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Pharmacokinetics, intraoperative effect and postoperative analgesia of tramadol in cats

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ABSTRACT

Tramadol is a synthetic codeine analogue used as an analgesic in human and veterinary medicine, but not approved for use in cats. Tramadol (2 mg/kg) was administered intravenously (IV) as preoperative analgesic in 12 cats (6 males) undergoing surgical gonadectomy. The pharmacokinetic profile of the drug and its *O*-desmethyl metabolite were determined in 8 animals (4 males), while intraoperative effects and postoperative analgesia, estimated by subjective pain score (0–24), were evaluated in all. Mean intraoperative isoflurane consumption was reduced, but hypoventilation was not observed. Sex-related differences were not observed, particularly in terms of postoperative analgesia: rescue analgesic was never administered. Concentrations of the active *O*-desmethyl metabolite were persistently high in all the animals. Considering the results obtained in this study, tramadol, at the dose of 2 mg/kg IV, did not produce any evident intraoperative cardiorespiratory side effects and with additional investigation may prove to be an appropriate intraoperative analgesic in cats undergoing gonadectomy.

1. Introduction

Tramadol is a synthetic codeine analogue that acts centrally as an analgesic. It was registered in 1977 in Germany, in 1994 in the UK, in 1995 in the USA (Grond and Sablotzki, 2004) and recently authorized for use in dogs in Europe (for example in Italy as Altadol, Formevet). The drug is supplied as a racemic mixture of the hydrochloride salts which are more effective than either enantiomer alone (Scott and Perry, 2000). The analgesic effects of tramadol result from complex interactions with opiate receptors and activation of descending inhibitory pathways (Scott and Perry, 2000). The stimulation of periaqueductal grey matter, nucleus raphe magnus, nucleus reticularis gigantocellularis and locus coeruleus inhibits norepinephrine and serotonin reuptake by neurons projecting from these nuclei to the spinal cord (Mayer, 1984); as a result, the activity of these neurons is increased, and pain transmission is modulated at the level of the dorsal horn. Tramadol inhibits neuronal norepinephrine and serotonin reuptake thereby increasing the activity of these pathways (Kayser et al., 1992; Raffa et al., 1992). Tramadol has no demonstrated affinity for δ or κ opioid receptors (Raffa et al., 1992) and its affinity for μ opioid receptors is about 10 times lower than that of codeine and about 6000 times lower than that of morphine (Grond and Sablotzki, 2004). However, the *O*-demethylated metabolite (*O*-desmethyl tramadol or M1) has a 300-fold greater affinity for μ opioid receptors than tramadol and thus contributes significantly to the analgesic effect of tramadol (Hennies et al., 1988; Frink et al., 1996; Gillen et al., 2000). In humans, tramadol is mainly metabolized to glucuronides and sulphates via *O*- and *N*-demethylation and conjugation reactions. Both tramadol and M1 are mainly excreted via the kidney (Scott and Perry, 2000). Hepatic demethylation to M1 occurs at different rates in dogs, goats, cats and horses (Kukanich and Papich, 2004; Vettorato et al., 2006; Zonca et al., 2006; Giorgi et al., 2007; Shilo et al., 2008; De Sousa et al., 2008; Pypendop and Ilkiw, 2008; Vettorato et al., 2010). As far as we are aware, limited information are available on how tramadol is metabolised in the cat. Previous studies (Cagnardi et al., 2006; Pypendop and Ilkiw, 2008) showed a high persistence of M1 and this seems to be related to cats' poor ability to glucuronidate compounds. Moreover the metabolite was present at levels over 0.01 μg/ml, i.e. above the lowest concentration associated with therapeutic efficacy in humans (Lehmann et al., 1990).

In humans, the analgesic effect of tramadol following parenteral administration is about 10% that of morphine (Grond and Sablotzki, 2004). Although the analgesic properties of tramadol

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have been investigated in laboratory conditions in various animals such as rat (Guneli et al., 2007; Kamerman et al., 2007), horse (Natalini and Robinson, 2000) and cat (Steagall et al., 2008; Pyendop et al., 2009; Castro et al., 2009), only few clinical trials have been published. The analgesic efficacy of tramadol has been studied in dogs after intravenous (IV) and extradural administration (Mastrocinque and Fantoni, 2003; Vettorato et al., 2010), and in cats after subcutaneous (SC) administration (Brondani et al., 2006, 2009a,b).

The present study reports (a) the pharmacokinetic profile of tramadol and its M1 metabolite in cats after IV administration at 2 mg/kg prior to surgical gonadectomy, and (b) a clinical evaluation of the efficacy of tramadol as postoperative analgesic. The aim of the study was to generate data for the rational dosing of this substance in cats.

2. Materials and methods

2.1. Animals

The study was performed on 12 healthy domestic shorthair cats, age 0.5–1.5 years, 6 males and 6 females, weighing between 2.5 kg and 4.3 kg, undergoing gonadectomy at the Department of the Clinical Veterinary Sciences, University of Milan. All animals were judged healthy (ASA status I) on the basis of physical examination and results of routine blood tests, and were enrolled in the study after written consent from their owners, as required by Italian law (D.L. 116/1992). Subsequently, the protocol was approved by the Ethical Committee of the University of Milan.

2.2. Anaesthetic and surgical procedures

All animals received atropine sulphate (0.03 mg/kg) and acepromazine maleate (0.05 mg/kg) intramuscularly (IM), as pre-anaesthetic medications. Anaesthesia was induced with isoflurane in oxygen (100%) using an anaesthetic chamber. After intubation anaesthesia was maintained with the same gases, delivered by a non-rebreathing system (Mapleson C). Tramadol (2 mg/kg) was administered IV as a bolus over 15 s through a cephalic catheter (22 gauge) 5 min after intubation and 20 min prior to beginning surgery. During surgery lactated Ringer's solution was administered at 5 ml/kg/h through the same catheter. Female cats underwent ovariectomy and the males underwent orchiectomy according to standard surgical procedures. During surgery, heart rate, electrocardiogram (lead II), respiration rate, oxyhaemoglobin saturation, end tidal carbon dioxide (CO_2), mean non-invasive arterial blood pressure, and end tidal isoflurane concentration were recorded every 5 min using a UT4000F Pro monitor (Goldway Inc.).

After extubation subjective pain scores were assessed by a trained observer using a method modified after Smith et al. (2004). The method involves assessment of behavioural indicators of pain (comfort, movement, appearance, unprovoked behaviour, interactive behaviour and vocalization) assigning a score of 0–4 for each. Thus a score of 24 indicates maximum pain and a score of zero no pain. Pain was assessed every 30 min up to 6 h. Buprenorphine (10 μg/kg IM) was administered if the pain score was 9 or above.

2.3. Collection, purification and analysis of serum samples

For 8 animals (4 males and 4 females), venous blood samples (2 ml) were collected into non-heparinized tubes from a jugular vein catheter: before tramadol administration (time 0) and 5, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 h after tramadol administration. The samples were centrifuged (1500*g*, 10 min at room temperature) soon after collection and the serum stored at −80 °C pending assay.

Serum samples were purified by solid phase extraction on Isolute SPE C2 (100 mg/ml) cartridges (International Sorbent Technology Ltd., UK) previously activated with 2 ml of methanol followed by 2 ml of 0.05 M sodium chloride. Five hundred μl of 0.05 M sodium hydrogen phosphate dodecahydrate solution was added to 500 μl of serum and briefly vortexed. The sample was then loaded onto the cartridge followed by washing with 2 ml of 0.05 M sodium chloride. The compounds were eluted with 1 ml of methanol. The eluate was evaporated to dryness under nitrogen at 45 °C and the residue dissolved in 100 μl of mobile phase.

Residues were analysed for tramadol and M1 by HPLC. The apparatus included a binary pump, auto sampler, Peltier column oven (all Perkin–Elmer Series 200, Italy) at 20 °C, and a fluorescence detector (Perkin–Elmer LC240, Italy) with excitation and emission wavelengths 200 nm and 301 nm, respectively. The column was an ODS Hypersil C18 250 × 4.6 mm 5 μm column with Hypersil 5 μm 4.6 mm pre-column (Supelco, Italy). The mobile phase was 15 mM aqueous sodium hydrogen phosphate dodecahydrate with 45 mM triethylamine pH 3 and acetonitrile (82:18, v:v). Flow rate was 1.0 ml/min and injection volume was 50 μl.

Solutions for the calibration curve were prepared diluting stock solutions of tramadol and M1 (1 mg/ml) to obtain concentrations in the range 0.05–10 μg/ml in blank cat serum.

HPLC retention times were 11.5 min for tramadol and 5.4 min for M1. The HPLC method was validated in our laboratory and found to be specific, linear (in the range 0.05–10 μg/ml) precise (CV 2.05–7.4% for tramadol and 3.8–9.6% for M1) and accurate (−13% to +0.1% for tramadol and −0.02% to +2.5% for M1), with limit of quantification 0.05 μg/ml and limit of detection 0.0008 μg/ml for both compounds investigated. The mean recoveries for tramadol and M1 were 98.6 ± 6.86% and 92.9 ± 4.6%.

Serum binding of tramadol and M1 in the range 0.5–1 μg/ml was determined in vitro. The serum-bound molecules were removed by ultrafiltration (Villa et al., 1994, 1997) using a disposable device (Amicon, Millipore, Italy) and free substances in the filtrate were analyzed by HPLC as described above.

Tramadol hydrochloride was kind gift from Formevet; M1 was purchased from Sigma. Other reagents and solvents were purchased from J.T. Baker (Italy).

2.4. Pharmacokinetic analysis

Pharmacokinetic parameters were deduced from serum concentration–time data using the WinNonLin 5.2.1 software (Pharsight Corporation, USA) which allows compartmental and non-compartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al., 1978) were used to choose the model that best fitted the data. All data points were weighted by the inverse square of the fitted value. Serum concentrations after IV tramadol administration were fitted to a standard bi-exponential curve (Gibaldi and Perrier, 1982) describing a two-compartment model with elimination from the central compartment.

Parameters estimated from the model were used to calculate pharmacokinetic variables for each animal. The volume of distribution in the central compartment (V_c) was calculated as:

$$V_c = Dose/C_0 \quad (1)$$

where *Dose* is dose of tramadol and C_0 is the extrapolated serum concentration of tramadol at time 0. The kinetics of M1 was determined by non-compartmental analysis. Mean residence time (MRT), body clearance (ClB) and volume of distribution at steady state (V_{dss}) were determined from the following equations (Gibaldi and Perrier 1982):