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Sublingual absorption of leuprolide: comparison between human and animal models

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Abstract

Leuprolide is a potent luteinizing hormone releasing hormone agonist used for the treatment of hormone-dependent diseases. It is a decapeptide drug currently administered by subcutaneous and intramuscular injection because it is not orally bioavailable. In the present study, sublingual gel formulations of leuprolide were administered to dogs, monkeys and humans. Plasma samples were analyzed by radioimmunoassay. Absorption and pharmacokinetics of leuprolide following sublingual administration were compared and evaluated. It was found that the extent and rate of absorption were similar between humans and monkeys following sublingual dosing of leuprolide formulations. A prolonged absorption of up to approximately 6 h after dosing was observed in both humans and monkeys. The rate and extent of absorption were significantly higher in dogs than in humans. The estimate of absolute bioavailability of leuprolide was 46.7% in dogs compared with 2.7% in monkeys at an equivalent dose of 0.45 mg/kg. Absolute bioavailabilities in humans were 2.0, 2.3 and 2.4% at doses of 1.125, 2.25 and 4.5 mg, respectively. Based on these results, the dog is not an appropriate animal model for evaluating sublingual absorption of leuprolide. This work suggests that monkey is a preferred model for the development and assessment of sublingual formulations of leuprolide. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Leuprolide; Sublingual absorption; Animal model; Pharmacokinetics

1. Introduction

Leuprolide, chemically known as 5-Oxo-Pro-His-Trp-Ser-Tyr-D-leu-Arg-Pro-ethylamide acetate, is a potent luteinizing hormone releasing hormone (LHRH) agonist. It is a decapeptide hormone effective in the treatment of hormone-dependent diseases such as prostate and mammary tumors and endometriosis (Adjei et al., 1993; Fu Lu and Reiland, 1994). Plasma levels of leuprolide following oral administration of an

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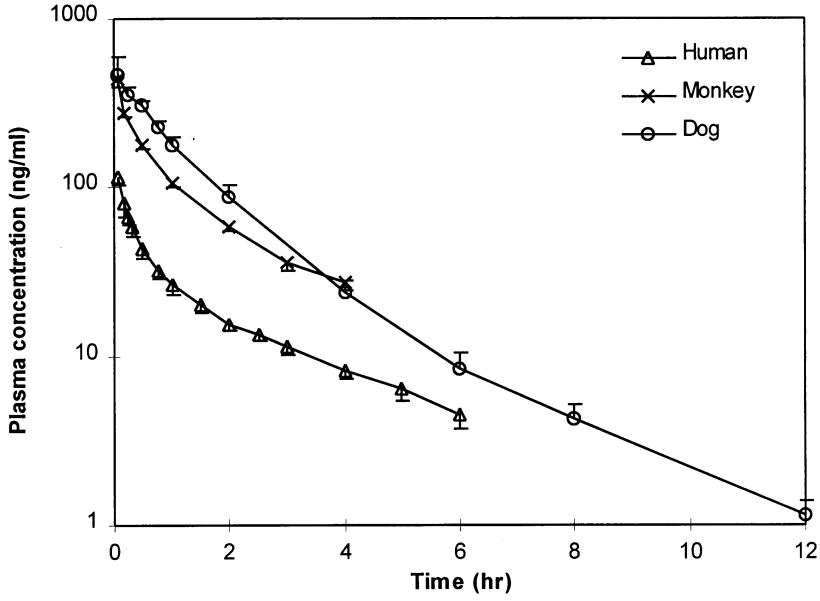

Fig. 1. Mean (± S.E.) plasma concentration–time profiles following intravenous administration of leuprolide to beagle dogs (0.1 mg/kg, $n = 4$), monkeys (0.1 mg/kg, $n = 4$) and humans (1 mg, $n = 6$).

aqueous solution to rats and humans are mostly below the limit of quantitation (0.2 ng/ml). The extremely poor oral bioavailability of this drug has been attributed to the first-pass metabolisms (Zheng et al., 1998a) and poor membrane permeability because of its large molecular size and hydrophilicity (Fu Lu et al., 1992; Zheng et al., 1998b). Presently, leuprolide is administered by parenteral routes: 11.25- and 22.5-mg, 3-month depot intramuscular injection (Lupron Depot®) and 1-mg daily subcutaneous injection (Lupron®).

Various nonparenteral routes that have been considered for systemic delivery include pulmonary, nasal, sublingual, oral, transdermal and iontophoresis (Okada et al., 1982; Chan et al., 1988; Adjei et al., 1990; Srinivisan et al., 1990; Fu Lu et al., 1992). Among the various alternative approaches, the sublingual route has substantial potential for administration of leuprolide. It is known that nonkeratinized lining areas like floor of the mouth and buccal mucosa are generally most permeable compared with the keratinized regions such as the hard palate and gingiva, because of different types of lipid making up the intercellular permeability barrier in the superficial layers of the epithelium (Squier, 1991). In addition, oral transmucosal drug delivery offers certain advantages in terms of patient acceptance and bypassing first-pass metabolism. Recent in vitro and in vivo studies have shown significant improvement in sublingual absorption of leuprolide over oral administration, especially in the presence of permeation enhancers, such as ethanol, benzoic acid and peppermint oil (Fu Lu and Reiland, 1994). During the formulation development process, formulations are often first evaluated in animal models. Thus, it is important to compare animal models with humans in order to determine the validity of an animal model. The objective of this work was to evaluate and compare absorption of leuprolide in dogs, monkeys and humans following sublingual administration.

2. Experimental

2.1. Materials and equipment

The following materials were used in the study: leuprolide acetate (TAP Pharmaceuticals,

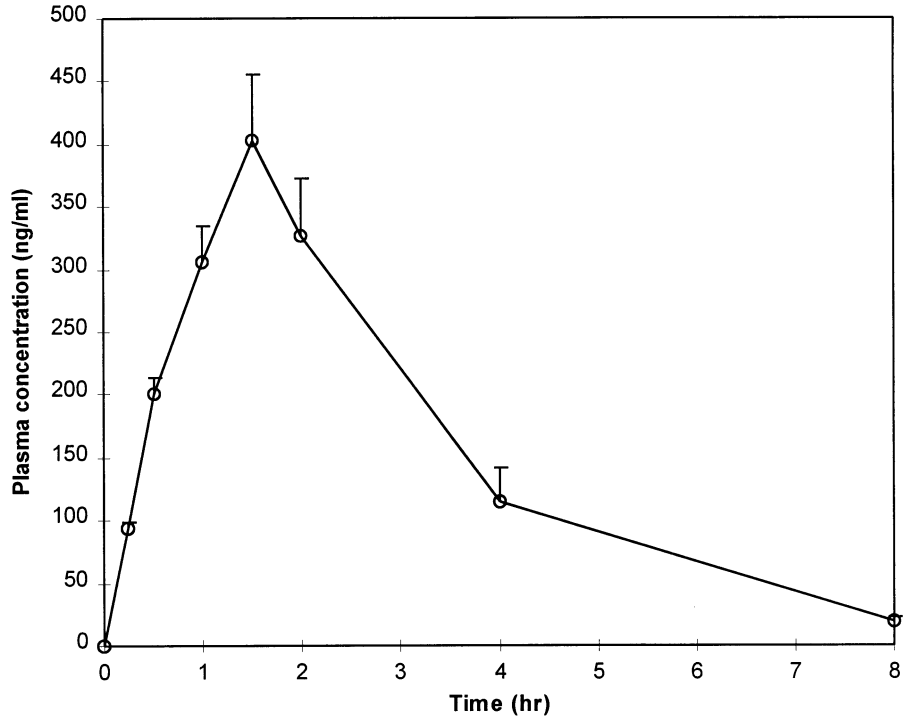

Fig. 2. Mean (± S.E.) plasma concentration–time profiles following sublingual administration of leuprolide to beagle dogs (0.45 mg/kg, $n = 4$).

Deerfield, IL), benzoic acid (Sigma Chemical, St Louis, MO), ethyl alcohol (Abbott Labs, North Chicago, IL), peppermint oil (Abbott Labs, North Chicago, IL), purified water (Abbott Labs, North Chicago, IL) and hydroxypropyl cellulose (Klucel LF; Hercules, Wilmington, DE), fentanyl citrate (Midwest Veterinary Supplies, Sun Prairie, WI), atropine (Midwest Veterinary Supplies, Sun Prairie, WI) and ketamine (Midwest Veterinary Supplies, Sun Prairie, WI).

2.2. Formulations

The sublingual formulations used for bioavailability studies in dogs, monkeys and humans were homogeneous gel solutions containing leuprolide acetate (45 mg/ml), benzoic acid (10%, w/v), Klucel LF (2.5%, w/v), ethanol (80%, v/v) and water (q.s.). All formulations were prepared and kept at 5°C prior to dosing. The potency and stability of the formulations were determined using a high performance liquid chromatography assay (Sutherland and Menon, 1987) and met the requirements for clinical studies in humans.

2.3. In vivo studies

2.3.1. Dog

Four beagle dogs weighing 9–11 kg were used in a bioavailability study of sublingual administration using intravenous injection as a reference. Dogs were fasted overnight and first anesthetized by intravenous injection of 0.64 mg/kg fentanyl citrate with droperidol, followed by additional anesthesia as needed to keep the dogs immobilized for approximately 45–60 min, to minimize the chance of swallowing. Animals were then placed in a dorsal recumbent position on a table. A volume of 100 μl of liquid formulation was administered underneath the tongue using a pipette. Serial blood samples were collected via the saphenous vein on the rear leg. After recover-

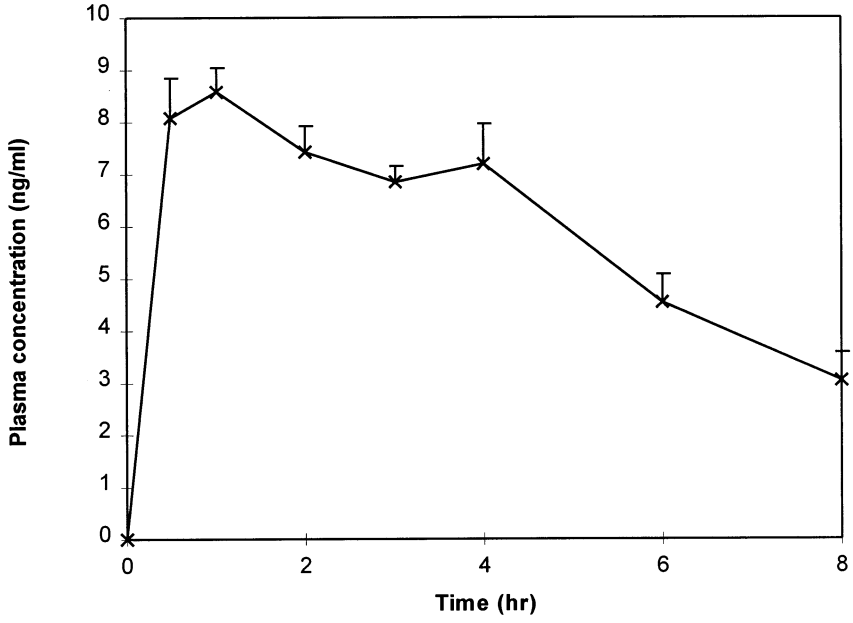

Fig. 3. Mean plasma concentration–time profiles following sublingual administration of leuprolide to monkeys (0.45 mg/kg, $n = 4$).

ing from the anesthetic, animals were returned to their cages. Blood sampling times were 0.0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0 and 8.0 h for the sublingual dosing of 0.45 mg/kg of leuprolide, and 0.083, 0.25, 0.5, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0 and 12.0 h for intravenous administration of leuprolide acetate

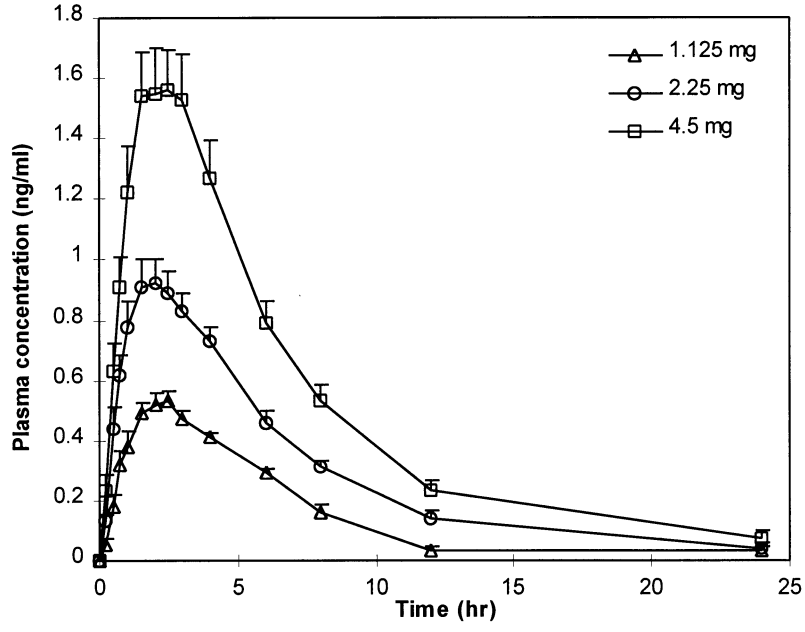

Fig. 4. Mean ($\pm$S.E.) plasma concentration–time profiles following sublingual administration of 1.125, 2.25 and 4.5 mg of leuprolide to humans ($n = 15$).

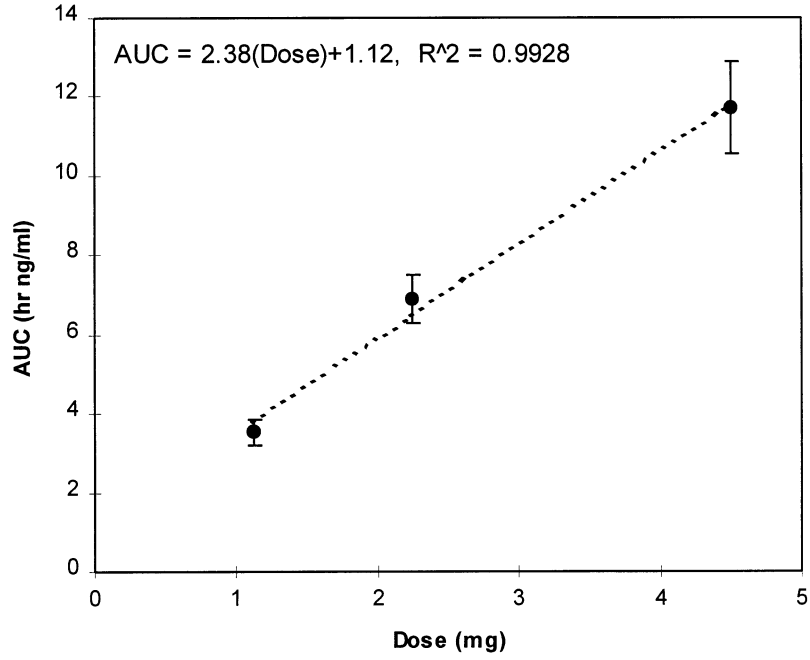

Fig. 5. Relationship between area under the plasma concentration curve ($AUC_{0-\infty}$) and dose following sublingual administration of 1.125, 2.25 and 4.5 mg of leuprolide to humans (mean ± S.E., $n = 15$).

in saline at a dose of 0.1 mg/kg, respectively. Plasma concentrations of leuprolide were determined by radioimmunoassay (Marshall and Odell, 1975).

2.3.2. Monkey

Sublingual absorption of the gel formulation of leuprolide was carried out in four Cynomolgous monkeys weighing 3–5 kg using intravenous injection as a reference. Before dosing, each monkey was fasted overnight and injected intramuscularly with 0.05 mg/kg of atropine to minimize saliva production. The monkeys were anesthetized by intramuscular injection of 10 mg/kg of ketamine and kept incapacitated for approximately 45–60 min. Monkeys were placed prone on a cart. Using a pipette, a volume of approximately 50 μl of the gel formulation was administered underneath the tongue. Blood samples were collected by placing the animals in a supine position. After recovering from the anesthetic, animals were placed in a restraining chair for up to 4 h and then returned to their cages. At each subsequent sampling, the monkeys were placed in restraining chairs for as long as needed to obtain the samples. Food was not resumed until the last sample was taken. Blood sampling times were 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 h for the sublingual dosing of 0.45 mg/kg of leuprolide, and 0.083, 0.17, 0.5, 1.0, 2.0, 3.0 and 4.0 h for intravenous administration of leuprolide acetate in saline at dose of 0.1 mg/kg, respectively. Plasma concentrations of leuprolide were determined by radioimmunoassay (Marshall and Odell, 1975).

2.3.3. Human

Fifteen sterilized female volunteers were used in a three-way crossover study of sublingual absorption of leuprolide at doses of 1.125, 2.25 and 4.5 mg. A solution of leuprolide acetate in saline was intravenously administered to six female subjects as a reference in a separate study. In the sublingual study, three volumes (25, 50 and 100 μl) of the gel formulation containing 45 mg/ml leuprolide acetate, representing the three doses, were administered quantitatively underneath the

Table 1
Average model-independent bioavailability parameters and pharmacokinetic parameters obtained from curve-fitting to polyexponetials following intravenous administration of leuprolide to dogs, monkeys and humans

Parameter	Species		
	Dogs (0.1 mg/kg)	Monkeys (0.1 mg/kg)	Humans (1.0 mg)
AUC_{0-t} (h ng/ml)	608.37(±95.94)	383.83(±20.08)	130.6(±32.4)
$AUC_{0-\infty}$ (h ng/ml)	611.52(±96.75)	448.33(±14.66)	130.6(±31.9)
k (h^{-1})	0.37±0.03)	–	–
β (h^{-1})	–	0.43(±0.03)	0. 17(±0.03)
tl/2 (h)	1.88(±0.16)	–	–
$t_{1/2(\beta)}$ (h)	–	1.64(±0.13)	4.23(±0.89)
Cl (l/h kg)	0.17(±0.03)	0.22(±0.0l)	0.14(±0.03)
V_d (l/kg)	0.45 (±0.09)	–	–
V_β (l/kg)	–	0.52 (±0.05)	0.89 (±0.34)
A_1(ng/ml)[a]	–	601.86 (±118.27)	111.57(±4.40)
α (h^{-1})[a]	–	12.686 (±2.87)	4.95(±0.41)
A_2(ng/ml)[a]	482.1 (±13.68)	237.3 (±24.55)	39.47(±3.31)
β (h^{-1})[a]	0.958 (±0.059)	0.678 (±0.098)	0.415(±0.044)
MRT (h)	1.04	1.31	1.98
MSC[a]	4.67	4.64	5.47
$t_{1/2}$ (h)[a]	0.72	–	–
$t_{1/2(\beta)}$ (h)[a]	–	1.02	1.67
$t_{1/2(\alpha)}$ (h)[a]	–	0.06	0.14
k_{10} (h)[a]	–	2.11	1.28
k_{12} (h)[a]	–	7.18	2.48
k_{21} (h)[a]	–	4.07	1.60

[a] Obtained from curve-fitting to polyexponentials.

tongue. The formulation was held in the mouth for a minimum of 2 min. Blood samples were collected at 0.0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after sublingual dosing. Blood sampling times following intravenous administration were 0.08, 0.17, 0.25, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h. Plasma concentrations of leuprolide were determined by radioimmunoassay with a limit of quantitation of 0.2 ng/ml (Marshall and Odell, 1975).

2.4. Data analysis

The area under the plasma concentration–time curve (AUC) from time zero to the last sampling time point t (AUC_t) was calculated by the trapezoidal rule. AUCs from time zero to time infinity (AUC) were obtained by extrapolation using elimination rate constants. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}) were obtained directly from the data without interpolation. A nonlinear least-squares program RSTRIP II (MicroMath® Scientific Software, Salt Lake City) was used to fit the plasma concentration data after intravenous administration to the polyexponential equation, $C(t) = \Sigma\ Ai\ e^{-Kit}$. The goodness of fit was assessed by model selection criteria (MSC), a modified Akaike information criterion, and examination of parameter redundancy. Elimination rate constants were estimated from the terminal phase of the plasma concentration–time profiles for individual dogs. The values of total clearance (Cl) of leuprolide were obtained based on:

$$Cl = \frac{FD}{\int_0^\infty C(t)\,dt} \quad (1)$$

where D is the drug dose and F is the fraction of drug absorbed.

Table 2
Average model-independent bioavailability and pharmacokinetic parameters of leuprolide following sublingual administration to dogs, monkeys and humans

Parameter	Dog (0.45 mg/kg)	Monkey (0.45 mg/kg)	Human (4.5 mg)	Human (2.25 mg)	Human (1.125 mg)
AUC_{0-t} (h ng/ml)[a]	1243.39 (±358.89)	47.64 (±5.02)	11.71 (±4.47)	6.88 (±2.34)	3.54 (±1.30)
$AUC_{0-\infty}$ (h ng/ml)[a]	1285.40 (±373.78)	54.76 (±6.84)	12.10 (±4.92)	7.18 (±2.66)	3.71 (±1.52)
F[b]	46.7%	2.7%	2.0%	2.3%	2.4%
F[c]	52.2%	2.9%	1.8%	2.1%	2.5%
C_{max} (ng/ml)	403.00 (±105.12)	8.75 (±0.66)	1.80 (±0.65)	1.00 (±0.36)	0.56 (±0.15)
t_{max} (h)	1.50 (±0.00)	0.75 (±0.29)	1.90 (±0.69)	1.82 (±0.79)	1.83 (±0.64)
k (h^{-1})	0.45 (±0.06)	–	–	–	–
$t_{1/2}$ (h^{-1})	1.55 (±0.20)	–	–	–	–
β (h^{-1})	–	0.19 (±0.07)	0.21 (±0.03)	0.19 (±0.04)	0.19 (±0.07)
$t_{1/2(\beta)}$ (h)	–	4.25 (±2.03)	3.38 (±0.44)	3.79 (±1.05)	4.25 (±1.87)
Cl/F (l/h kg)	0.37 (±0.11)	8.32 (±1.08)	7.83 (±3.07)	6.61 (±2.82)	6.48 (±2.75)

[a] Last data point at $t = 8$ h for dog, 8 h for monkey and 24 h for human.
[b] Fraction absorbed based on AUC
[c] Fraction absorbed estimated by deconvolution.

3. Results and discussion

3.1. Bioavailability and pharmacokinetics

Plasma concentration–time profiles of leuprolide in dogs, monkeys and humans following intravenous administration are shown in Fig. 1. Results of sublingual dosing in the three species are presented in Figs. 2–4, respectively. The absolute bioavailabilities of sublingual administration were 46.7% in dogs and 2.7% in monkeys at the equivalent dose of 0.45 mg/kg. Bioavailabilities in humans were estimated at 2.0, 2.3 and 2.4% at doses of 1.125, 2.25 and 4.5 mg, respectively. Linear dose–AUC proportionality is obtained with a coefficient of determination of 0.9928 for the average sublingual data in humans (Fig. 5). When the relationship between dose and AUC for individual subjects was examined, no curvature was observed at either end of the dose range, although the dosing volumes are different.

Model-independent bioavailability parameters (Wagner, 1993) are summarized in Tables 1 and 2. The results of pharmacokinetic analysis of the intravenous data in three species were also given in Table 1. In vivo disposition of leuprolide followed bi-exponentials in humans and monkeys. In dogs, the disposition kinetics are better described using a single exponential equation, suggesting different in vivo distribution characteristics of leuprolide between dogs and humans. For sublingual delivery, similar apparent elimination parameters, such as terminal phase rate constant and total body clearance (Cl/F) were found between monkeys and humans at all three different doses, although their intrinsic parameters based on intravenous data were different. When the terminal phase half-lives ($t_{1/2}$) were compared between intravenous and sublingual administration, the apparent $t_{1/2}$ was prolonged in sublingual administration in monkeys. This observation may be attributed to one or more of the following: (1) inadequate and short sampling times in monkeys may lead to underestimation of the terminal rate constant; (2) compared with humans, the relatively long duration of incapacitation of moneys (45–60 min) after dosing resulted in an increase in residence time of the gel formulation at the site of absorption; (3) the rate of drug permeation through the membrane may change due to anesthetization.

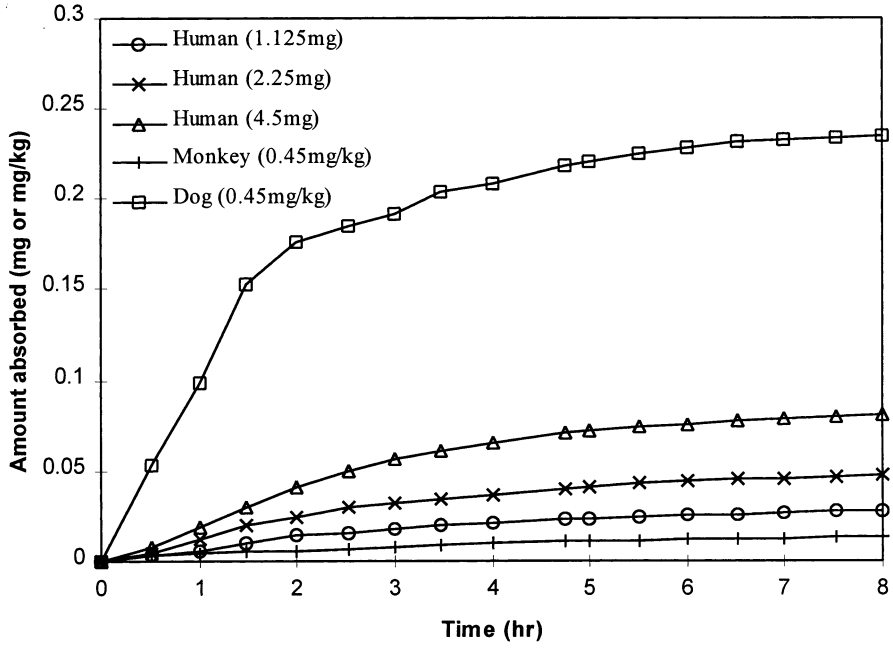

Fig. 6. In vivo absorption of leuprolide estimated by deconvolution after sublingual administration to dogs (0.45 mg/kg), monkeys (0.45 mg/kg) and humans at three doses: 1.125, 2.25 and 4.5 mg.

3.2. Absorption comparison

Linear system analysis is a model-independent method useful in evaluation of drug absorption processes (Cutler, 1978). Based on the superposition principle in a linear time-invariant system, a response, $C(t)$, to an input, $f(t)$ of a system is defined by the following convolution integral (Cutler, 1978):

$$C(t) = f(t)^* C_\delta(t) = \int_0^\infty C_\delta(t-\tau) f(\tau)\, \mathrm{d}\tau \qquad (2)$$

where $C_\delta(t)$ is the unit impulse response characteristic of the system. For most pharmacokinetic applications, $C(t)$ and $f(t)$ represent the plasma drug concentration and the rate at which the drug enters the system, respectively (Cutler, 1978). $C_\delta(t)$ is the plasma concentration resulting from the instantaneous input of a unit amount of drug into the system.

In the present study, plasma concentration profiles following intravenous administration were used as unit impulse responses $C_\delta(t)$. Drug plasma data from the sublingual formulations $C(t)$ were fitted to a smoothing cubic spline function followed by deconvolution with $C_\delta(t)$ using the Program PCDCON (W. Gillespie, FDA) to obtain apparent in vivo sublingual absorption profiles. The results are shown in Fig. 6. The in vivo absorption profiles of leuprolide showed the highest extent of absorption from oral mucosa in dogs, while a much lowered extent of absorption was observed in both monkeys and humans. The 'sublingual bioavailability' of leuprolide from the gel formulation can be estimated by the plateau values from respective in vivo apparent absorption profiles (Gillespie and Veng-Pedersen, 1985) in Fig. 6. The results agree with the bioavailability values obtained based on AUC data (see Table 2). Fig. 7 shows fractional sublingual absorption profiles of leuprolide in dogs, monkeys and humans. These are profiles normalized by the bioavailable dose, representing the rate of absorption in the three species. The absorption of leuprolide in dogs was characterized by a rapid uptake of about 75% of the bioavailable dose in the first 2 h, followed by slower absorption of the remaining drug over the next 3 h. Both monkeys

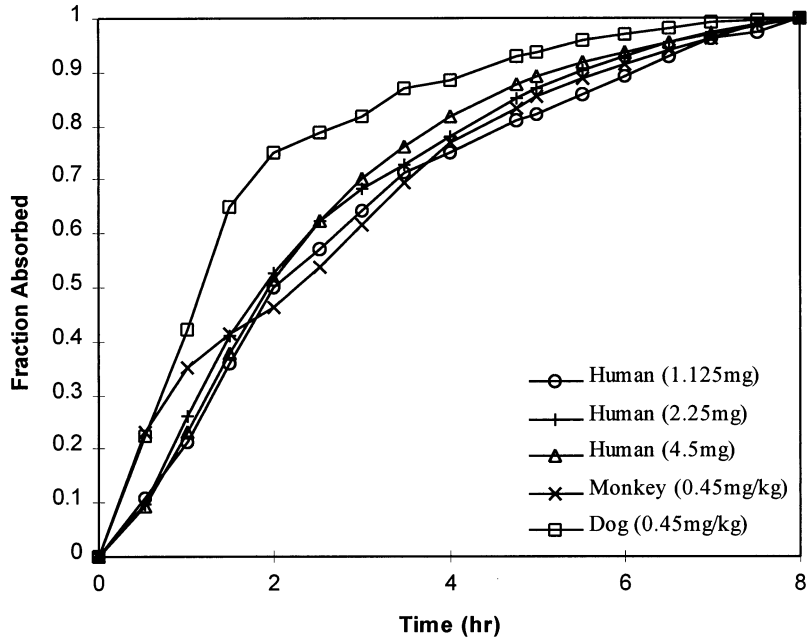

Fig. 7. Comparison of in vivo fraction of absorbed leuprolide estimated by deconvolution after sublingual administration to dogs (0.45 mg/kg), monkeys (0.45 mg/kg) and humans at three doses: 1.125, 2.25 and 4.5 mg.

and human subjects showed similar absorption prolonged up to approximately 6 h after dosing. To compare the absorption among different dosing groups, rate constants of absorption were estimated by fitting the data up to 94% of absorption to a first-order kinetic model. The results in Table 3 indicate similar rates of absorption between monkeys and humans, whereas the rate of absorption is significantly higher in dogs. In addition, similar absorption rate in humans at three different doses supports the linear dose–AUC relationship. It should be pointed out that the absorption in the first hour appeared to be more rapid in monkeys than in human subjects, which is also suggested by the difference in t_{max} values (Table 3). This may be related, at least in part, to the longer duration of intimate contact between the gel formulation and the sublingual region in monkeys (45–60 min.). Further experiments with more test subjects are needed for a conclusive comparison.

On the basis of this discussion, dog is not an appropriate animal model for studying sublingual absorption of leuprolide. Monkey appears to be a good model for evaluation of sublingual formulations, but animal handling and cost of using monkeys can be challenging for extensive formulation studies. In most transmucosal studies, the choice of species is based on the tissue structure, i.e. nonkeratinized mucosa, in order to obtain

Table 3
Results of curve fitting of fractional absorption to first-order kinetics

Parameter	Dog (0.45 mg/kg)	Monkey (0.45 mg/kg)	Human (4.5 mg)	Human (2.25 mg)	Human (1.125 mg)
k (h^{-1})	0.612	0.420	0.395	0.447	0.478
Intercept	0.051	0.143	0.144	0.168	0.176
R^2	0.9772	0.9760	0.9895	0.9928	0.9944

results comparable with humans. Canine and porcine tissues have been reported to compare favorably with human tissue in certain cases (de Vries et al., 1991). However, there is still no generalized model for the human because the keratinization state is only one criterion for choosing an animal model (de Vries et al., 1991). There are often differences across species in other characteristics, e.g. biochemical properties and enzymatic activities (de Vries et al., 1991). The impact of these factors on absorption can often be drug dependent. In the present study, the higher extent and rate of absorption from the dogs may be attributed to the higher apparent permeability of oral mucosal membrane compared with humans. In fact, Lesch et al. (1989) and Squier (1991) have reported much higher permeability in dog oral mucosa than in that of human. Similar differences in bioavailability between dogs and humans were also observed with another peptide (MW = 841.0) and a sparingly soluble nonpeptide (MW = 436.0) in our laboratories (unpublished data).

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