Plasma Concentrations of Tapentadol and Clinical Evaluations of a Combination of Tapentadol Plus Sevoflurane for Surgical Anaesthesia and Analgesia in Rabbits (*Oryctolagus cuniculus*) Undergoing Orchiectomy

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ABSTRACT

Pain is probably under-treated in animals, particularly in rabbits, due to a lack of familiarity with the species and limited information about analgesic dose, efficacy and safety. Tapentadol (TAP) is a novel opioid drug, with a proven efficacy and safety profile in humans, which could be useful as an analgesic in rabbits. In a clinical study, TAP was administered (5 mg/kg, IV) to seven male New Zealand White rabbits 5 min before anaesthetic induction with sevoflurane to perform orchiectomy. Monitoring of vital signs, including heart rate, electrocardiogram, respiratory rate, invasive blood pressure, oxygen saturation, righting reflex, palpebral reflex, jaw tone and tongue retraction, was performed throughout surgery. Pain was assessed for 8 h following surgery, using previously validated parameters, physiological assessments and behavioural assessments. Blood was also collected at regular intervals to assess the pharmacokinetic profile of the drug. TAP was rapidly distributed and eliminated in rabbits. Apnoea did not occurred in any subject. Following surgery, there were very few observable signs of pain in four rabbits and all resumed normal activities within a few hours. In conclusion, this is the first study about the clinical effects and potential utility of TAP as an adjunct drug for anaesthesia and analgesia in the rabbit. However, further studies are still needed before its use in the veterinary clinical practice.

Keywords: Tapentadol, Rabbit, Pain, Opioid, Sevoflurane.

INTRODUCTION

Rabbits are common household pets in the United States and many European countries. The population of pet rabbits is estimated at several million and this number is thought to be rapidly increasing (1). Veterinarians can now expect to see rabbits routinely and be required to perform both routine and increasingly complex procedures on this species (2). Paralleling this trend is the increasing need to ensure adequate and reliable analgesia. Pain is probably under-treated in small animals, such as rabbits (3, 4), due to a lack of familiarity with the species, limited information about analgesic dose, efficacy and safety, and the difficulties associated with assessing pain and efficacy of pain management in rabbits (5). However, rabbits are likely to experience pain following trauma, surgery or disease and it is ethically and morally desirable to provide analgesia.

Opioids are frequently used for management of moderate to severe pain. Classical opioid drugs produce analgesia by binding to mu and/or kappa opioid receptors, although stimulation of these same receptors can result in adverse effects, including sedation, respiratory depression and re-

duced gastrointestinal motility. Atypical opioid drugs, tramadol and tapentatol (TAP), have a lower affinity for the mu and/or kappa opioid receptors (thereby inducing less adverse effects) but their actions also inhibit serotonin and or norepinephrine reuptake, further contributing to the pain relief (6).

Anecdotally, tramadol has been used in the clinical management of pain in rabbits, but the only published study to date (7) showed that an oral dose (11 mg/kg) resulted in plasma concentrations of the parent drug and its active metabolite, *O*-desmethyltramadol, below levels considered analgesic in humans. The desired therapeutic plasma levels and the effective dose and dosing interval have not been established in rabbits.

TAP is a novel opioid drug with features similar to tramadol which was launched on the European market for human use at the end of 2011. Several clinical studies in humans have shown that TAP provides analgesia of equal magnitude to classical opioids, morphine and oxycodone, but with lower adverse effects (8, 9). TAP could potentially overcome some of the disadvantages associated with tramadol, since TAP exists as a single stereoisomer and the parent compound is solely responsible for its pharmacological activity. Accordingly, the relative contributions of the two mechanisms, mu opioid receptor (MOR) activation and noradrenalin reuptake inhibitor (NRI), do not vary during metabolic transformation. MOR agonism allows a reduction in spinal pain transmission as well as actions at supraspinal sites through descending projections that further reduce sensory transmission. The inhibition of noradrenalin reuptake enhances the descending inhibition of pain likely via alpha 2 adrenoceptors (10). Furthermore, TAP does not affect (induce or inhibit) cytochrome 450 (CYP450), a common metabolic pathway for opioid analgesics, while 5-hydroxytryptamine receptors (5HT) reuptake inhibition (which triggers adverse effects) is negligible (11). Due to a lack of serotonergic activity in tapentadol, pain facilitation via the descending transmission system is not enhanced, and the side effects caused by increased serotonin in the central and the enteric nervous systems (constipation, nausea, vomiting, and diarrhoea) are avoided (10). The aim of the present study was to assess the Phramacokinetic-Pharmacodynamic (PK-PD) profile of TAP intravenously administered to rabbits undergoing orchiectomy.

MATERIAL AND METHODS

Materials

Pure powder (>99.8% purity) of TAP hydrochloride was supplied by Bepharm (Shanghai, China). A pure solution of *O*-desmethyltramadol (M1), used as internal standard (IS), was purchased from LCG Promochem (Bologna, Italy). HPLC grade acetonitrile (ACN), dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), were purchased from Scharlau (Barcelona, Spain). Analytical grade acetic acid and sodium tetraborate decahydrate were obtained from BDH (Milan, Italy). HPLC grade water was obtained by distilling deionised water produced by a Milli-Q Millipore Water System (Billerica, MA, USA). All the other reagents and materials were of analytical grade and supplied from commercial sources.

Animals and experimental design

Seven male New Zealand White rabbits (Harlan, Udine, Italy) aged 12-16 months, with a mean bodyweight (BW) of 3.1 kg (2.8-3.5 kg), were used. The animals were housed in single cages in adjacent floor pens (1 m×1 m), under conventional conditions of ventilation, temperature (18-22°C) and lighting (12 h light/day). They were acclimatised for a 2-week period prior to commencement of the study. During this period the rabbits received food and water *ad libitum*. The rabbits were previously determined to be clinically healthy on physical examination, serum chemistry and haematological analyses. Animal care and handling was performed according to the provision of the EC Council Directive 86/609 EEC. The study was approved by the ethical committee for animal welfare of the University of Pisa (authorisation number 7920).

Rabbit surgical procedure

A 6-h period of pre-surgery fasting was applied. After clipping away hair, a local anaesthetic ointment (EMLA; AstraZeneca, Milan, Italy) was applied to both ears of each rabbit, in order to avoid discomfort associated with placement of two catheters. An IV polyurethane catheter (JelcoI.V. Catheter Radiopaque; Smiths Medical International Ltd, UK) was placed aseptically into the left external ear vein for the drug administration. The size of the catheter (22 G) was chosen to fit with the size of the vein of the animals. An extension ("T"-connector (Luer-lock)

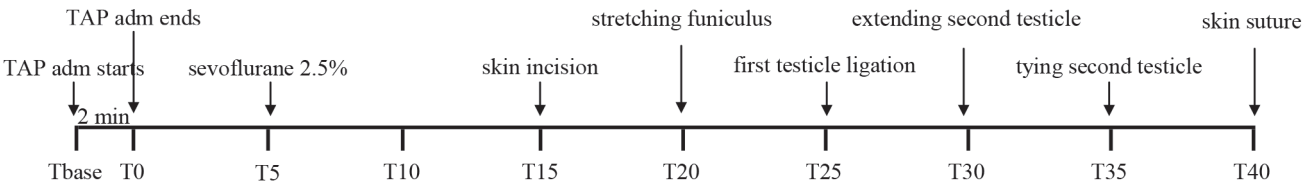


Figure 1: Timing of drug administrations and pain evaluation.

with Bionector; Vygon, UK) was connected to the catheter to allow IV drug administration without touching or interfering with the ear. The second catheter was inserted into the right median ear artery for the blood pressure measurement. As a control and to ensure patency before administration of TAP, the catheter was flushed with 2 mL saline 0.9% (Viaflo, sodium chloride 0.9% w/v; Baxter, UK). TAP was prepared by dissolving pure TAP hydrochloride powder into saline to give a 5 mg/mL solution, which was then passed through a 0.45 μm filter before being administered at a dose of 5 mg/kg (IV) over 2 min. The T0 min was taken at the end of drug administration (Figure 1). Five minutes later (T5 min), the rabbits were induced with sevoflurane (2.5% via face mask) and maintained at 3.5% (Sevorane, Abbott Laboratories, UK) using a calibrated 'out-of-circuit' vaporizer (Penlon Sigma Delta, Abingdon, UK) set at 2.5-3.5%. At T5 min physiological parameters were measured before sevoflurane administration. During anaesthesia, Ringer's lactate (10 mL/kg/h) was administered. The fluids were heated and the subjects were placed on a heating pad to avoid hypothermia.

Perioperative parameter evaluations

The following parameters were monitored during anaesthesia: heart rate (HR), electrocardiogram (ECG), invasive blood pressure (systolic arterial pressure (SAP)); diastolic arterial pressure (DAP); mean arterial pressure (MAP), oxygen saturation (SpO_2) with the probe positioned on the tongue, using a multiparametrical monitor (Mindray BeneView T5, China), respiratory rate (RR, via direct observation), righting and palpebral reflex (absent, weak, strong), jaw tone and tongue retraction. The above parameters were monitored at Tbase (prior T0) on a fully conscious patient prior to TAP administration, T0 and then every 5 min for the duration of the surgical procedure. Periods where the animals were exposed to surgical stimuli of a greater magnitude (T15 min: skin incision; T20 min: stretching funiculus; T25 min: first ligation testicle; T30 min: extending the second testicle; T35 min: tying the second testicle; T40 min: suturing skin) (Figure 1) were measured, recorded and studied.

At the end of the surgical procedure, sevoflurane was discontinued and the time until the return of righting reflex was recorded. Each rabbit was also administered enrofloxacin (Baytril, Bayer; Milan, Italy) at 10 mg/kg (SC) at the end of the procedure.

Analgesic rescue contingency plans included: increasing sevoflurane concentration for reaction to the stimulus on the skin (Backhaus positioning, skin incision) and an increase in sevoflurane concentration and/or infiltration of lidocaine into the spermatic cord for reaction to the visceral stimulus (stretch cord, tying testicles). However, Lidocaine was never used. Atropine, adrenaline, naloxone, intubation kit, and Ambu-bag were also available for other likely complications.

Post-surgical pain evaluation

After the return of righting reflex, rabbits were transferred to hospital cages and provided with food and water. Pain assessment was based on evaluation of ear position, face, abdomen, back and hind limbs (5). Briefly, the rabbits' body was divided into 5 regions; (i) face (face, head and neck, but excluding the ears); (ii) ears (ears only) evaluated according to the grimace scale (12); (iii) abdomen; (iv) back; and (v) hindquarters, evaluated for pain-indicating behaviours and posture (e.g. back arching, skin twitches, muscle contracting, unusual position, etc. (13)). In addition, behavioural assessments (including reduced activity, excessively slow/fast response to external stimuli, increased aggression), as previously reported, were performed (13, 14). These body and behavioural parameters, were scored separately from 0 to 10 according to the Numerical Rating Scale (NRS) of pain intensity (0 corresponds to the absence of pain and 10 maximum pain) in each subject for each observation time. Then all the scores were averaged

for each time point for each subject. The physiological assessments (such as food and water intake, urination and defecation) were considered as "yes" or "no" (first food or water consumption or faeces production) and did not affect the overall score but were considered important parameters to point out the rabbits' wellbeing. These assessments were carried out every hour for a total of 8 h (T2 h – T10 h) after administration of TAP. Each evaluation was 10 minutes long. These evaluations were always carried out using the same criteria and the same experienced observers, to reduce the bias.

Blood withdrawals for HPLC analysis

Blood samples (1 mL) were collected at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10 and 24, h after administration of TAP, and placed in collection tubes containing lithium heparin. The blood samples were centrifuged at 1,000 *g* within 30 min of collection, and the harvested plasma was stored at -20°C until use (within 15 days of collection).

High performance liquid chromatography (HPLC)

The concentrations of TAP in plasma were evaluated using HPLC with fluorescence detector (Jasco Europe, Modena, Italy), according to the method previously described by Giorgi et al. (15), with slight modifications. Briefly, the chromatographic separation assay was performed with a SunFire C18 analytical column (150×4.6 mm inner diameter, 5 µm particle size) (Waters, Milan, Italy) maintained at 25°C. The mobile phase consisted of ACN (A): 0.2% acetic acid (B) at a flow rate of 1 mL/min. Excitation and emission wavelengths were set at 273 and 298 nm, respectively. The linear gradient elution system was performed as follows; 5-95% A (0-20 min), 95-5% A (20-25 min) and finally 5% A isocratically (25-32 min).

Pharmacokinetic evaluation

The pharmacokinetic calculations were carried out using WinNonLin v 5.3 (Pharsight, Sunnyvale, CA, USA). Maximum concentration (Cmax) of TAP in plasma, and the time required to reach Cmax (T_{max}) were predicted from the data. The concentration at time 0 (C_0) was estimated by back-extrapolating from the first two concentration values. 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. 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 ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule.

Changes in plasma concentration of TAP were evaluated using the standard non-compartmental analysis, and the relative pharmacokinetic parameters were determined using standard non-compartmental equations (16).

Statistical analysis

Graph-Pad Prism 4.0 (La Jolla, CA, USA) performed the statistical analysis of the parameters (Graph-Pad Software). Data were analysed for the normal distribution using the Kolmogorov-Smirnov test. The analysis of variance (ANOVA), with Tukey's test as *post hoc*, was used to evaluate the likely differences between subjects. Additionally, ANOVA tests for repeated data with a test of Dunnett's was carried out to evaluate differences in clinical parameters at Tbase and other recorded times for each subject. Values $P < 0.05$ were considered significant.

RESULTS

Pharmacokinetic parameters

After TAP administration, concentrations were detectable in plasma for up to 4 h (Figure 2). In this period, the concentrations exceeded or were within the effective concentration range reported for humans (5-300 ng/mL). The clearance was rapid (2093 mL/h/Kg) and the volume of distribution was large (1522 mL/Kg). The mean pharmacokinetic values are reported in Table 1.

Pharmacodynamic parameters

The induction of anaesthesia was smooth and uncomplicated. At T0, all the subjects showed attenuation of righting and eyelid reflexes, tongue retraction and jaw tone becoming completely absent at T5 min.

The mean HR was almost constant in all subjects (Figure 3). Significant increases in this parameter were recorded in relation to surgical stimuli at T20 min and T30 min in all subjects except one. The sevoflurane concentration was increased to 4-4.5% and dropped down to 3.5% as soon the HR decreased. All rabbits showed polypnoea at Tbase and T0. The RR decreased significantly to T5 min and was constant from T10 min to T40 min (Figure 4). Only two subjects showed a slight increase in RR in relation to surgical

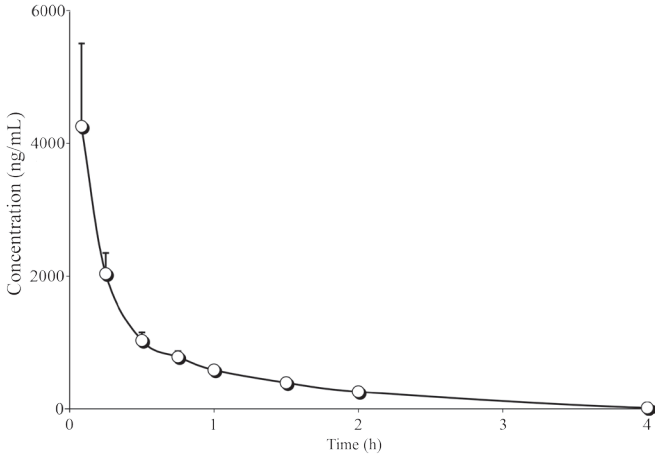


Figure 2: Plasma concentration vs. time mean curve of TAP, following single IV administration at 5 mg/kg in New Zealand rabbits (*n*=7). Bars represent the standard deviation.

Table 1: Main pharmacokinetics parameters of TAP following single IV administration at 5 mg/kg in New Zealand White rabbits (*n*=7).

Parameters	Mean	SD	CV%
R^2	0.990	0.009	0.9
λz (1/h)	1.44	0.38	26.6
$T_{1/2}$ λz (h)	0.52	0.17	31.8
C_{max} (ng/mL)	4257	1247	29.3
C0 (ng/mL)	6260	2737	43.7
$AUC_{0-\infty}$ (h ng/mL)	2446	411	16.8
VZ (mL/Kg)	1522	400	26.3
CL (mL/h/Kg)	2093	345	16.5
$AUMC_{0-\infty}$ (h h ng/mL)	1739	355	20.4
MRT (h)	0.71	0.11	15.6

R^2 = correlation coefficient; λz = terminal phase rate constant; $T_{1/2}$λz = terminal half-life; C_{max} = peak plasma concentration; C_0 = calculated concentration at T_0; $AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity; VZ = volume of distribution; CL = clearance; $AUMC_{0-\infty}$ = area under the first moment curve from zero to infinity; MRT = mean resident time.

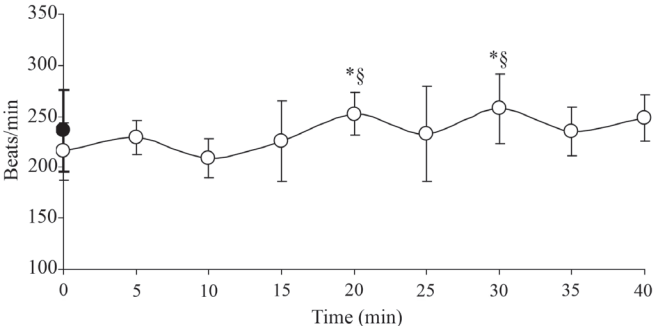


Figure 3: Mean beats/min vs. time curve (-○-) following single IV administration at 5 mg/kg in New Zealand rabbits (*n*=7). Mean Tbase value is indicated by ●. Bars represent the SD values. * Significantly different from T0; § significantly different from T5 ($P<0.05$).

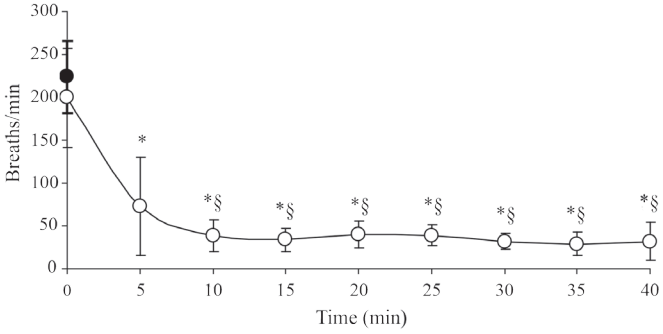
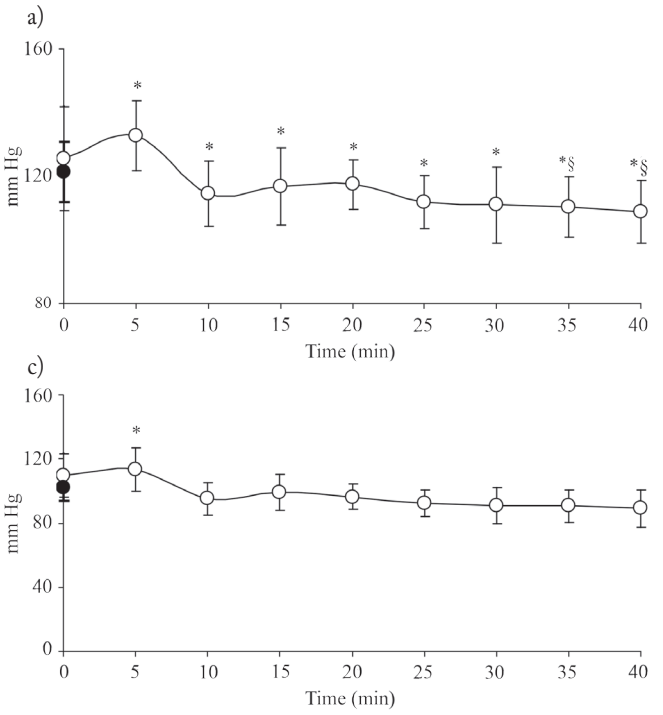


Figure 4: Mean respiratory rate vs. time curve (-○-) following single IV administration at 5 mg/kg in New Zealand rabbits (*n*=7). Mean Tbase value is indicated by ●. Bars represent the SD values. * Significantly different from T0; § significantly different from T5 ($P<0.05$).

stimuli. Apnoea was not recorded during the procedure. The percentage of SpO_2 was stable between 97% and 100% in all subjects throughout orchiectomy.

There was evidence of an increase in SAP between T0 and T5 min (Figure 5a). Significant reductions in this parameter were recorded for the whole surgical period. A continuous slight, but non-significant decrease in DAP was reported (Figure 5b). This parameter was constant during the surgical stimuli. MAP increased at T5 min in all subjects, except one (Figure 5c). However, this parameter did not change significantly in relation to surgical stimuli.

All the rabbits regained consciousness quickly and quietly; reappearance of the righting reflex was recorded between 5 and 15 min after discontinuation of sevoflurane, and sedation disappeared within 30 min. The assessment of post-surgical pain was carried out using the NRS (Table 2). Four rabbits were scored with the lightest value of pain after 2 h from the TAP administration. After the 3 h evaluation point, animals were assessed as not affected by pain. In line to these evaluations, most of the rabbits ate, drank, urinated and defecated at the first evaluation point (2 h), while all the animals did so by 4 h after TAP administration.



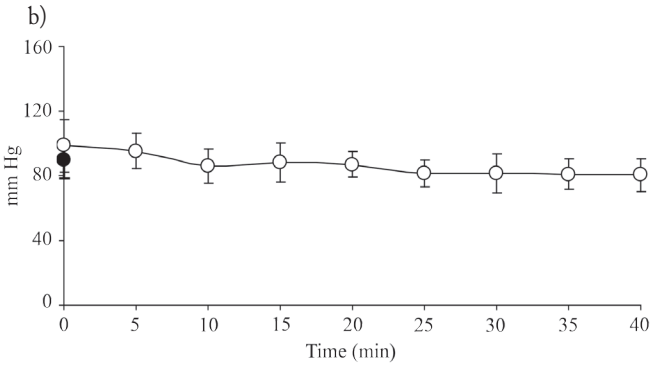


Figure 5: a) Mean systolic arterial pressure vs. time curve (-○-) following single IV administration at 5 mg/kg in New Zealand rabbits (*n*=7). Mean Tbase value is indicated by ●. Bars represent the SD values. * Significantly different from T0; § significantly different from T5 ($P<0.05$); b) Mean diastolic arterial pressure vs. time curve (-○-) following single IV administration at 5 mg/kg in New Zealand rabbits (*n*=7). Mean Tbase value is indicated by ●. Bars represent the SD values; c) Mean arterial pressure vs. time curve (-○-) following single IV administration at 5 mg/kg in New Zealand rabbits (*n*=7). Mean Tbase value is indicated by ●. Bars represent the SD values. * Significantly different from T0 ($P<0.05$).

Table 2: Individual pain assesment by NRS (number).

	Rabbits						
Time (h)	**A**	**B**	**C**	**D**	**E**	**F**	**G**
2	1[a]	1[a]	1[ab]	0	1	0[b]	0[ab]
3	0	0[b]	0	0[b]	0[b]	0	0
4	0[b]	0	0	0[a]	0[a]	0[a]	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0

[a]1st food consumption; [b]1st feaces production.

DISCUSSION

The pharmacokinetic analysis showed that TAP, when administered via an IV route in rabbits at the dose of 5 mg/kg, was rapidly and widely distributed to tissues with a distribution volume of 1.5 L/kg. This value was lower compared to that observed both in rats (10.4 L/kg) administered with similar dose (17) and dogs (9.0 L/kg) administered with half the dose (6). In contrast, the half-life ($T_{1/2}$ λz) was similar to the values reported in rats (17) and dogs (6). TAP showed rapid elimination with a clearance value seven times lower than in rats (17) and four times lower than in dogs (6, 17), which may reflect species differences in metabolism. This is not a pure PK study because TAP was administered with sevofluorane but, although some parameters could be slightly modified, it is unlikely that sevofluorane co-administration may extensively affect the PK of the opioid (18).

In humans, the minimum effective concentration (MEC) range is between 5-300 ng/mL (Prof. Rolf Terlinden, personal communication, 2007). A following study reported as MEC, a more accurate value of 0.67 mM/L, equivalent to 148 ng/mL (19). The extrapolation of the MEC value from human to rabbits is not ideal and caution should be used. In the present study, rabbits were also administered with sevoflurane that could have affected the effectiveness of TAP. However, the behavioural and physiological assessments showed that TAP was effective in providing post surgical analgesia. The mean plasma concentration of TAP after 2 h (first evaluation time of post surgical pain) was 260 ng/mL and 16 ng/mL at 4 h (which was also the limit of detection for analysis in plasma (10 ng/mL)). These low concentration values might suggest the rabbit is an animal species more susceptible to TAP (as already reported for other opioids (20)) than humans, although further pure PK/PD studies in rabbits are required to confirm this.

The reason why orchiectomy was chosen as the surgical procedure in order to assess the efficacy of TAP as an analgesic, was because castration is the most frequent surgical procedure in the rabbit in our Veterinary school. TAP

provided good intra-surgery analgesia. However, to assess properly the pain relief produced by TAP a control rabbit group should have been used, but was not possible for ethical reasons. To avoid this concern, an historical comparison with the institution's standard anaesthetic protocol in rabbits (ketamine (15 mg/kg) and medetomidine (0.5 mg/kg) IM) for routine surgery (21), such as orchiectomy, was performed. The clinical records of 19 rabbits over the last 2 years showed that the combination of TAP and sevoflurane resulted in a very rapid and smooth recovery (15-30 min), in contrast to ketamine/medetomidine (86-120 min (22)). If the effects of medetomidine was reversed by the specific antagonist, righting reflex returned within 5 min, but sedation lasted for over 1 h (23). The righting reflex ranged within 5 and 15 min after the sevoflurane discontinuation in the present study, but sedation also disappeared rapidly (within 30 min).

The increase in HR was not accompanied by an increase in MAP during the surgical procedure. Typically, following a pain stimulus, an early increase in MAP followed by an increase of the RR and HR is expected. Hence, the observed variation in HR may be related to an unstable or light anaesthesia regimen. Moreover, the stretching of the gonads can cause sympathetic stimulation, which accounts for the increase in HR (24). A combination of the present anaesthetic protocol with a sedative drug (e.g. alpha-2-agonist), could evoke a deeper anaesthesia, although further studies to support this hypothesis are required. The increase in MAP at T5 min could be explained by the mechanism of action of the drug. TAP is an inhibitor of norepinephrine reuptake and might stimulate a sympathetic response (25).

Respiratory depression and apnoea are the most common complications encountered with classical more potent opioids in humans (26) and animals (24). In the present study apnoea did not occurred in any subject. This was very significant for rabbits since they are anatomically difficult to intubate and frequently exhibit trauma, laryngeal spasm and tracheal injury following intubation (27). Given the moderate affinity of TAP at the mu opioid receptor and the opioid-sparing effect of TAP's norepinephrine reuptake inhibitor component, it seems logical that TAP would produce fewer opioid-related side effects than classical MOR agonists, such as morphine. In contrast, classical opioid drugs, in addition to limited efficacy, induce hypotension, respiratory depression and prolonged recovery time in rabbits (24).

There does not appear to be a validated pain measurement scale based on objective parameters published for the rabbit. As such, assessment of pain in rabbits is therefore more subjective than in other domestic species where validated pain scales are currently used. Recent studies (5,13) reported an insensitivity to pain assessment in rabbits when facial responses only were evaluated, while responses in areas such as the ears, abdomen, back and hind limbs were considered more significant. In the present study, pain assessment was based on evaluation of areas described by Leach *et al*. (5,13), in addition to physiological assessments (such as food and water intake, urination and defecation) and behavioural assessments (such as reduced activity, slow/fast response to external stimuli, increased aggression), as previously reported by Flecknell (14). Food and water intake, considered with urine and stool production, did not affect the NRS but were considered physiological changes that assist pain assessment in the rabbit and would not take place if the subjects were experiencing pain (28,29).

At T2 h, four subjects showed the slightest sign of pain (body's and behavioural) and they resumed normal activities (food intake, urination, defecation) within a few hours. These observations suggested that TAP provided sufficient post-surgery analgesia in each subject. It should be noted that sevoflurane has no specific analgesic effect, which is therefore only provided by TAP. Moreover, the institution's routine anaesthetic protocol for rabbits (ketamine-demetomidine) usually required additional analgesia (multimodal approach) during the postoperative recovery. This is also in agreement with other previous evaluations (21).

CONCLUSIONS

This is the first PK/PD study of TAP in rabbits. TAP proved an effective analgesic agent in this species on the basis of the parameters used as pain markers in the present study. TAP elicited a good analgesic plane that resulted in a better post-operative course with a more rapid recovery of patients, compared to the institution's standard anaesthetic protocol for rabbits (ketamine-medetomidine). No complications occurred, particularly apnoea or reduced blood pressure. Further studies are required before TAP can be used routinely in veterinary clinical practice, but these preliminary findings can pave the way for its use as a useful analgesic in rabbits.

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REFERENCES

1. Anonymous: American Veterinary Medical Association. U.S. Pet Ownership & Demographics Sourcebook (2007 Edition). Available at: http://www.avma.org/reference/marketstats/sourcebook.asp. Accessed May 30, 2012.
2. Barter, L.S.: Rabbit analgesia. Vet. Clin. North Am. Exotic. Anim. Pract. 14:93-104, 2011.
3. Capner, C.A., Lascelles, B.D. and Waterman-Pearson, A.E.: Current British veterinary attitudes to perioperative analgesia for dogs. Vet. Rec. 145:95-99, 1999.
4. Lascelles, B.D., Capner, C.A. and Waterman-Pearson, A.E.: Current British attitudes to perioperative analgesia for cats and small mammals. Vet. Rec. 145:601-604, 1999.
5. Leach, M.C., Coulter, C.A., Richardson, C.A. and Flecknell, P.A.: Are we looking in the wrong place? Implications for behavioural-based pain assessment in rabbits. Plos One 6:3, 2011.
6. Giorgi, M., Meizler, A. and Mills, P.C.: Pharmacokinetics of the novel atypical opioid tapentadol after oral and intravenous administration in dogs. Vet. J. 194:309-313, 2012.
7. Souza, M.J., Greenacre, C.B. and Cox, S.K.: Pharmacokinetics of orally administered tramadol in domestic rabbits (*Oryctolagus cuniculus*). Am. J. Vet. Res. 69:979–982, 2008.
8. Kleinert, R., Lange, C., Steup, A., Black, P., Goldberg, J. and Desjardins, P.: Single dose analgesic efficacy of tapentadol in post-surgical dental pain: the results of a randomized, double-blind, placebo-controlled study. Anaest. Analg. 107:2048-2055, 2008.
9. Etropolski, M., Kelly, K., Okamoto, A. and Rauschkolb, C.: Comparable efficacy and superior gastrointestinal tolerability (nausea, vomiting, constipation) of tapentadol compared with oxycodone hydrochloride. Adv. Ther. 28:401-417, 2011.
10. Kress, H.G.: Tapentadol and its two mechanisms of action: Is there a new pharmacological class of centrally-acting analgesics on the horizon? Eur. J. Pain 14:781-783, 2010.
11. Giorgi, M.: Tramadol vs Tapentadol: a new horizon in pain treatment? Am. J. Anim. Vet. Sci. 7:7-14, 2012.
12. Keating, S.C.J., Thomas, A.A., Flecknell, P.A. and Leach, M.C.: Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses. PLoS ONE 7:e44437, 2012.
13. Leach, M.C., Allweiler, S., Richardson, C.A., Roughan, J.V., Narbe, R. and Flecknell, P.A.: Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory housed rabbits. Res. Vet. Sci. 87:336-347, 2009.
14. Flecknell, P.A.: Advances in the assessment and alleviation of pain in laboratory and domestic animals laboratory and domestic animals. J. Vet. Anaest. 21:98-105, 1994.
15. Giorgi, M., Meizler, A. and Mills, P.C.: Quantification of tapentadol in canine plasma by HPLC with spectrofluorimetric detection: Development and validation of a new methodology. J. Pharm. Biomed. Anal. 67-68:148-153, 2012.
16. Gabrielsson, J. and Weiner, D.: Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, Swedish Pharmaceutical Press, Stockholm, 2002.
17. Tzschentke, T.M., De Vry, J., Terlinden, R., Hennies, H.H., Lange, C., Strassburger, W., Haurand, M., Kolb, J., Schneider, J., Buschmann, H., Finkam, M., Jahnel, U. and Friderichs, E.: Tapentadol hydrochloride: Analgesic mu-opioid receptor agonist noradrenaline reuptake inhibitor. Drugs Future. 31:1053-1061, 2006.
18. Payne, K.A., Roelofse, J.A. and Shipton, E.A.: Pharmacokinetics of oral tramadol drops for postoperative pain relief in children aged 4 to 7 years--a pilot study. Anesth. Progr. 49:109-112, 2002.
19. Tzschentke, T.M., Christoph, T., Kogel, B., Schiene, K., Hennies, H.H., Englberger, W., Haurand, M., Jahnel, U., Cremers, T.I., Friderichs, E. and De Vry, J.: (-)-(1R,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride (Tapentadol HCl): a novel mu-opioid receptor agonist/norepinephrine reuptake inhibitor with broadspectrum analgesic properties. J. Pharmacol. Exp. Therap. 323:265-276, 2007.
20. Rivier, J. and Papich, M.G.: Opioid analgesic drug. In: Riviere J.E. and Papich, M.G. (Eds): Veterinary Pharmacology and Therapeutics. Whiley Blackwell, Ames. pp. 301-336, 2009.
21. Orr, H.E., Roughan, J.V. and Flecknell, P.A.: Assessment of ketamine and medetomidine anaesthesia in the domestic rabbit. Vet. Anaest. Analg. 32:271-279, 2005.
22. Hedenqvist, P., Roughan, J.V., Antunes, L., Orr, H. and Flecknell, P.A.: Induction of anaesthesia with desflurane and isoflurane in the rabbit. Lab. Anim. 35:172-179, 2001.
23. Allweiler, S., Leach, M.C. and Flecknell, P.A.: The use of propofol and sevoflurane for surgical anaesthesia in New Zealand White rabbits. Lab. Anim. 44:113-117, 2010.
24. Weaver, L.A., Blaze, C.A., Linder, D.E., Andrutis, K.A. and Karas, A.Z.: A model for clinical evaluation of perioperative analgesia in rabbits. J. Am. Assoc. Lab. Anim. Sci. 49:845-851, 2010.
25. Tzschentke, T.M., Jahnel, U., Kögel, B., Christoph, T., Englberger, W., De Vry, J., Schiene, K., Okamoto, A., Upmalis, D., Weber, H., Lange, C., Stegmann, J.U. and Kleinert, R.: Tapentadol hydrochloride: A next-generation, centrally acting analgesic with two mechanisms of action in a single molecule. Drugs Today. 45:483-496, 2009.
26. Koo, C.Y. and Eikermann, M.: Respiratory effects of opioids in perioperative medicine. Open Anesthesiol. J. 5:S23-34, 2011.
27. Bateman, L., Ludders, J.W., Gleed, R.D. and Erb, H.N.: Comparison between facemask and laryngeal mask airway in rabbits during isofluorane anaesthesia. Vet. Anaest. Analg. 32:280-288, 2005.
28. Kohn, D.F., Martin, T.E., Foley, P.L., Morris, T.H., Swindle, M.M., Vogler, G.A. and Wixon, S.K.: Guidelines for the assessment and management of pain in rodents and rabbits. www.aclam.org/Content/files/files/Public/Active/position_pain-rodent-rabbit.pdf accessed March 20, 2013.
29. Stasiak, K.L., Maul, D., French, E., Hellyer, P.W. and VandeWoude, S.: Species-specific assessment of pain in laboratory animals. J. Am. Assoc. Lab. Anim. Sci. 42:13-20, 2003.