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Intranasal bioavailability of buprenorphine in rabbit correlated to sheep and man

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Abstract

The purpose of the present study of buprenorphine is to add information about the correlation between various animal models and nasal bioavailabilities in man. PEG 300 was added to one formulation to study whether the addition of the co-solvent results in the same absorption pattern as seen for sheep. The bioavailability of intranasal buprenorphine 0.6 mg in PEG 300 and 5% dextrose was assessed in a cross-over study in six rabbits. The mean bioavailabilities, T_{max} and C_{max} were 46% (S.D. $\pm$ 13) and 53% (S.D. $\pm$ 17), 8 and 12 min, 28 and 27 ng/ml for 30% PEG 300 and 5% dextrose, respectively. No significant differences were found between the nasal buprenorphine formulations. The bioavailabilities in rabbit and sheep, respectively, were $\approx$ 2.5 and four times higher than for man. The absorption rate was faster for rabbit and sheep than for man. It appears that rabbit and sheep bioavailability differ from humans, especially with respect to rate. PEG 300 do not increase the bioavailability of buprenorphine.

Keywords: Buprenorphine; Intranasal; Polyethylene glycol 300; Bioavailability

1. Introduction

Buprenorphine, a partial agonist derivative of thebaine, has long-acting and potent analgesic effect and commercial formulations for intravenous and sublingual administration are available. Sublingual administration of buprenorphine, suggested for treating opiate dependence, has the advantage of avoiding first pass liver metabolism (Hand et al., 1990), as long as the drug is not swallowed. T_{max} is beyond 1 h (Mendelson et al., 1997; Martindale 1999) with an onset of action of 30 min. In treatment of acute pain, where injections are not wanted or possible, it would be desirable to have a much shorter T_{max} than that obtained by sublingual administration. Additionally, some publications indicate substantial variations in sublingual bioavailability of buprenorphine (Bullingham et al., 1982; Kuhlman

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et al., 1996), resulting in difficulties in the dose regimen of the drug. Based on these disadvantages associated with oral formulation and sublingual application, an alternative route of administration, e.g. intranasal, is of potential interest.

Dogs, monkeys, rabbits and sheep are the preferred animal models for pharmacokinetic and formulation studies in nasal drug delivery (Gizurarson 1990). A series of bioavailability studies have been performed on rabbits (Baldwin et al., 1990; Fisher et al., 1991; Schipper et al., 1993; Marttin et al., 1997; Bechgaard et al., 1996, 1997, 1999). However, the number of publications describing nasal bioavailability studies in sheep is limited, probably because of the need for special stable facilities for the relatively big animals. However, the sheep model is also expected to be promising in respect to correlation to man (Illum, 1996). In general, only a few correlation studies of nasal bioavailability in different animal species are published, (e.g. Baldwin et al., 1990; Fisher et al., 1991). Even more limited are studies where the animal data are correlated to human data (Merkus et al., 1996).

In an earlier study, Lindhardt et al. (2000) dosed sheep intranasally with buprenorphine formulated in 30% polyethylene glycol 300 (PEG 300) and in 5% dextrose, the same vehicles as used in this study. The bioavailabilities in sheep after 1 h were 70 and 89%, respectively. The sheep bioavailability study was correlated to a human study performed by Eriksen et al. (1989). In the human study, 0.3 mg per dose in 5% dextrose resulted in 48% bioavailability after 12 h.

In the present study, an intranasal dose of 0.6 mg buprenorphine was chosen, to increase the analytical accuracy of the plasma data and because it was expected to be the most relevant for a future nasal formulation.

The purpose of the present bioavailability study of buprenorphine is to add information about the correlation between various animal models and man. PEG 300 was added to one formulation to study whether addition of the co-solvent results in the same absorption pattern as seen for sheep.

2. Materials and method

2.1. Chemicals

Buprenorphine, HCl used for nasal formulations was obtained from Sigma (St. Louis) whereas a commercial formulation, Anorfin, from GEA (Copenhagen, Denmark) was applied for intravenous injection. PEG 300 and dextrose was from Union Carbide Chemicals and Plastics Company Inc. (Danbury) and Merck (Darmstadt, Germany), respectively.

2.2. RIA analysis of buprenorphine rabbit plasma

A double antibody RIA method containing I^{125} labelled buprenorphine from Diagnostic Products Corporation (Los Angeles) with prior liquid extraction (phosphate buffer pH = 7 and cyclohexane) was used for the analysis of buprenorphine in rabbit plasma. This specific method was a modification of a method described for human plasma (Lee and Dockham, 1991). The plasma concentrations were calculated from a standard curve, freshly prepared every day. To 50 μl plasma sample, 75 μl of phosphate buffer (pH = 7) was added and whirled for 10 s in 75 × 10 mm glass centrifuge tubes from Bie og Berntsen (Copenhagen, Denmark). The plasma was extracted with 1 ml cyclohexane, analytical grade, from Sigma (St. Louis), by rotating 1 h at 30 rpm. Phase separation was then allowed to occur and 10 min after 500 μl of the organic phase, was transferred to a new set of glass tubes and evaporated at room temperature with pressurised atmospheric air. A buprenorphine radioimmunoassay kit KBPD from Diagnostic Product Cooperation (CA) provided the possibility of measuring the sample concentration in a gamma counter, LKB-Wallac 1272 Clini Gamma (Wallac Oy, Turku, Finland). As discussed by Lindhardt et al. (2000), the cross reactivity to the major metabolites of the analytical method is only $\approx 10\%$ at the relevant concentrations and is not expected to have significant influence on the analysis. The same analytical method has also been applied for studies in sheep (Lindhardt et al., 2000) and a similar method was used in man (Eriksen et al., 1989).

2.3. In vivo study

Six New Zealand White rabbits, obtained from Hvidesten (Allerød, Denmark), with a mean weight of ≈ 3.9 kg, were used in a cross-over study ($n = 6$) with a wash out period of 1 week. Since it was found that the solubility curve of buprenorphine had a maximum at 30% PEG 300 (Lindhardt et al., 2000), this vehicle, which is expected to be clinically acceptable, was applied in the bioavailability study. The design consisted of three legs with two rabbits receiving one out of three formulations in each run: 12 mg/ml buprenorphine in 5% dextrose (intranasal), 12 mg/ml buprenorphine in 30% PEG 300 (intranasal) and 0.6 mg (2 ml) Anorfin (intravenous). All nasal preparations were administered to the rabbit while in a supine position and the rabbit was kept in this position for 1 min after administration. A total of 50 μl was administered in one nostril with an Eppendorf multipipet.

Blood samples of 1–1.5 ml withdrawn from the marginal ear vein were collected in 1.5 ml microcentrifuge tubes coated with heparin HEP-19 from Bie og Berntsen (Copenhagen, Denmark). The samples were taken just before and 2, 5, 10, 15, 20, 25, 30, 45 and 60 min after administration of buprenorphine. Plasma was obtained after centrifugation at $3300 \times g$ and 4°C for 10 min and stored at -20°C until analysis. The study was performed according to permission (journal number, 1999-561-182) approved by the Danish committee for animal experiments.

2.4. Calculations

The area under the curve (AUC) was calculated using the trapezoidal rule. AUC from 0–2 min for intravenous administration was determined by extrapolation of the zero value from 2 and 5 min. On average, AUC_{0-2min} accounted for $< 10\%$ of the $AUC_{0-60min}$.

In the cross-over study, serum concentrations were corrected for differences in body weight during the test period by a factor *f:*

$$f = W/W_{mean}$$

where W is the body weight of the individual rabbit and W_{mean} denotes the average body weight of the rabbits in the first leg ($n = 6$). Student's *t*-test was applied for the statistical analysis.

3. Results and discussion

The bioavailabilities in rabbits after nasal administration of two formulations ($n = 6$) containing buprenorphine in 30% PEG and 5% dextrose was 46 ± 13 and 53 ± 17%, respectively, see Table 1. The ranking between the two formulations was

Table 1
Mean time (T_{max}) to maximal serum concentration (C_{max}) and bioavailability of intra-nasal buprenorphine formulations from 0 to 60 min (30% PEG 300 and 5% dextrose) for rabbits compared to intravenous (iv)[a]

Formulation	Species	Dose (mg)	T_{max} (min)	C_{max} (ng/ml)	Bioavailability (%)
Iv	Rabbit	0.6	–	203 ± 195	100
	Sheep	0.6	–	46 ± 21	100
	Man	0.3	–	61 ± 25	100
PEG 300	Rabbit	0.6	8 ± 6	28 ± 11	46 ± 13
	Sheep	0.9	10 ± 5	37 ± 17	70 ± 27*
Dextrose	Rabbit	0.6	12 ± 6	27 ± 7	53 ± 17
	Sheep	0.9	9 ± 6	48 ± 10	89 ± 23**
	Man	0.3	31 ± 7	1.8 ± 0.5	21 ± 10***

[a] Data for sheep (Lindhardt et al., 2000) and man (Eriksen et al., 1989) may also be found.
* Significance level in relation to nasal buprenorphine bioavailability in rabbit of the same vehicle ($P < 0.05$).
** Significance level ($P < 0.01$).
*** Significance level ($P < 0.001$).

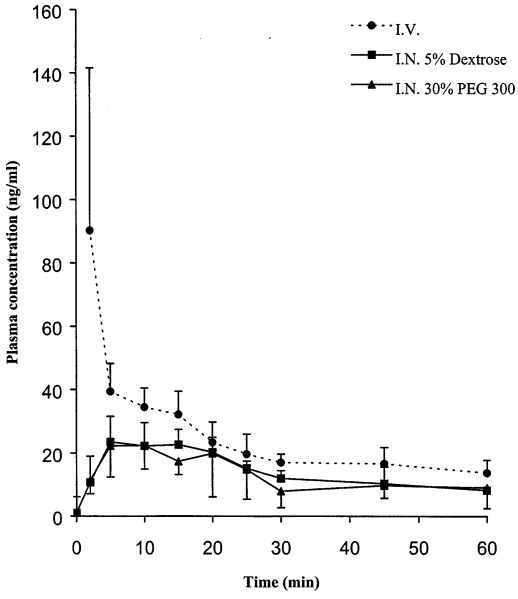

Fig. 1. Mean plasma concentration-time profiles in rabbits after intranasal administration of 0.6 mg buprenorphine in two formulations containing 12 mg/ml buprenorphine in 30% PEG 300 (■) or in 5% dextrose (▲). The plasma concentration-time profile after intravenous administration is represented by a dotted line with symbol (●).

similar to that found in sheep, but likewise, the difference was not statistical. Whether the lower bioavailability of buprenorphine from the PEG formulation is due to higher retention in the vehicle is not known. As it appears from Fig. 1, the bioavailabilities of the two nasal formulations are similar. The absorption rate was very fast (mean $T_{max} = 10$ min) and C_{max} was 27.6 ± 10.7 and 26.5 ± 7.3 ng/ml for PEG 300 and dextrose, respectively.

The bioavailabilities in sheep (Lindhardt et al., 2000), after nasal application of buprenorfin in 30% PEG 300 and 5% dextrose, were 70 ± 27 and $89 \pm 23\%$, respectively. The absorption rate in sheep was also fast, yielding plasma profiles similar to that found in rabbits. The correlation, found in this study, between rabbit and sheep is relatively good, but the sheep mean bioavailability is a factor of 1.5 ($P < 0.05$) higher than rabbit. The blood samples of the sheep study was withdrawn from the jugular vein, as described by Illum (1996). The blood from the nasal cavity is drained in the jugular vein (Khamas and Ghoshal, 1982), which may result in over-estimation of bioavailability after nasal administration.

As described, the human nasal bioavailability (Eriksen et al., 1989) with 5% dextrose was found to be 48%, but the study was carried out for 720 min, although it may be questionable if the plasma profiles exceeding 1 h is relevant, especially when the focus is on acute situations. Recalculating from 0–60 min gives a human bioavailability of 21% indicating a significantly higher absorption in rabbits and sheep by a factor of ≈ 2.5 and 4, respectively, according to data found in Table 1. The differences between animal and man bioavailabilities are even bigger when shorter periods than 0–60 min are used in the calculations, see Fig. 2. The purpose of a nasal spray containing buprenorphine is to obtain an analgesic effect as fast as possible, while various initial observation periods, such as the first 10–20 min, may be the most relevant. Rather than stating one absolute bioavailability, a series of initial periods may give information about the development of the absorption, relative to the fastest alternative, the intravenous administration. A bioavailability/time profile is further illustrated in Fig. 3, where the fraction of bioavailability ($Bioavailability_{t/min}/Bioavailability_{60min}$) at various times relative to the 60 min bioavailability (for each species) is illustrated. It appears that rabbit and sheep are similar with respect to rate, but the correlation to man is not optimal.

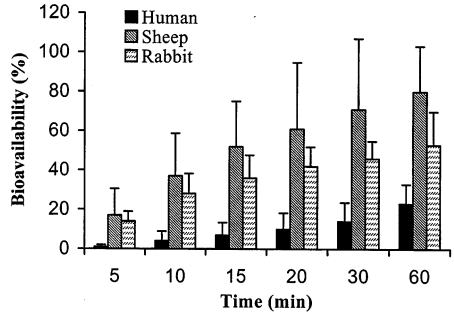

Fig. 2. The rabbit bioavailabilities ($\pm$ S.D.) of buprenorphine, at various initial periods (0–5, 0–10, 0–15, 0–20, 0–30 and 0–60 min) after nasal administration of an aqueous vehicle (5% dextrose). Data for sheep (from Lindhardt et al., 2000) and man (Eriksen et al., 1989) are also illustrated.

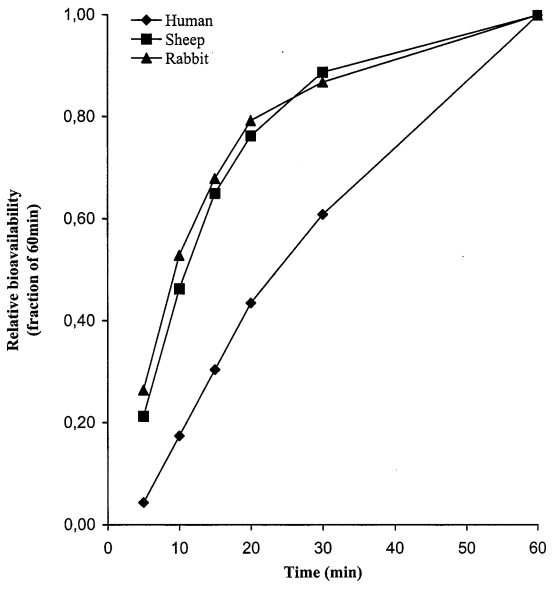

Fig. 3. Relative bioavailability (fraction of the bioavailability of 60 min, each species its own reference) after nasal application of buprenorphine in 5% dextrose to man (◆), sheep (■) and rabbit (▲). (Data from sheep and man are from Lindhardt et al. (2000) and Eriksen et al. (1989), respectively).

These differences between the animal models and man may partly be explained by the difference in T_{max}, which was significantly longer for man having an average peak plasma concentration after ≈ 30 min.

The pharmacokinetics of buprenorphine in rabbits may be relatively complex, but can possibly be described by a three-compartment model where half-lives of 2.5, 20 and 100 min. The pharmacokinetics in rabbits is in good agreement with man, where half-lives of ≈ 2.5, 15 and 140 min have been observed (Bullingham et al., 1980).

The low T_{max} indicate that nasal administration of buprenorphine is likely to give a faster effect than, for example sublingual, which would be convenient for the patient. The variations in nasal bioavailability are moderate, which may be an advantage to, for example, sublingual administration.

The co-solvent, PEG 300, does not increase the bioavailability of buprenorphine. On the contrary, the bioavailability seems to be slightly lower for the PEG formulation both in rabbit and sheep. This may be due to buprenorphine staying in solution longer when PEG 300 is present. It appears that rabbit and sheep bioavailability is similar with respect to rate but differs substantially from humans.

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