PRELIMINARY STUDY: PLASMA BUPRENORPHINE CONCENTRATIONS AFTER THE APPLICATION OF A 70 µg/h TRANSDERMAL PATCH IN DOGS ♦

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RUNNING TITLE: TRANSDERMAL ABSORPTION OF BUPRENORPHINE IN DOGS

ABSTRACT

Objective: The objective of the present study was to evaluate the plasma concentrations of buprenorphine after transdermal application in dogs.

Animals: Four healthy, intact male Beagles.

Material and methods: On the day of the study, a 70 µg/h trandermal buprenorphine patch (Transtec, Grünenthal) was applied to the ventral abdomen of each Beagle. Blood samples were collected through a preplaced jugular catheter before and at 1, 2, 4, 8, 12, 24, 36, 48 and every 6h until 108 h after the patch application. Plasma concentrations of buprenorphine were measured using a ^{125}I-labelled radioimmunoassay (RIA) assay.

Results: No adverse effects were observed in any of the dogs. Buprenorphine plasma concentrations increased during the first 36 h and then remained in the 0.7- 1.0 ng/mL range during the interval of time studied. The concentrations obtained in our study were similar to those obtained in human patients after the application of the 70 µg/h patch. A decrease in plasma buprenorphine concentration was not observed during the time studied. **Conclusion and Clinical relevance**: Concentrations of buprenorphine were detected in plasma after the application of a transdermal buprenorphine pach on the four experimental animals. On the basis of the results of the present study, transdermal buprenorphine could be a useful alternative to other available opioids in many postoperative pain conditions. However, in addition to the data obtained in the present study, clinical studies are needed to evaluate the role of transdermal buprenorphine among current treatment strategies for pain management in dogs.

Keywords: Buprenorphine, dogs, pharmacokinetics, transdermal, patch, radioimmunoassay (RIA).

INTRODUCTION

Buprenorphine transdermal delivery system is widely used in human medicine for the treatment of moderate to severe cancer-associated pain and severe pain unresponsive to non-opioid analgesics (Evans and Easthope, 2003; Sorge and Sittl, 2004). Transdermal delivery of opioid analgesics offers an attractive means of maintaining analgesia for extended periods of time in veterinary patients and avoids most of the drawbacks of parenteral or oral chronic administrations. If effective, transdermal administration of analgesics should be ideal for veterinary patients because it allows ambulatory treatments, avoids frequent dosing, and minimizes some of the side effects observed with periodic administration (Egger et al., 1998; Pascoe, 2000; Magnusson et al., 2001; Riviere and Papich, 2001). Nowadays transdermal fentanyl patch systems are widely used in veterinary patients. They have been shown to be useful for the pain management in dogs (McKelvey and Hollingshead, 2000). However, fentanyl is not the preferred opioid in some patients due to adverse effects such as respiratory depression, sedation, euphoria and hypothermia (Egger et al., 1998). At present, transdermal buprenorphine patch systems are available in human medicine. 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 with a molecular weight of 467.64 (Budd, 2002). It is long acting and its cardiovascular adverse effects are not clinically important in healthy animals.

To the author's knowledge, no studies have been performed to evaluate the absorption of buprenorphine by the transdermal route in dogs. The purpose of this study was to evaluate the plasma buprenorphine concentrations after the transdermal administration in healthy dogs.

MATERIAL AND METHODS

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. Four Beagles between 3 and 4 years of age and weighting 13.35± 2.8 kg were used in the study. On the day before the study, the dogs were anaesthetised with a single bolus of propofol (4 mg/kg Propofol-Lipuro; B.Braun, Rubí, Barcelona, Spain) administered through a catheter placed in the cephalic vein (Vasocan; B. Braun Rubí, Barcelona, Spain). The animals were intubated and anaesthesia was maintained with isofluorane (Isoflo, Laboratories Esteve, Barcelona, Spain), 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, Rubí, Barcelona, Spain) was inserted into the left jugular vein and secured. Once the dogs regained their reflexes, they were returned to their boxes for a minimum of 24 h. During this period, the venous catheters were flushed with heparinised saline (0.9% saline with 5 U heparine/mL) every 8h to avoid catheter occlusion. On the day of the study, a 70 µg/h trandermal buprenorphine patch (Transtec; Grünenthal, Madrid, Spain) was applied to the ventral abdomen of each dog. The area for patch application was prepared by clipping, gently washing with warm water, and air drying. After patch application, a light wrap of comfortable bandage was applied (Askina; B.Braun, Rubí, Barcelona, Spain). Blood samples were collected before and at 1, 2, 4, 8, 12, 24, 36, 48 and, then every 6h, until 108 h after the patch application. For one of the dogs samples were only collected until 48 h after patch application. The volume of blood was 2.0 mL per sample so less than 10 per cent of the dog's

118 total blood volume was removed through all the study. An equal volume of normal saline
119 was injected after each sample was withdrawn. Blood samples were collected in lithium
120 heparin tubes (Tapval; Aquisel, Abrera, Barcelona, Spain) and immediately centrifuged at
121 2600*g* for 10 min. Plasma was separated and stored at -20º C until analysis.
122
123 **Plasma concentrations of buprenorphine**
124 The plasma concentrations of buprenorphine were measured with a ^{125}I-labelled RIA assay
125 (Buprenorphine double antibody RIA kit; Diagnostic Products Corporation, Los Angeles,
126 CA, California, USA). The RIA, commercially developed for human urine samples, was used
127 for dog plasma samples. Calibration (0, 0.5, 1, 2 and 5 ng/mL of buprenorphine) and control
128 (1 ng/mL of buprenorphine) samples were prepared using blank dog plasma loaded with the
129 adequate volume of a methanolic solution of buprenorphine to obtain the desired
130 concentrations. The protocol of analysis recommended by the manufacturer for urine samples
131 was applied to plasma samples.
132 The mathematical model and transformations suggested by the manufacturers were used for
133 fitting the signal with the concentration of analyte. Regression analysis was applied to the
134 logit transformation of the signal (B/Bo %, percentage of corrected counts per minute)
135 versus logarithmic buprenorphine concentration. As a measure of the goodness of fit, the
136 error (%) in the back-calculated concentration of the calibration samples was monitored.
137 Samples with buprenorphine concentrations higher than 5 ng/mL were diluted 1/10 using
138 blank dog plasma and reanalyzed.
139 Up to five replicates of one control sample were analysed for the determination of intra-assay
140 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).

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 transdermal buprenorphine for each dog is shown in Fig. 1. As can be seen plasma buprenorphine concentrations remained low range in one dog whereas in the other three animals plasma concentrations remained around similar values. Buprenorphine plasma concentrations increased during the first 36 h and then remained around the 0.7- 1.0 ng/mL range during the interval of time studied. A decrease in plasma buprenorphine concentration was not observed during the time studied.

No adverse effects were observed in any of the dogs.

DISCUSSION

Most relevant advantages of buprenorphine include its long action (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). Nowadays, buprenorphine transdermal delivery system is available in human medicine for the treatment of moderate to severe cancer-associated pain and severe pain unresponsive to non-opioid analgesics (Evans and Easthope, 2003; Sorge and Sittl, 2004). Nevertheless, these patches had not been tested in animals.

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.

In our study, buprenorphine plasma concentrations increased during the first 36 h and then remained around the 0.7- 1.0 ng/mL range during the interval of time studied. The low concentrations detected in one of the animals may be due to individual variability as well as movement or accidental detachment of the patch.

A decrease in plasma buprenorphine concentration was not observed during the time studied, in contrast with results obtained in human subjects. This result could indicate that buprenorphine patch in dogs could provide clinical effect for more than 108h.

The concentrations obtained in our study were similar to those obtained in human patients after the application of the 70 µg/h patch (Terlinden and Stadler, 2000). In that study,

plasma concentrations of buprenorphine gradually increased after the application of a 70 µg/h patch and after 11 h the minimum effective concentration (MEC) of 100 pg/mL was reached in humans. Plateau levels of about 624 pg/mL were detected between 36 and 60 h (Terlinden and Stadler, 2000; Evans and Easthope, 2003). Although the MEC of buprenorphine for dogs has not been described, previous clinical studies have shown that an intravenous dose of 0.02 mg/ kg provides a clinical analgesia for 6-8 h, with a peak affect around 45-60 min after the intravenous administration (Pascoe, 2000). During this time period buprenorphine plasma concentrations were between 0.26-2.36 ng/mL (non published studies).

On the basis of the results of the present study, transdermal buprenorphine could be a useful alternative to other available opioids in many postoperative pain conditions. However, in addition to the data obtained in the present study, clinical studies are needed to evaluate the role of transdermal buprenorphine among current treatment strategies for pain management in dogs.

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FIGURE LEGENDS

Fig. 1: Plasma buprenorphine after the application of a 70 µg/h transdermal patch.

TABLE LEGENDS

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

TABLES

Table 1:

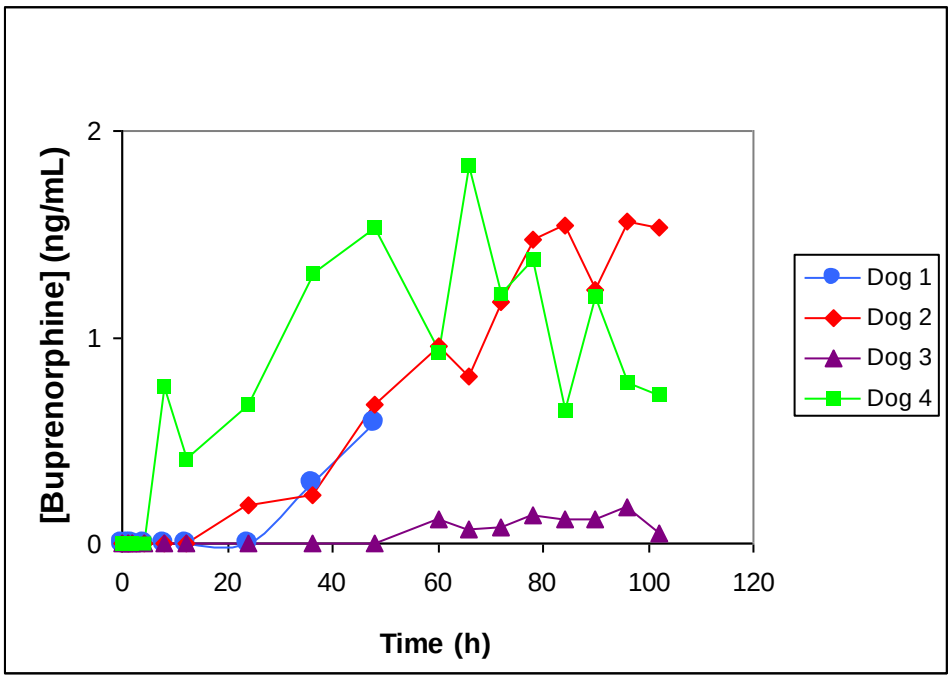
* Measured as relative standard deviation (RSD).

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

		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					

FIGURES

Fig. 1.


[Buprenorphine] (ng/mL)
2
1
0
0
20
40
60
80
100
120
Time (h)
Dog 1
Dog 2
Dog 3
Dog 4