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Research Paper

Evaluation of the Clearance of a Sublingual Buprenorphine Spray in the Beagle Dog Using Gamma Scintigraphy

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Purpose. The aim of this study was to evaluate clearance from the buccal cavity and pharmacokinetic profiles of a sublingual spray formulation in the dog, to assist in interpretation of future pharmacokinetic studies.
Methods. Radiolabelled buprenorphine in a spray formulation (400 μg/100 μl in 30% ethanol) was administered sublingually to four beagle dogs, and the residence in the oral cavity was determined using gamma scintigraphy. Pharmacokinetic sampling was performed to facilitate correlation of location of dose with significant pharmacokinetic events.
Results. Scintigraphic imaging revealed that clearance of the formulation from the oral cavity was rapid, with a mean $T_{50\%}$ clearance of 0.86±0.46 min, and $T_{80\%}$ clearance of 2.75±1.52 min. In comparison, absorption of buprenorphine was relatively slow, with a T_{max} of 0.56±0.13 h. Good buccal absorption despite short residence time can be explained by lipophilicity of buprenorphine enabling rapid sequestration into the oral mucosa, prior to diffusion and absorption directly into systemic circulation.
Conclusion. This study demonstrated rapid clearance of a sublingual solution from the canine oral cavity, with $T_{50\%}$ similar to results previously reported in man, providing initial confidence in using a conscious dog model to achieve representative residence times for a sublingual solution.

KEY WORDS: buccal; buprenorphine; gamma scintigraphy; sublingual.

INTRODUCTION

Drug delivery by the sublingual route has been reported in the literature for a variety of different applications (1–3), as the buccal and sublingual mucosae are known to be permeable to a number of compounds, although sometimes the aid of permeation enhancers is required (4,5). This permeability is a result of the non-keratinised nature of these areas of the oral mucosa, and has led researchers to investigate the route as a means to deliver larger molecules such as leuprolide (2), which are either unabsorbed orally or are destroyed by the harsh environment of the gastrointestinal tract. For such drugs the sublingual route is an attractive alternative to injection, and the patient acceptability of this route is demonstrated by the range of preparations currently available commercially (4).

The sublingual mucosa is also highly vascularised (6), meaning that when a drug is absorbed sublingually it will rapidly enter the systemic circulation, providing there is no retention and distribution into the mucosal tissues. As a result, the sublingual route is considered to be useful for administration of therapeutic agents for which a rapid onset of action is required, such as fentanyl citrate for breakthrough pain (7), and thiocolchicoside for analgesia and muscle relaxation (3). However, the speed of onset of a sublingual dose may also be influenced by the distribution of the compound within the tissues following absorption, as the literature reports a range of T_{max} values of between 4.2 and 110 min for various drugs administered sublingually in humans (8–10).

The high degree of vascular perfusion in this area combined with direct access to systemic circulation also makes the sublingual route desirable as a means of delivering compounds which exhibit low oral bioavailability due to the effect of first pass hepatic metabolism. One such compound, which is susceptible to this effect is the highly lipid soluble partial μ-opioid agonist buprenorphine (11,12). In contrast to the low oral bioavailability of around 15% (13,14), studies on sublingual formulations have shown bioavailability of 55% and 51.4% for liquid and tablet formulations, respectively (14,15). Sublingual buprenorphine is currently available in the UK in tablet form (Temgesic® and Subutex®) for treatment of pain relief and opioid dependence, respectively. As a compound with well documented sublingual absorption

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ABBREVIATIONS: $AUC_{(0–8)}$, area under the plasma concentration curve from 0 to 8 h; $AUC_{(0–\infty)}$, area under the plasma concentration curve from 0 h extrapolated to infinity; C_{max}, maximum plasma concentration; GI, gastrointestinal; MBq, megabequerels; ROI, region of interest; T_{max}, time to reach maximum plasma concentration; $T_{1/2}$, plasma half life; ^{99m}Tc-DTPA, technetium-99*m*-diethylenetriaminepentaacetic acid.

(14–16), buprenorphine is ideal for use in a pharmacoscintigraphic sublingual study.

In the investigation of potential oral mucosal formulations, *in vitro* methods of estimating permeability and drug diffusion such as Franz cells or Ussing chambers are available (17), however there are inevitable limitations to *in vitro* methods in predicting *in vivo* exposure, such as loss of enzymatic activity, difficulties associated with obtaining sufficient quantities of tissue, the potential for damage or alteration of the test tissue, and surface area effects, which often makes the use of a suitable animal model the first *in vivo* step towards developing a formulation for human use. The dog has been used for the evaluation of new formulations for human use (18) as the oral mucosa is reported as being similar to that of man (4). However, one major difference when carrying out sublingual studies in human volunteers is that it is possible to ask the subject to retain the dose in the mouth for as long as possible without swallowing, maximising absorption from the sublingual area. This is evidently not feasible with a conscious dog, and it therefore remains undefined with such studies exactly how long the administered dose remains in the buccal cavity of the dog. This may present a problem when assessing a compound which is not only absorbed sublingually, but is also absorbed from lower down the gastrointestinal (GI) tract, as it may be very difficult without the use of a charcoal block to distinguish from the pharmacokinetic profiles which section of the absorption phase observed is due to sublingual absorption, and which can be explained by other GI absorption. This is of obvious consequence when attempting to define the sublingual bioavailability of a new formulation intended to be administered sublingually. Previous studies have reported the use of anaesthesia on dogs in an attempt to overcome the problem of swallowing (2), however the use of anaesthesia is generally best avoided where possible to reduce risk to the animal and potential interference with absorption and distribution kinetics, and the period reported in this example (45–60 min) is longer than any human subject would retain a formulation without swallowing (although a briefer period of sedation would be possible), introducing the potential for overestimating bioavailability. It has also been reported that the pH of canine saliva is around pH 9 (19), compared to pH 6–7 in humans, which depending on the pKa of the compound may affect the percentage ionised/unionised drug and therefore extent of absorption.

Gamma scintigraphy is a long established technique for non-invasive monitoring of dosages *in vivo* (20), and its use in the dog has been previously reported for gastrointestinal formulation studies (21–23). The use of pharmacoscintigraphy to evaluate the clearance of a buprenorphine sublingual spray dose was therefore investigated, with the intention of obtaining information, which could aid in interpretation of pharmacokinetic data obtained from such sublingual studies. In addition, to compare and validate if the dog would be a useful sublingual absorption model in relation to man, it was essential to understand the typical residence time for a solution formulation. It is unlikely the conscious sublingual dog model would be useful for more solid dosage formulations due to inability to hold these in the mouth.

MATERIALS AND METHODS

Materials

Buprenorphine hydrochloride was obtained from Sigma (Poole, UK). Ethanol was obtained from VWR (Lutterworth, UK), and Technetium-99*m*-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) was obtained from the West of Scotland Radionuclide Dispensary, Glasgow, UK. PF-446,687 was obtained from Pfizer Global Research and Development (Sandwich, UK). The Pfeiffer spray device was obtained from Pfeiffer (Pfeiffer GmbH, Radolfzell, Germany) and lithium-heparin monovette tubes and luer adaptors were from Sarstedt (UK).

Methods

Preparation of Buprenorphine Sublingual Spray

Radiolabelled buprenorphine sublingual spray was prepared by dissolving an appropriate weight of buprenorphine in 30% *v/v* ethanol/70% *v/v* water to give a concentration of 4 mg/ml, accounting for the addition of a small volume of ^{99m} technetium on each study occasion to give an activity of approximately 5 MBq at the time of dosing. A volume of 130 μl of this solution was then added to each spray device, and the device sealed prior to administration. The spray dosing device delivered a volume of 100 μl, resulting in a buprenorphine dose of 400 μg per spray.

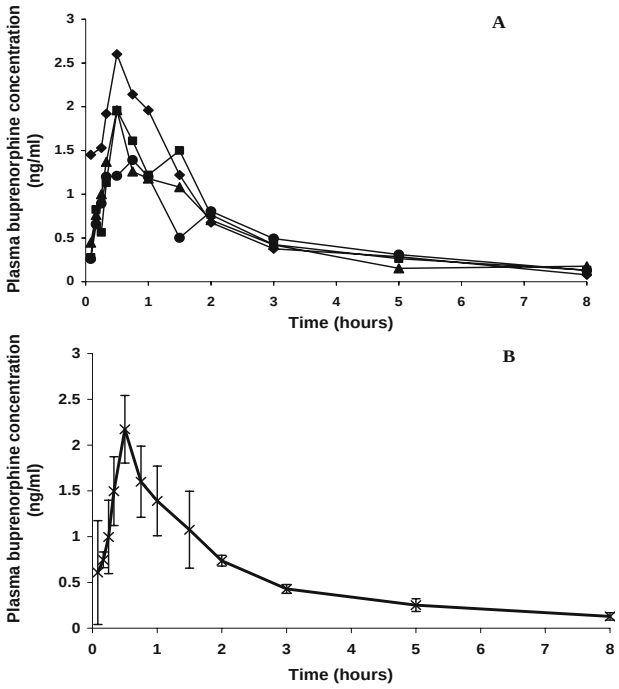

Fig. 1. Individual (**A**) and mean (**B**) plasma profiles following sublingual administration of 0.4 mg buprenorphine in 30% ethanol. Subjects 1 (*filled diamonds*), 2 (*filled squares*), 3 (*filled triangles*), 4 (*filled circles*), and mean (×).

Table I. Pharmacokinetic Parameters Following Sublingual Administration of Buprenorphine 0.4 mg in 30% Ethanol

Subject	Weight (kg)	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	$T_{1/2}$ (h)	$AUC_{(0-8)}$ (ng h/ml)	$AUC_{(0-8)}$ (ng h/ml)	Cl_{SL} (ml/min/kg)	Estimated bioavailability (%)
1	11.8	0.034	0.50	2.29	2.06	4.36	4.58	124	21.0
2	12.5	0.032	0.50	1.84	3.00	4.10	4.64	115	22.6
3	14.0	0.029	0.50	1.96	2.07	3.87	4.19	115	22.6
4	13.5	0.030	0.75	1.41	2.55	3.96	4.45	112	23.2
Mean	13.0	0.031	0.56	1.87	2.42	4.07	4.46	116	22.4
SD	1.0	0.002	0.13	0.37	0.45	0.21	0.20	5.1	0.94

Apparent plasma clearance following sublingual administration, Cl_{SL}, is calculated from dose/$AUC_{(0-\infty)}$.
Sublingual bioavailability can be estimated from $F = \frac{Cl}{Cl_{SL}}$ where Cl is the plasma clearance following IV administration, which is assumed to be 26 ml/min/kg for buprenorphine.

In vivo Study

Four male beagle dogs (11–14 kg) were given a single buprenorphine sublingual dose. The animals were fasted overnight prior to the study day, with free access to water overnight and on the day of the study. A standard canine meal was given at 4 h post-dose. On the morning of each study day a temporary in-dwelling cannula was placed in the saphenous vein of the hind leg of each animal for collection of blood samples during the study period. The animal was then placed in a standing position in a sling device, which was designed to encourage the animal to remain relatively still for acquisition of scintigraphic images. Sealed markers containing approximately 0.1 MBq ^{99m}technetium were placed on the back of the head, and on the back directly above the stomach as positional references.

The spray device was used to deliver one dose of 400 μg buprenorphine sublingually to the floor of the mouth, under the tongue, and the subject was immediately placed next to a gamma camera for the acquisition of scintigraphic images. Blood samples of 2.6 ml were collected in Lithium-Heparin tubes at 0, 0.083, 0.166, 0.25, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 5, and 8 h post-dose, and were centrifuged at 2,000 rpm for 15 min at 4°C within 1 h of sampling. Plasma was then separated from the samples, and stored at −20°C until required for analysis. All procedures were carried out under a valid Home Office Animals (Scientific Procedures) Project Licence and adhered to the Principles of Laboratory Animal Care.

Imaging using a gamma camera equipped with a low energy collimator (MIE Systems, Germany) was carried out from the left lateral aspect, initially acquiring a dynamic series of 1 s images until most of the activity was observed to have passed from the mouth to the stomach. Static images were then acquired as required until the activity was observed to have left the stomach. Images were stored electronically for subsequent analysis.

Scintigraphic Data Analysis

The scintigraphic images obtained were analysed electronically using Scintron analysis software (MIE Systems, Germany). Study of the images allowed definition of regions of interest (ROIs), which were drawn around the mouth, oesophagus and the stomach, and the counts in each region were recorded. All data was corrected for background counts and radioactive decay. The parameters assessed for the scintigraphic data were the time taken for 50% and 80% ($T_{50\%}$ and $T_{80\%}$) of the total activity to clear from the mouth, and the combined mouth, oesophagus and stomach ROIs.

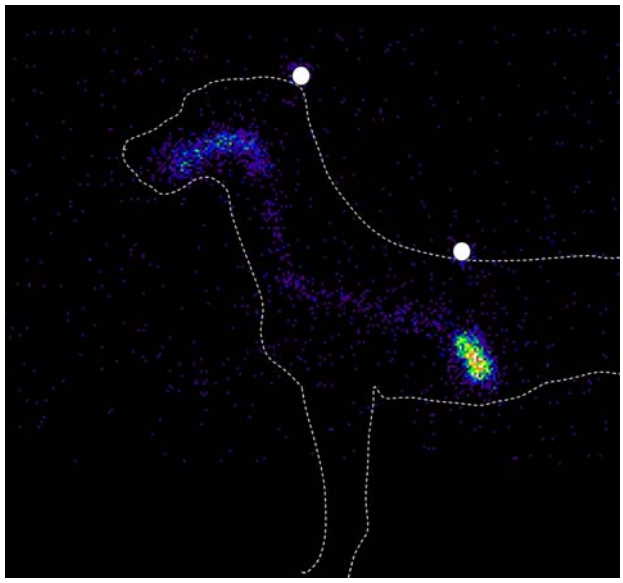

Fig. 2. Cumulative scintigraphic image (*left lateral view*) from animal 2 over 10 s (1 s image) following sublingual dosing with ^{99m}Tc-DTPA labelled solution.

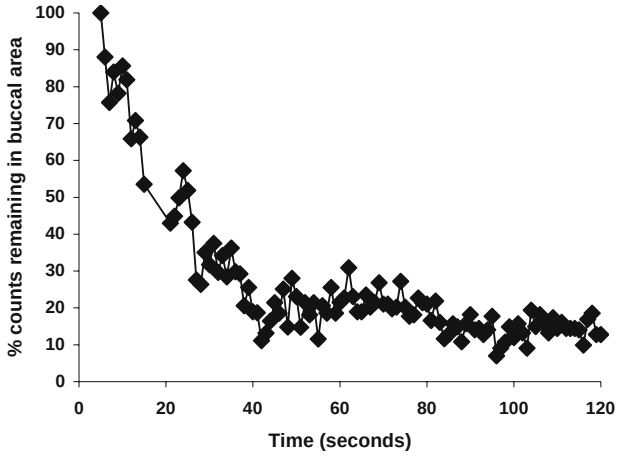

Fig. 3. Sublingual clearance profile of radiolabelled solution from subject 2.

Plasma Analysis

Calibration standards were prepared by adding known amounts of buprenorphine to blank dog plasma. The wells on a Waters OASIS MCX μ-elution plate were conditioned by the addition of 200 μl methanol, followed by 200 μl water. The spiked plasma calibration standards and samples were loaded onto the wells, followed by the addition of 400 μl of 4% phosphoric acid and 25 ng of internal standard (PF-446,687). Samples were washed with 200 μl of 4% phosphoric acid and eluted with two 100 μl aliquots of 40:60:5 acetonitrile:IPA:5% ammonia. The samples were then evaporated to dryness, and reconstituted in 20 μl of 90:10 methanol:water with 0.027% formic acid and 2 mM ammonium acetate. Samples were analysed for buprenorphine content using a Nanomate MS/MS.

Pharmacokinetic Parameters

The pharmacokinetic data obtained was analysed using WinNonlin v 5.0.1 analysis software (Pharsight), with a non-compartmental model, and was evaluated for C_{max}, T_{max}, $T_{1/2}$ and area under the curve (AUC). Pharmacokinetic parameters were calculated on an individual basis for each animal, and then combined to obtain a mean value.

RESULTS

Plasma Analysis

The individual and mean plasma profiles obtained from the administration of the radiolabelled solution are shown in Fig. 1, and overall it was observed that the pharmacokinetic data showed a relatively low degree of inter-subject variability. Plasma levels of buprenorphine increased until approximately 0.5–0.75 h post-dose, following which there was a relatively rapid decrease in plasma concentration, with levels at 8 h post-dose of just over 5% of that of peak concentrations. The pharmacokinetic parameters obtained for sublingual buprenorphine are shown in Table I, with the mean half life value of buprenorphine determined in this study in dogs of 2.42±0.45 h being very similar to that reported in the literature for an intravenous dose in human subjects of 2–3 h (24).

Table II. Clearance Times (min) of Radiolabelled Sublingual Solutions from Buccal Area, and Combined Buccal, Oesophageal and Stomach Area

Subject	Clearance from Buccal Area (min)		Clearance from Combined Area (min)	
	$T_{50\%}$	$T_{80\%}$	$T_{50\%}$	$T_{80\%}$
1	1.33	3.03	7.76	16.3
2	0.25	1.37	6.0	19.6
3	0.81	1.84	0.96	3.43
4	1.05	4.77	8.52	12.57
Mean	0.86	2.75	5.81	12.97
SD	0.46	1.52	3.40	6.98

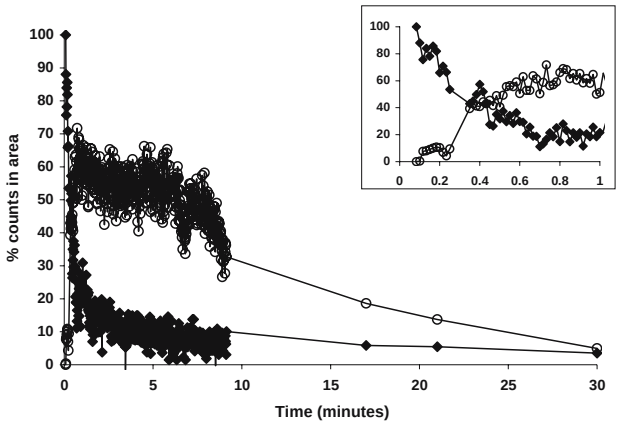

Fig. 4. Distribution of radioactive counts in the sublingual area (*filled diamonds*) and stomach (*open circles*) in subject 2 with activity moving between the mouth and the stomach during first 1 min post-dose, magnified *inset*.

Scintigraphic Analysis

Analysis of the scintigraphic data revealed the clearance pattern of the sublingual solution from the mouth, and subsequently from the gastric area. A representative image is shown in Fig. 2, showing the visualisation of the swallowing of ^{99m}Tc-DTPA labelled solution, with the solution progressing from the buccal area to the stomach. An example of the clearance pattern from the mouth is shown in Fig. 3. The mean data for clearance of the activity from the mouth in Table II shows that the clearance of the radiolabelled sublingual solution was extremely rapid, occurring in a timeframe of the order of seconds. When expressed as a percentage of the initial counts in the mouth ROI, the time to 50% and 80% clearance of activity was 0.86±0.46 and 2.75±1.52 min, respectively.

When analysed in combination with the activity present in the stomach it was possible to graphically observe the 'movement' of the dose from mouth to stomach as shown in Fig. 4. The data displayed in Fig. 4 also demonstrates the dynamic process occurring in the stomach, as some activity that has entered the stomach begins to empty from this area (activity located in oesophagus is omitted for clarity).

DISCUSSION

The T_{max} of 0.56 h determined in this study is in general agreement with the value of 0.71 h as reported by Kuhlman *et al.* (15) who administered 4 mg buprenorphine sublingually to patients in a 30% alcohol solution. The slightly longer T_{max} in humans can be partially explained by the reported elimination half life of 27.72 h in the clinical study by Kuhlman *et al.* (15), which is longer than the 2.42 h established in dogs in the current study. The alcoholic formulation is thought to enhance both the availability and permeation of drugs through the buccal mucosa. However, also relevant to the current dog study is that although the oral mucosa of the dog is relatively similar to man compared to many other animal species, it may be slightly more permeable (25), facilitating easier and therefore more rapid drug absorption. Buprenorphine is a weak base with a p*K*a of 8.42 (13), which means that in canine saliva at pH 7.5–8.5 (26) more buprenorphine will be in the unionised state compared to

human saliva (pH 6.2–7.4) (27). This may affect the absorption of buprenorphine in the dog compared to humans.

The mean elimination half life of buprenorphine in the dog for the 8-h study period was found to be 2.42±0.45 h. It has been suggested that the blood sampling period may influence the elimination half life determined, as plasma levels were found to plateau at 13 h following administration to human subjects (15), thus extending the calculated elimination half life value. This was proposed to be a result of the lipophilic buprenorphine being sequestered into depot 'pockets' in the oral mucosa, slowly releasing buprenorphine to the systemic circulation, an effect previously quantified by measuring saliva buprenorphine concentration (28). A similar model of sublingual absorption has also been proposed for propranolol (29).

The mean $T_{50\%}$ for clearance of the activity from the mouth in Table II of 0.86±0.46 min (52±28 s) is similar to data reported in a scintigraphic study of a fast dissolving (15 s) sublingual dosage form in human volunteers who were instructed to swallow normally (30), where $T_{50\%}$ clearance of one formulation studied was 50±20 s. The scintigraphic data obtained here confirms the general conclusion reached by Wilson *et al*., that following administration of a sublingual solution or fast dissolving dosage form, there is a relatively short time frame in which the major portion of sublingual absorption can occur, with the current study suggesting that clearance behaviour in the dog is similar to that of man. It will always be possible for sublingual studies in humans to request that formulations are held in the mouth for several minutes, which is not possible in the dog model. In practice however, this is extremely challenging for an individual for anything >2–3 min without swallowing.

One source of variability in the scintigraphic data observed in Table II is the movement of the animal immediately post-dose, as in the absence of any major physical restraint the animal was able to turn its head towards or away from the camera on occasion. This movement would ideally have been corrected for by the placement of a radioactive marker on a location such as the tip of the nose, which would move with the head movement, however placement of a marker so close to the sublingual area may have caused interference with the counts in the oral area, and was therefore avoided. However, as a large volume of scintigraphic data was gathered over a short period of time, overall clearance profiles could easily be observed.

In this study a small quantity of radioactivity (approximately 5%) was observed to remain in the region of the mouth for at least 8 h following dosing. This is thought likely to be a small amount of activity, which has been deposited on some other area of the mouth such as the gums or teeth, due to the fact that it was not cleared from the mouth for the duration of the study. In fact, it was observed from the mean scintigraphic data that 50% of the total counts in the mouth, oesophagus and stomach regions combined had been cleared from this region into the small intestine by 5.81±3.4 min, and 80% clearance had occurred by 12.97±6.98 min. It is therefore possible that a proportion of the absorption phase of the plasma profile observed could be a result of absorption from the small intestine rather than sublingual absorption, although the low oral bioavailability of this compound in comparison with the potentially higher sublingual absorption in dogs may mean that this is less significant. In two cases the individual plasma profiles following the sublingual dose show evidence of a possible double peak that could suggest oral absorption, however in the other two cases this effect was not observed, making it difficult to conclude that this was a 'real' event.

A previous pharmacokinetic study (data not shown) in dogs compared an identical sublingual dose and formulation with an intravenous dose. In this study plasma clearance of buprenorphine was found to be 26±8 ml/min/kg. This is virtually the same as liver plasma flow in the dog (24 ml/min/kg, based on a liver blood flow of approximately 40 ml/min/kg (31) and a haematocrit value of 0.4) suggesting that the majority of buprenorphine absorbed from the intestine would be cleared on its first pass through the liver, resulting in a low oral bioavailability. This agrees with the low reported value for oral bioavailability of buprenorphine in the dog (32). The mean bioavailability of buprenorphine in the dog following sublingual administration in the present study was estimated to be 22.4±0.9%, see Table I for details, which is much higher than the reported oral bioavailability of 3–6%. This suggests that despite the rapid clearance of the radiolabelled solution from the buccal area observed in this study, a large proportion of the absorption observed was likely to be sublingual rather than oral.

It is ultimately impossible to determine the exact proportion of absorption, which occurs orally without using deconvolution methods or a charcoal block, however the determination of clearance using the scintigraphic data obtained allows useful insight into the length of time the formulation was resident in the sublingual area to contribute to the overall percentage absorbed.

CONCLUSION

The use of gamma scintigraphy in combination with pharmacokinetic data has demonstrated that a sublingual radiolabelled solution was rapidly cleared from the mouth of conscious beagle dogs, in a similar manner to that reported for human subjects, and that despite this rapid clearance T_{max} was not observed until 0.56 h post-dose. The information obtained from this study provides confidence in using a conscious dog model to achieve a representative residence time for a solution formulation in order to predict the potential for sublingual absorption in man. However when conducting such predictions to man, it is also important to consider species differences in metabolism, dose size, formulation, saliva pH and volume, mucosal permeability and retention in relation to compound physicochemistry, as all these factors will affect the overall bioavailability.

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