MPR (open squares) (right Y axis) vs. time curves in rabbits (n = 6) after IV grapiprant administration (2 mg/kg).

COLOR



15 **Fig. 5.** Mean (±SD) experimental plasma concentrations vs. mean (±SD) % MPR curve. Numbers in the brackets represent time of collection/reading and are expressed in hours.

from one species to another species is not appropriate (Giorgi, 2012).

Carrageenan-induced inflammation in the animal paw represents a classical model of oedema formation and hyperalgesia, which has been extensively used in the development of nonsteroidal anti-inflammatory drugs and selective COX1-2 inhibitors. Evidences suggest that the COX-2-mediated increase in prostaglandin (PG) E2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model. COX-2 is rapidly induced in the spinal cord and other regions of the CNS following carrageenan injection in the paw (Ichitani *et al.*, 1997). These features should also make this method useful for testing PG receptor antagonists such as grapiprant. Although these inflammation models most commonly use rats and mice, a recent study has validated the carrageenan-induced inflammation in the rabbit, showing that this animal species is also suitable for such experiments (Dong *et al.*, 2008).

There is great potential for use of grapiprant in veterinary species (Giorgi, 2015). Its PK profiles have been already published in dogs (Lebkowska-Wieruszewska *et al.*, 2016; Nagahisa & Okumura, 2016) and cats (Rausch-Derra & Rhodes, 2016), but no PK or PD profiles have been assessed in rabbits. Grapiprant is an novel active ingredient that might theoretically overcome a number of the disadvantages reported for classical NSAID and COX-2 selective inhibitors. Grapiprant targets the EP4 receptor and does not inhibit the production of prostanoids. As prostanoids are important in a variety of physiological functions, the adverse effects associated with the inhibition of the cycloxygenase enzymes such as renal, gastrointestinal and hepatic toxicity and coagulopathies are minimized. This drug has been shown to have a very safe and effective profile in dogs (Rausch-Derra *et al.*, 2016a,b) and cats (Rausch-Derra & Rhodes, 2016). However, lagomorphs may react to grapiprant differently to cats and dogs; hence, a PK/PD study in rabbits is essential to understand the effectiveness of this drug. 7

Several nociceptive tests have been established for use in laboratory animals, but only a few are available for use in rabbits. In the present study, the TWL was evaluated using a noxious heat radiant model with an automatic motion sensor device. This method is easy, fast and noninvasive compared with other methods, and rabbits can escape the stimuli immediately by moving their hindlimb. Due to these advantages, many nociceptive tests have been carried out by this method (Ren & Dubner, 1999). The TWL evaluated by Hargreaves's device has proven to be reproducible measure of complex nociceptive behaviour in rodents (Dirig *et al.*, 1997) as well as other veterinary species such as dogs (Kögel *et al.*, 2014), cats (Lascelles & Robertson, 2004), birds (Guzman *et al.*, 2014) and rabbits (Barter & Kwiatkowski, 2013). It has also been extensively used for pain assessment in reptiles (Sladky *et al.*, 2008, 2009; Fleming & Robertson, 2012). However, thermal (anti-)nociception may be different from clinical (anti-)nociception and from chronic pain. For this reason, clinical studies are warranted to assess if grapiprant may or may not be useful in clinical settings at the dose studied here.

After IV injection of 2 mg/kg grapiprant, plasma drug concentrations were detectable up to 10 h. This persistence was similar to those reported in dogs despite lower doses being used (Lebkowska-Wieruszewska *et al.*, 2016 [0.5 mg/kg]; Nagahisa & Okumura, 2016 [1 mg/kg]). Grapiprant is a drug intended for oral administration but IV administration was chosen because of the stress that oral gavage might have induced in the animals, and because no data on the oral bioavailability of grapiprant in rabbits are known thus far. The Vd value in this study was similar to that reported in dogs (Lebkowska-Wieruszewska *et al.*, 2016 [median 3763 mL/kg]), while the clearance value was twice those reported in canine species (Lebkowska-Wieruszewska *et al.*, 2016 [median 460 mL/h/kg]; Nagahisa & Okumura, 2016 [mean 348 mL/h/kg]). However, the extraction ratio was similar to that reported in dogs (7.7–8.6%, Lebkowska-Wieruszewska *et al.*, 2016) indicating that the overall ability of the rabbit to eliminate grapiprant is mainly driven by the cardiac output. The half-life value found in this study was shorter than those reported in dogs (Lebkowska-Wieruszewska *et al.*, 2016 [median 5.68 h]; Nagahisa & Okumura, 2016 [mean 4.2 h]) after IV administration.

In a previous clinical study, 2 mg/kg grapiprant (administered per OS once a day for 4 weeks) produced effective antinociception in dogs with natural osteoarthritis (Rausch-Derra *et al.*, 2016a,b). In the present study, grapiprant produced thermal antinociception from 1 h up to 10 h. This is in line with former studies using rat models to demonstrate garpiprant's ability to reduce acute and chronic pain and inflammation (Nakao *et al.*, 2007; RaQualia, 2007a,b). Grapiprant in the present study showed an onset time that was shorter than meloxicam (1 h vs 4 h). This might be due to the different injection routes used for the two drugs. The % of antinociception was not significantly different between the grapiprant and meloxicam groups in the period 4 to 8 h after drug administration. Another earlier study showed that grapiprant reduced paw swelling in rats to a similar degree to rofecoxib and piroxicam (RaQualia, 2007a). Other studies using different experimental selective EP4 antagonists have shown similar results (Clark *et al.*, 2008; Murase *et al.*, 2008). Grapiprant in the present study showed significantly more efficacy than meloxicam at 10 h after drug administration. This might be due to the different doses of drugs administered, to a wider counterclockwise hysteresis loop or to an active metabolite of grapiprant that might have prolonged the treatment effect. Also the different mechanisms of action of the two drugs might play a role in this effect.

Concerning the value of % MPR at 24 h shown in the grapiprant group, caution should be taken in interpreting these data. Indeed, 27 h after the carrageenan injection, the inflammation is likely to be physiologically resolved. This has been previously reported (Dong *et al.*, 2008) and is evident in the present data (Fig 2). Hence, the carrageenan-induced inflammation model probably does not produce hyperalgesia at that time and the % MPR value might not be valid (Barter & Kwiatkowski, 2013). Although this study used *in vivo* PK and *in vivo* PD endpoints to determine the hysteresis loop, the PK/PD correlation was not easy. The general assumption is that the drug in the surrogate biological matrix, such as plasma, and the drug at the biophase are at equilibrium (Campbell, 1990). However, this assumption may not be correct because the drug concentrations change as a result of the innate pharmacokinetics of the drug, and the pharmacodynamics could also change independently or in an opposite direction to the drug concentration. A variety of factors (distribution delay into the site of effect, slow receptor kinetics, delayed or modified pharmacological activity, the presence of active agonist metabolites and indirect physiological response) that might have affected the hysteresis shape have been previously reported in the literature (Louizos *et al.*, 2014). Further studies are warranted to clarify this issue.

CONCLUSION

Compared to its behaviour in dogs, grapiprant, when administered to rabbits, showed a number of similarities in pharmacokinetic parameters. The thermal antinociceptive effect occurred within 1 h and lasted up to 10 h. Grapiprant appears to be an attractive option for antinociception in rabbits, due to its rapid onset and long duration of effect. Grapiprant administered at 2 mg/kg IV has shown a maximal thermal antinociceptive effect not significantly different to 0.5 mg/kg SC meloxicam. Studies with more doses and routes need, however, to assess the dosage regime in rabbits. As the oral administration is the only formulation available on the market for grapiprant, the oral bioavailability should be considered along with a sound assessment of drug safety, identification and testing of likely active metabolite(s) and the tissue cage study to estimate the concentration–time curve at the site of inflammation, before its use in lagomorph clinical practice.

CONFLICT OF INTEREST

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the manuscript.

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