

**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 transdermal 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 ¹²⁵I-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 patch 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.

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95 MATERIAL AND METHODS

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97 All procedures were performed under the authorisation of the Ethical Commission of
98 Animal and Human Experimentation (Spanish Government, Authorisation Number DARP
99 2981) and under the control of the Ethical Commission of Autonomous University of
100 Barcelona. Four Beagles between 3 and 4 years of age and weighting 13.35 ± 2.8 kg were
101 used in the study. On the day before the study, the dogs were anaesthetised with a single
102 bolus of propofol (4 mg/kg Propofol-Lipuro; B.Braun, Rubí, Barcelona, Spain) administered
103 through a catheter placed in the cephalic vein (Vasocan; B. Braun Rubí, Barcelona, Spain).
104 The animals were intubated and anaesthesia was maintained with isoflurane (Isoflo,
105 Laboratories Esteve, Barcelona, Spain), vaporizer setting 2% in oxygen (150 mL/kg/min) in
106 a Bain coaxial breathing system. A 20G polyurethane central venous catheter (Certofix
107 Mono; B.Braun, Rubí, Barcelona, Spain) was inserted into the left jugular vein and secured.
108 Once the dogs regained their reflexes, they were returned to their boxes for a minimum of 24
109 h. During this period, the venous catheters were flushed with heparinised saline (0.9% saline
110 with 5 U heparine/mL) every 8h to avoid catheter occlusion. On the day of the study, a 70
111 $\mu\text{g/h}$ transdermal buprenorphine patch (Transtec; Grünenthal, Madrid, Spain) was applied to
112 the ventral abdomen of each dog. The area for patch application was prepared by clipping,
113 gently washing with warm water, and air drying. After patch application, a light wrap of
114 comfortable bandage was applied (Askina; B.Braun, Rubí, Barcelona, Spain). Blood samples
115 were collected before and at 1, 2, 4, 8, 12, 24, 36, 48 and, then every 6h, until 108 h after the
116 patch application. For one of the dogs samples were only collected until 48 h after patch
117 application. The volume of blood was 2.0 mL per sample so less than 10 per cent of the dog's

total blood volume was removed through all the study. An equal volume of normal saline was injected after each sample was withdrawn. Blood samples were collected in lithium heparin tubes (Tapval; Aquisel, Abrera, Barcelona, Spain) and immediately centrifuged at 2600g for 10 min. Plasma was separated and stored at -20° C until analysis.

Plasma concentrations of buprenorphine

The plasma concentrations of buprenorphine were measured with a ¹²⁵I-labelled RIA assay (Buprenorphine double antibody RIA kit; Diagnostic Products Corporation, Los Angeles, CA, California, USA). 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).

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,

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

197 On the basis of the results of the present study, transdermal buprenorphine could be
198 a useful alternative to other available opioids in many postoperative pain conditions.
199 However, in addition to the data obtained in the present study, clinical studies are needed to
200 evaluate the role of transdermal buprenorphine among current treatment strategies for pain
201 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.

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275 **TABLES**

276 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	15	0.98	0.09	8.8	6.8
2	5	0.99	0.05	5.5	4.3					
3	5	0.97	0.14	14.3	11.4					

FIGURES

Fig. 1.

