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Intranasal bioavailability of diazepam in sheep correlated to rabbit and man

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Abstract

The purposes of the present study were to estimate the nasal bioavailability of diazepam in sheep and to compare this to earlier results in rabbits and humans. Additional, to compare the absorption during various initial periods in the two animal models and man, due to the importance of early absorption in emergency treatment. In a cross-over design, diazepam was nasally administered (7 mg) and intravenously (3 mg), respectively, to six sheep. Diazepam was solubilised in polyethylene glycol 300 in the nasal formulation. The mean nasal bioavailability, t_{max} and C_{max} were 15% (S.D. $\pm$ 8), 5 min (S.D. $\pm$ 3) and 934 ng/ml (S.D. $\pm$ 593), respectively. Sheep bioavailability was lower than rabbit 54% ($P < 0.001$) and man 34% ($P < 0.05$). In conclusion, the nasal absorption of diazepam was found to be fast, indicating the potential of nasal delivery in acute treatment. The initial (30 min) nasal bioavailability (30 min) for sheep and rabbit is a factor of 2.3 lower and 1.6 higher than man, respectively. The correlation of bioavailability was not optimal between sheep, man and rabbit with differences both in relation to extend and rate.

1. Introduction

The preferred drugs for the treatment of epileptic seizures are diazepam or clonazepam, intravenously administered. These administrations are effective for hospitalised or institutionalised patients, since the application cannot be performed by an untrained person and the treatment may be associated with hypotension, cardiac dysrhytmias or central nervous systemic depression. Rectal administration of diazepam is applied in paediatric therapy, however, the inconvenience of this type of administration may limit the success of this route in adult treatment (Gizurarson et al., 1999).

Nasal application of diazepam is a potential alternative to intravenous injections and rectal administration in treatment of acute seizures (Bechgaard et al., 1991; Gizurarson et al., 1999). A nasal spray may be a beneficial delivery system

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in acute situations, where the time of effect onset needs to occur within minutes or seconds. Rabbit experiments have shown that intranasal administration of diazepam result in a pharmacodynamic effect within 2–4 min. Additionally, a peak plasma concentration was found to be about 5 min (Bechgaard et al., 1991, 1997). Li et al. (2000), made a similar rabbit study, with a potentially more irritating vehicle, and found about the same fast onset of effect (about 2 min). As diazepam is a very low soluble drug (about 0.05 mg/ml in water), a good solubilising and relatively non-toxic excipient is required for a nasal formulation. Polyethylene glycol 300 (PEG 300), a promising vehicle, was able to solubilise an expected clinical relevant dose (about 4–10 mg) of diazepam, in the limited volume of 150–300 μl, applicable for nasal administration (Gizurarson, 1990).

In order to increase the value of animal experiments (Lindhardt et al., 2000, 2001a) it is important to establish a correlation of nasal bioavailability between animal and man. Dogs, monkeys, rabbits and sheep are the preferred animal models for pharmacokinetic and formulation studies in nasal drug delivery (Gizurarson, 1990). The number of publications, which describe nasal bioavailability studies in sheep, is limited, probably because of the considerable size of the animals, requiring special stable facilities. However, the sheep model is expected to be promising concerning correlation to man (Illum, 1996). Only very few bioavailability studies, where the same nasal formulations administered to different animals as well as to man, are available.

Electroencephalographic effect measurements have indicated that a nasal diazepam dose of about 7 mg may be of clinical relevance (Lindhardt et al., 2001a), and the same dose was chosen for this study. These measurements showed a very fast onset of effect (within 2 min) on the event-related potential difference, P300-N100, after intranasal administration of diazepam, also solubilised in PEG 300.

Human and rabbit nasal bioavailability during the initial 30 min was found to be about 37 and 54%, respectively. Bioavailabilities to various times, from the initial blood levels up to e.g. 60 min, may give important information about the correlation between absorption rates in various animals (Lindhardt et al., 2001b). The present paper focuses on bioavailabilities calculated from 0 to 30 min. A longer period may not be relevant for acute treatment, because most intranasally administered drugs are cleared towards the nasopharynx and swallowed in approximately 20–30 min after administration (Gizurarson et al., 1999).

The purposes of the present study were to estimate the nasal bioavailability of diazepam in sheep and to correlate this to earlier results in rabbits and humans. Additional, to compare the absorption at various initial periods in the two animal models and man, due to the importance of early absorption in emergency treatment.

2. Materials and method

2.1. Chemicals

Stesolid Novum®, a commercial intravenous formulation of diazepam, and PEG300 were from Dumex-Alpharma A/S (Copenhagen, Denmark) and Union Carbide (Danbury, CO, USA), respectively. Diazepam was from Nycomed Pharma A/S (Roskilde, Denmark)

2.2. Preparations

Diazepam was solubilised in PEG 300 to a concentration of 47 mg/ml and filled into the nasal device. The spray volume was 75 μl for each device. The pH of the formulations was about 5.5. Two devices were applied for each animal, one for each nostril. 600 μl (3 mg) of Diazepam Novum® (5 mg/ml) was applied for each intravenous administration.

2.3. In vivo study

Six Icelandic sheep, 1–2 years old, weighing 35–40 kg, was obtained from Keldur, Institute of Experimental Pathology, University of Iceland (Reykjavik, Iceland). The sheep were used for a cross-over study ($n = 6$) with a wash out period of

10 days. They received food and water ad libitum. During each of the two study days, three sheep were selected receiving 7 mg diazepam in PEG 300 or 3 mg diazepam intravenously.

All nasal preparations were administered with the sheep in a fixed standing position forcing its head slightly backward while administrating the nasal solution. The device, a Pfeiffer Unit Dose, was modified enabling the device to spray the viscous vehicle. Two strokes were administered, corresponding to a total of 150 μl. Blood samples of 4 ml were withdrawn from the jugular vein, applying 8 ml Vacuette® tubes from Greiner (Bel Air, MD, USA). Samples were taken at -2, 2, 5, 10, 15, 20, 30, 45 and 60 min after drug administration and centrifuged $3200 \times g$ for 10 min. Serum was transferred to 1 ml cryotubes from NUNC (Copenhagen, Denmark) and stored at -80 °C until analysis.

2.4. Serum analysis of diazepam

An enzyme-immunoassay (EIA) based analysis for diazepam in serum from STC Technologies, Inc (Bethlehem, PA, USA) was applied for serum analysis. The same analytical method was used for human serum in an earlier study (Lindhardt et al., 2001a).

2.5. Calculations

The area under the curve (AUC) was calculated using the trapezoidal method. AUC from 0 to 2 min for intravenous administration was determined by extrapolation to a zero value by the mean of linear regression analysis. In average, $AUC_{0-2\ min}$ accounted for less than 5% of the $AUC_{0-60\ min}$. The bioavailability (F%) was calculated using:

$$F(\%) = \left(\frac{AUC_{i.n.(0-60min)} \times 3}{AUC_{i.v.(0-60min)} \times 7}\right)100\%$$

Statistically significant differences between the nasal formulations were tested by a Student's t-test. The results from one of the six sheep were discarded, because the nasal bioavailability was more than three S.D.'s higher than the total average.

3. Results and discussion

The sheep serum diazepam concentration profiles (Fig. 1) show higher blood levels after intravenous administration of 3 mg than after intranasal administration of 7 mg, indicating a relatively low nasal bioavailability. The initial bioavailability (30 min), t_{max} and C_{max} in sheep were found to be 15% (S.D. $\pm$ 8), 5 min (S.D. $\pm$ 3) and 934 ng/ml (S.D. $\pm$ 593), respectively (see Table 1). The bioavailability after 60 min was similar (14% $\pm$ 7). The very fast absorption found both in sheep, rabbit and man is beneficial in treatment of e.g. epileptic seizures, and emphasises one of the most important advantages of nasal delivery for acute purposes.

As mentioned, earlier studies of nasal administered diazepam in man (4 mg) and rabbit (3 mg) resulted in nasal bioavailabilities after 30 min of 34% (S.D. $\pm$ 18) and 54% (S.D. $\pm$ 11), respectively (Table 1). The initial (30 min) nasal bioavailability for sheep and rabbit is a factor of 2.3 lower and 1.6 higher than man, respectively. This significantly lower bioavailability found in sheep, $P < 0.05$ and $P < 0.001$ for man and rabbit, respectively, may be due to precipitation in the nose, limiting the free fraction of diazepam available for absorption as suggested by Lindhardt et al. (2001a). This may result in a very short (< 2 min) absorption phase.

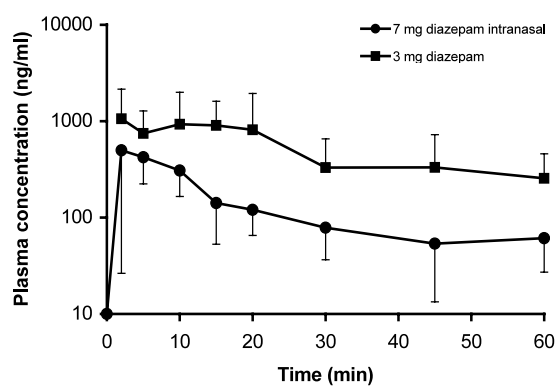

Fig. 1. Mean plasma concentration-time ($\pm$S.D.) profiles in sheep after intranasal administration of 7 mg diazepam PEG300 (●) and after intravenous administration of 3 mg diazepam (■).

Table 1
Mean time (t_{max}) to maximal serum concentration (C_{max}) and bioavailability of intra-nasal diazepam formulations from 0 to 30 min

	Species	Dose (mg)	Concentration mg/ml	N	t_{max} (min)	C_{max} (ng/ml)	Bioavail. (%)
i.v	Sheep	3	–	5	–	1618 ± 1016	100
	Rabbit	3	–	4	–	422 ± 146	100
	Man	2	–	9	–	132 ± 71	100
	Man	5	–	7	–	821 ± 567	100
i.n	Sheep	7	47	5	5 ± 3	934 ± 593	15 ± 8
	Rabbit	3	30	4	9 ± 3	235 ± 49	54 ± 11***
	Man	2	13	9	18 ± 11	39 ± 17	37 ± 17**
	Man	4	27	7	18 ± 9	99 ± 21	34 ± 18*
	Man	7	47	7	42 ± 23 #	179 ± 72	27 ± 20

Data for rabbit (Bechgaard et al., 1997) and man (Gizurarson et al., 1999 (2 mg), Lindhardt et al., 2001a (4 and 7 mg)) are also presented. The vehicle used for sheep and man was PEG300 and the vehicle used for rabbit was PEG200, which is very similar. *Significance level in relation to sheep ($P<0.05$), **Significance level ($P<0.01$), ***Significance level ($P<0.001$). # The t_{max} was relatively high, due to a second absorption phase, possibly in the mouth. Time to first peak was about 12 min.

Bioavailability data were calculated for the initial 30 min, because this period may be the most relevant period with respect to acute medical treatment. In acute situations, absorption rate is probably more important than the total bioavailability in a defined time period, why the finding of a good animal model for prediction of rate in man is of importance.

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 diazepam is to obtain an anti-convulsive effect as fast as possible, why 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 (Lindhardt et al., 2001b). Steadily increasing bioavailabilities, with time, are found for all doses in man, but the profiles for rabbit and sheep are more complex. The sheep availability is higher than man, in the early initial phase, but after 30 min the bioavailability appears higher for man. Therefore, the influence of observation period with respect to rate of availability is illustrated in Fig. 3. This profile was suggested by Lindhardt et al. (2001b), illustrating the fraction of bioavailability $(f(t) = (AUC_{i.n,\ t\ min.}/AUC_{i.v,\ t\ min})/(AUC_{i.n,\ 60\ min.}/AUC_{i.v,\ 60\ min.})$ at various times relative to the 60 min bioavailability (each species its own reference). It appears that all three nasal formulations administered to man have similar profiles with respect to rate. However, the correlation to sheep and rabbit

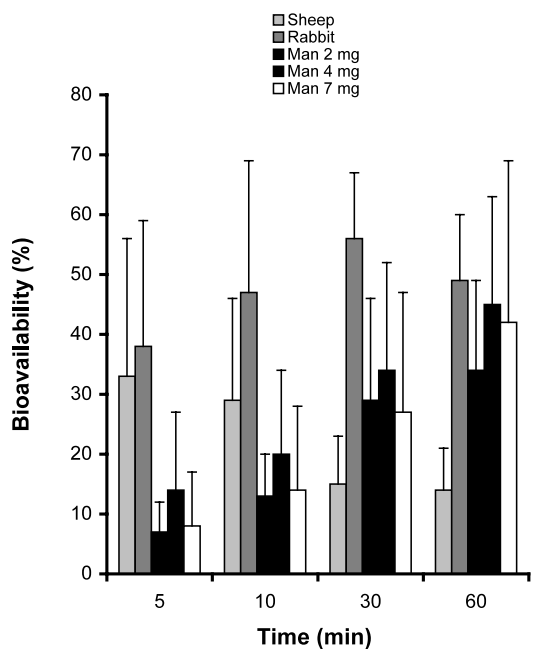

Fig. 2. The intranasal bioavailabilities in sheep after nasal administration of diazepam, solubilised in PEG 300, calculated for various initial periods up to 0–60 min. Data for rabbit (Bechgaard et al., 1997) and man (Gizurarson et al., 1999 and Lindhardt et al., 2001a) are also illustrated.

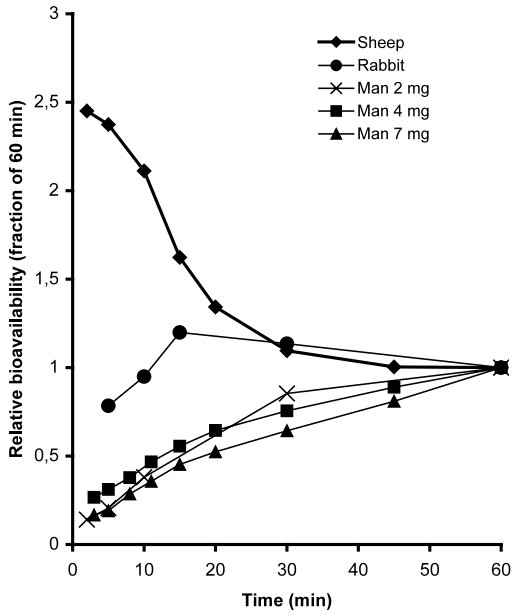

Fig. 3. Relative bioavailability at various times (fraction of the bioavailability after 60 min, each species its own reference) after nasal application of diazepam in PEG 300 (PEG 200 for rabbit) to sheep (♦), rabbit (●) and man (×, ■, ▲) for 2, 4 and 7 mg, respectively. Data for rabbit obtained from Bechgaard et al. (1997) and from man Gizurarson et al. (1999) and Lindhardt et al. (2001a).

is not optimal. The blood sampling from the jugular vein, for sheep, may explain the unusual fall in nasal bioavailability with time. This vein drains blood from the nasal mucosa and as diazepam has a high volume of distribution this effect may be very fast, resulting in an initial overestimation of nasal bioavailability (Lindhardt et al., 2001b). However, as the total bioavailability is low, blood sampling from another vein would probably make this discrepancy between sheep and man even more pronounced.

Li et al. (2000) used vehicles containing ethanol (30–60%) added to PEG400 and water mixtures to solubilise diazepam. The nasal bioavailabilities obtained were about 60% and t_{max} 2–4 min. It is questionable, however, whether this vehicle is clinically relevant with respect to local irritation.

The vehicle in the rabbit study was PEG 200, which is very similar to the PEG 300 used in man and sheep, and this small difference is not expected to influence the absorption. The EIA analysis was found to be as suitable for sheep serum as for human serum. As the sampling period is limited, and the specificity to diazepam is >5 times higher than to the major metabolite, the analytical method is suggested to be sufficiently specific (Lindhardt et al., 2001a).

In conclusion, the nasal absorption of diazepam in sheep was found to be fast ($t_{max} = 5$ min), indicating the relevance in acute treatment. The initial (30 min) nasal bioavailability for sheep and rabbit is a factor of 2.3 lower and 1.6 higher than man, respectively. Whether this low absorption could be due to precipitation in the nose is not known. The correlation of bioavailability was not optimal between sheep, man and rabbit, with differences both in relation to extend and rate, but the rabbit showed higher similarity to the human data than was the case for sheep.

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