PHARMACOKINETIC OF BUPRENORPHINE AFTER AN INTRAVENOUS ADMINISTRATION IN DOGS ♦

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ABSTRACT

The purpose of this study was to evaluate the plasma concentrations and pharmacokinetic parameters of buprenorphine in dogs after intravenous administration. An intravenous bolus of 0.02 mg/kg of buprenorphine was administered to six healthy Beagle dogs. Blood samples were collected through a jugular catheter before and at 1, 5, 10, 15, 20, 30 and 45 min, and 1, 2, 4, 6, 8 and 12 h after administration of the drug. Plasma buprenorphine concentrations were measured using a commercial radioimmunoassay. The plasma concentrations of buprenorphine decreased following a three-exponential curve. The two distribution and the elimination half-lives were 3.1 ± 4.1 min, 20.2 ± 8.3 min, and 269.6 ± 199.3 min, respectively; the clearance was 365.6 ± 77.8 mL/min; and volume of distribution was 86112.8 ± 62346.1 mL.

Keywords:

Buprenorphine, dogs, pharmacokinetics, intravenous, radioimmunoassay,

INTRODUCTION

During the last decade, the effective management of pain in animals has undergone a remarkable advance in veterinary medicine and may also increase in the near future. Currently, there are many analgesic drugs commercially available which modes of actionare different . Opiates have been widely used to control both acute and chronic pain, and are used as agents to relief moderate or severe pain.

Buprenorphine is one of the opioid drugs most commonly used in the UK (Lascelles et al., 1999) and in Australia (Watson et al., 1996). It has high analgesic potency as compared with morphine and is an effective analgesic for the management of moderate to severe pain.

Buprenorphine is a potent, semisynthetic opioid with mixed agonist/antagonist properties- a partial agonist at μ-opioid receptors and an antagonist at the kappa-receptor. It is a highly lipophilic opiate derived from thebaine (Budd, 2002). In dogs and cats, the dose of buprenorphine varies from 0.01 to 0.02 mg/kg (Thurmon et al., 1996). When administered intramuscularly or intravenously provide postoperative analgesia for 6 to 8 hours (Brodbelt et al., 1997; Taylor et al., 1984). Its long action and unusual receptor kinetics, lead to particular interest in kinetics.

The analysis of buprenorphine in biological samples has been extensively described in the literature. Different methods based on liquid chromatography (LC), gas chromatography (GC) and mass spectrometric (MS) detection have been applied for the analysis of buprenorphine in plasma or serum, faeces, urine or hair (Garret and Chandran, 1990; Ho et al., 1991; Ohtani et al., 1995; Taylor and Robertson, 2001; Gopal et al., 2002; Ceccato et al., 2003; Robertson et al., 2005; Yassen et al., 2005). The clinical dose of buprenorphine in animals is quite low and, as a result, the plasma

concentrations of buprenorphine and norbuprenorphine are in the sub-ng/ mL level. Due to the low concentration level, the development of methods to analyse burprenorphine and metabolites in biological fluids is a challenging task for analysts (Ceccato et al., 2003).

Some studies described the pharmacokinetics of buprenorphine in dogs after an IV bolus by LC. These studies were performed using doses within a supra therapeutical range (between 0.7-2.6 mg/kg) (Garret and Chandran, 1990). To the author's knowledge, a part of these studies at high doses, pharmacokinetics of buprenorphine has never been reported using therapeutical doses in dogs.

Plasma buprenorphine concentrations were measured in cats after a clinical IM or IV doses (Taylor et al., 2001; Robertson et al., 2003) using radioimmunoassay (RIA), showing adequate sensitivity and reproducibility. Yassen et al., (2005) applied an LC/MS/MS method to determine plasma concentrations of buprenorphine in rats in pharmacokinetic/pharmacodynamic experiments.

The objective of the present study was to characterize the pharmacokinetics of buprenorphine after single IV clinical doses in dogs.

MATERIALS AND METHODS

Animals

All procedures were performed under the authorisation of the Ethical Commission of Animal and Human Experimentation (Spanish Government, Authorisation Number DARP 2981) and under the control of the Ethical Commission of Autonomous University of Barcelona.

Six Beagle dogs between 3 and 4 years of age weighting between 12 and 15 kg were included in the study. Health status was established based on clinical examination, haematological and biochemical analyses.

Drugs

The buprenorphine formulation used for the animal treatment was Buprenorphine hydrochloride (Buprex, Schering-Plough). The drugs administered during the anaesthetic procedure were: Propofol 4 mg/kg (Propofol- Lipuro, B.Braun), Isofluorane (Forane, Abbott), heparin (Heparina Rovi 5%, Rovi) and 0.9% physiologic saline (Fisiologico Braun, B. Braun).

Pure standard buprenorphine chlorhydrate (99% purity) was supplied by Laboratories Esteve.

Study Design

On the day before each study, the dogs were anaesthetised with a single bolus of propofol (4 mg/kg) administered through a catheter placed in the cephalic vein (Vasocan, B. Braun). The animals were intubated and anaesthesia was maintained with isofluorane (vaporizer setting 2%) in oxygen (150 mL/kg/min) in a Bain coaxial

breathing system. A 20G polyurethane central venous catheter (Certofix Mono, B.Braun) was inserted into the left jugular vein and secured with suture material and an elastic bandage. When the dogs had regained their reflexes, they were returned to their box for a minimum of 24 h. During this period, the venous catheters were flushed with heparinised saline (0.9% saline with 5U heparine/mL) to avoid occlusion.

On the day of the study, an intravenous dose of 0.02 mg/kg of buprenorphine was administered to each dog through the cephalic vein. Blood samples were taken through the jugular catheter before and at 1, 5, 10, 15, 20, 30 and 45 min, and 1, 2, 4, 6, 8 and 12 h after drug administration. The volume of blood collected was 2.0 mL per sample so that less than 10% of the dog's total blood volume was drawn. An equal volume of normal saline was injected after each sample was withdrawn. The blood samples were transferred to lithium heparin tubes (Tapval, Aquisel) and centrifuged at 2600*g* for 10 minutes. Plasma was separated and stored at -22º C until analysis. Food but not water was withheld during all the study period.

Plasma concentrations of buprenorphine

The plasma concentrations of buprenorphine were measured with a ^{125}I-labelled RIA (Buprenorphine double antibody RIA kit; Diagnostic Products Corporation). The RIA, commercially developed for human urine samples, was used for dog plasma samples. Calibration (0, 0.5, 1, 2 and 5 ng/mL of buprenorphine) and control (1 ng/mL of buprenorphine) samples were prepared using blank dog plasma loaded with the adequate volume of a methanolic solution of buprenorphine to obtain the desired concentrations. The protocol of analysis recommended by the manufacturer for urine samples was applied to plasma samples.

The mathematical model and transformations suggested by the manufacturers were used for fitting the signal with the concentration of analyte. Regression analysis was applied to the logit transformation of the signal (B/Bo %, percentage of corrected counts per minute) versus logarithmic buprenorphine concentration. As a measure of the goodness of fit, the error (%) in the back-calculated concentration of the calibration samples was monitored.

Samples with buprenorphine concentrations higher than 5 ng/mL were diluted 1/10 using blank dog plasma and reanalyzed.

Up to five replicates of one control sample were analysed for the determination of intra-assay precision and accuracy, while the inter-assay precision and accuracy were determined using the concentration values of the control sample obtained along three independent experimental assays. Precision was expressed as the relative standard deviation (RSD%) of the measurements performed, and accuracy was expressed as the relative error (%) of the value obtained with respect to the assigned value for the control sample.

To calculate the limit of quantification, a blank calibration sample (absence of analyte) was analysed five times in the same run. The standard deviation of the values obtained was taken as the measure of the noise. Limit of quantification was defined as the mean value obtained for the blank sample less ten times the estimated value of the noise (due to the decrement sign of the slope of the calibration curve).

Pharmacokinetic analysis

Compartimental pharmacokinetic analysis of the concentration-time data was carried out using WinNonlin Software Package (version 4.1). Ordinary least square criteria was chosen for fitting procedures to minimize the differences between the observed and predicted concentrations. The goodness of fit was checked by diagnostic plots and analysis of residuals. F-test, Akaike information criteria and Schwartz criteria were used for discrimination between models.

The pharmacokinetic parameters describing the equation were calculated for each dog. The following parameters were calculated: the area under the concentration-time curve (AUC^{∞}_{0}), the systemic clearance, the distribution and elimination half-lives, and the distribution volume.

RESULTS

The results of the validation study of the RIA assay for dog plasma samples are shown in Table 1. The errors between the assigned concentration of the calibration samples and the re-calculated values obtained with the equations were always lower than 10%, showing a good fitting of the calibration curve. As can be seen in Table 1, the intra- and inter-assay precisions and accuracies were lower than 15%. The limit of quantification was estimated in 0.04 ng/ mL.

The concentration-time plot for buprenorphine in plasma after an IV bolus is shown in Fig. 1. Buprenorphine was detected in plasma for 12 h after administration in 4 dogs, for 6 h in one animal, and for 4 h in the last dog. The plasma concentrations were fitted to a three-compartment model. A weighing function of the reciprocal of concentration was used. As can be seen, there is a first phase where the buprenorphine plasma concentration falls rapidly, followed by two additional slower phases of elimination. The equation describing the plasma concentration behaviour is $C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$, where A, B and C are the intercepts, and α, β and γ are the first-order rate constants for three compartments. The mean values for all those coefficients are listed in Table 2. The calculated pharmacokinetic parameters for buprenorphine are also shown in Table 2.

No adverse effects were observed after the administration of buprenorphine to any of the dogs.

DISCUSSION

Buprenorphine is a partial agonist better than the other opioids available for use in animals. Most relevant advantages of buprenorphie are that it is long acting (therefore it does not need to be administered at short intervals), does not tend to induce vomiting and its cardiovascular adverse effects are negligible in healthy animals (Thurmon et al., 1996; Budd, 2002; Elkader and Sproule, 2005). For these reasons buprenorphine is one of the opioid drugs most commonly used in the UK (Taylor et al., 2001; Robertson et al., 2003). Its use has been described in both, small and large animals but few data is available about its pharmacokinetic profile after the administration of a clinical dose.

In the present study, a jugular catheter was used in order to avoid several traumatic blood extractions to animals. For catheter placement, administration of anaesthetic was needed. Short-action anaesthetics with fast metabolism, such as propofol and isofluorane, were used in order to obtain minimal effects in the animal (Thurmon et al., 1996). Opioid analgesics were not used in order to avoid analytical interferences during buprenorphine determinations.

Several studies have been performed to determine plasma buprenorphine concentrations in humans and in animals. In dogs Garret and Chandran (1990) determined the pharmacokinetics after an intravenous bolus of buprenorphine. In that study the dose used was higher than the clinical dose described for dogs (Thurmon et al., 1996) (0.7- 2.6 mg/kg vs. 0.01-0.02 mg/kg). Moreover in their study the analysis of plasma buprenorphine concentrations was performed using LC and the determination of some important pharmacokinetic parameters was not feasible due to the poor detection limit (Garret and Chandran, 1990).

In the present study, a dose within the clinical range described in dogs was used (0.02 mg/kg) (Taylor and Houlton, 1984; Thurmon et al., 1996). The plasma collections times were selected according to the results of a study undertaken by Robertson et al., (2003) for cats, with slight modifications.

The validation results demonstrated the feasibility of the RIA assay to analyze buprenorphine in dog plasma samples. Intra and inter-assay accuracy and precision obtained were adequate, and the limit of quantification for this RIA assay allowed the detection of very low concentrations of buprenorphine.

No side-effects were observed in any of the dogs after IV administration of buprenorphine in agreement with previously published data (Thurmon et al., 1996; Martinez et al., 1997).

After IV administration, buprenorphine plasma concentrations decreased with a three-phase kinetic profile. These findings correspond to the physicochemical characteristics of buprenorphine, and have been described previously for cats (Taylor et al., 2001; Robertson et al., 2003), rabbits (Ho et al., 1991), humans (Ho et al., 1991) and rats (Gopal et al., 2002; Ohtani et al., 1995; Yassen et al., 2005). Due to the differences in the doses and in the animal species the comparison of data between different studies is difficult.

The pharmacokinetic data obtained in dogs are similar to those described in people, rabbits and cats. During the initial phase, buprenorphine concentrations showed a fast decrease, as indicated by the low $t_{1/2}$ α observed in our study (3.12 min). The value of $t_{1/2}$ α obtained in dogs is similar to those described in human and rabbit studies (Ho et al., 1991). After the fast initial decrease, buprenorphine concentrations remained in a

low concentration range with a long $t_{1/2}$ γ, showing a slow elimination rate from the peripheral tissue. The elimination half-lives for the slow elimination phase were similar to those obtained in rabbits and humans (Ho et al., 1991).

In our study buprenorphine concentrations remained in a low range from one hour after its IV administration. The same pharmacokinetic profile has been previously described in humans and in other animals (Ho et al., 1991; Gopal et al., 2002; Ohtani et al., 1995; Taylor et al., 2001; Robertson et al., 2003).

The low plasma concentration range obtained after 1h of IV administration of buprenorphine seems to be not concordant with published data regarding the clinical effect. The peak effect of buprenorphine does not occur until about 45-50 min after IV administration. In dogs, Taylor and Houlton (1984) described duration of the clinical effect of 4 h whereas some other authors described longer duration of action (McKelvey and Hollingshead, 2000; Gaynor and Muir, 2002). Pharmacokinetics, however, do not always predict the clinical duration of effect. The duration of action is longer than one would predict from concentrations in plasma. The concentration in the compartment where the effect takes place do not have the same time course as plasma concentrations. The prolonged duration of effect of buprenorphine (6-8 h) is caused by a high affinity for the μ-receptor and a slow dissociation. An evaluation of the duration of the clinical effect of buprenorphine was not performed in our study. However, as the dose used was within the clinical range for dogs (0.01-0.02 mg/kg), it is expected that the clinical effects would remain for at least the same time as described in the literature.

In summary, after IV administration of clinical doses of buprenorphine in dogs, buprenorphine showed a three-compartmental model plasma kinetics with two distribution and one elimination phase, as described in humans and in other animals.

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TABLES

Table 1: Intra and interassay precisions and accuracies for buprenorphine in dog plasma obtained by RIA assay.

Intra-assay						Inter-assay				
Assay	N	Mean (ng/mL)	SD (ng/mL)	Precision* RSD (%)	Accuracy** Error (%)	n	Mean (ng/mL)	SD (ng/mL)	Precision† RSD (%)	Accuracy** Error (%)
1	5	0.98	0.06	6.0	4.6					
2	5	0.99	0.05	5.5	4.3	15	0.98	0.09	8.8	6.8
3	5	0.97	0.14	14.3	11.4					

* Measured as relative standard deviation (RSD).

** Measured as the relative error respect the assigned control sample value.

Table 2: Pharmacokinetic parameters for buprenorphine after an IV bolus to six dogs.

Parameter	Units	mean	sd
A	**(ng/mL)**	25.9	18.3
B	**(ng/mL)**	9.1	2.6
C	**(ng/mL)**	1.8	1.2
a	**(L/min)**	0.5	0.3
b	**(L/min)**	0.04	0.02
g	**(L/min)**	0.004	0.003
t1/2(a)	**(min)**	3.1	4.6
t1/2(b)	**(min)**	20.2	8.3
t1/2(g)	**(min)**	269.6	199.3
t1/2	**(min)**	16.6	5.8
AUC	**(ng.min/mL)**	775.4	196.0
Cmax	**(ng/mL)**	36.8	18.27
Clearance	**(mL/min)**	365.6	77.8
Vd	**(mL)**	86112.8	62346.1

FIGURE LEGENDS

Fig 1: Plasma concentration-time profile for buprenorphine (mean ± sd) in six dogs after an IV bolus of buprenorphine.

FIGURES