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Pharmacokinetic and Pharmacodynamic Response after Intranasal Administration of Diazepam to Rabbits

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Abstract

Nasal application of drugs might be an alternative to intravenous administration in acute situations such as epileptic or fever seizures. In the search for a nasal formulation leading to a peak plasma concentration of diazepam at a $t_{max} \leq 5$ min bioavailability in rabbits has been studied after intranasal administration of the drug in ten vehicles of different polarity.

The animals were dosed with 3 mg diazepam, dissolved in 100 μL vehicle, the solution being administered into both nostrils. The bioavailability, measured during the first 30 min, because periods after this are not relevant for acute treatment, was found to be between 49 and 62% for the four most promising vehicles, pure glycofurol 75, tetraethyleneglycol, poly(ethylene glycol) 200 and 30% glycofurol in tetraethyleneglycol. The t_{max} for these vehicles was achieved after 5 min, and they induced a very rapid pharmacodynamic response after 1·5 to 3·5 min. The bioavailability was reduced when more polar liquids such as ethanol and tween 20, or lipid oils, e.g. vegetable oil and miglyol 840 were added to the glycofurol. There was a good correlation between t_{max} and the induction of pharmacodynamic response.

These results suggest that nasal application of diazepam in a water-free low-molecular-weight glycol might be of clinical importance as an alternative to intravenous injection, especially in acute situations.

The treatment of choice for epileptic seizures is intravenous administration of diazepam or clonazepam, followed by phenytoin or phenobarbital (Lott 1990). Intravenous administration, however, requires skilled personnel in an acute care facility. The facility requirements are important, because intravenous treatment is often associated with hypotension, cardiac dysrhythmias or central nervous system depression, including respiratory failure as a result of the high peak concentration. All this increases medical cost and family stress. Rectal administration of diazepam solution (clysma) might be an alternative to conventional therapy in children, but is not as practical for adults or outdoors. Kriel et al (1991) have found that rectal administration of diazepam was effective in controlling seizures (in 85% of patients) and improving the quality of life (in 58% of patients).

Various studies have been conducted in which benzodiazepines have been administered intranasally to animals and man. For man, the most common benzodiazepine has been midazolam, used to induce sedation in children (Wilton et al 1988; Kupietzky et al 1996; Zedie et al 1996). The drug was usually administered as an injectable parenteral formulation. Studies in which the plasma-concentration profiles are presented show that the time to maximum plasma concentration for midazolam, triazolam and flurazepam in dogs was achieved within about 15 min (Lui et al 1991); this is similar to results obtained for midazolam in children, when t_{max} was also approximately 15 min (Malinovsky et al 1993; Rey et al 1991; Fosel et al 1995). Studies with diazepam and lorazepam, however, show that t_{max} was achieved after about 1·4 and 2·3 h, respectively (Lau & Slattery 1989). Studies on rectal administration of diazepam show that suppositories are of limited value in emergency treatment. The therapeutic plasma concentration is not reached until after approximately 20 min (Knudsen 1979) whereas clysma can cause anticonvulsant activity after approximately 12 min, which is sufficiently rapid to control seizures in most patients suffering from multiple seizures, and thus to improve the quality of life (Kriel et al 1991). Therefore, an effective treatment within 5 min is an attractive goal.

Few formulation studies of solubility have been performed (Lau & Slattery 1989; Nielsen 1996). For nasal administration one clinical dose of, e.g., 10 mg diazepam must be contained in less than 300 μL because the largest volume which can be instilled into one nostril without excessive overflow is approximately 150 μL; above this the formulation will be drained out into the pharynx and swallowed. Therefore, the solubility required is 50 mg mL^{-1} or more. As diazepam is poorly water-soluble (0·05 mg mL^{-1} in water), good non-toxic excipients are required. No water can be added to reduce the local irritative potential of the formulation, although some local irritation might be acceptable if the cost-benefit ratio favours the use of the formulation. A group of excipients able to fulfil these criteria are low-molecular-weight glycols such as poly(ethylene glycol) 200, tetraethyleneglycol and glycofurol (Bechgaard et al 1997). The aim of this study is to estimate the bioavailability and t_{max} in rabbits for the most promising formulations, and to develop a pharmacodynamic model for estimating the onset of muscle relaxation.

Materials and Methods

Animals

Healthy New Zealand white rabbits, 2·8–3·2 kg, were used in all experiments. They were kept in single cages with free

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access to food and water. Most rabbits received more than one formulation, but with at least 3 weeks between experiments.

Chemicals

Diazepam and stesolid injection were kindly provided by Dumex (Copenhagen, Denmark); glycofurol 75 was from Hoffman-la Roche (Basle, Switzerland) and desmethyldiazepam from Ferrosan (Soeborg, Denmark). Poly(ethylene glycol) 200, tetraethyleneglycol, phosphoric acid, hexane, ethyl acetate, sodium carbonate and anhydrous sodium bicarbonate were purchased from Merck-Schuchard (Darmstadt, Germany); ethanol, tween 20 and vegetable oil from Mecobenzon (Copenhagen, Denmark); miglyol 840 from Eyvind Gunst (Dynamit Nobel, Troisdorf, Germany); acetonitrile and methanol from Rathburn Chemicals (UK) and nitrogen from Novo Nordisk (Bagsværd, Denmark). Other chemicals were of analytical grade.

Formulations

Stesolid for intravenous injection (5 mg mL^{-1}) was used as received. Diazepam was dissolved in: poly(ethylene glycol) 200, glycofurol, tetraethyleneglycol, 30% glycofurol in tetraethyleneglycol, 15% tween 20 in poly(ethylene glycol) 200, 30% glycofurol in vegetable oil, 30% glycofurol in miglyol 840, 10% ethanol in vegetable oil, 10% ethanol in miglyol 840 and 25% miglyol 840, and 25% poly(ethylene glycol) 200 in glycofurol; the concentration in each was 30 mg mL^{-1}. One group received pure diazepam powder.

Experimental procedures

The intravenous injection (0·66 mL) was administered into the exterior ear vein. Intranasal administration was performed while the rabbits were restrained in a pillory with their heads and bodies manually fixed. They received a 50 μL solution into each nostril by means of an Eppendorf pipette (Eppendorf, Hamburg, Germany). After each application, the pipette was examined for loss of dose. Experimental results were discarded if loss of dose was estimated at more than 20%. Venous blood samples were collected from the exterior ear vein into heparinized tubes before dosing and at 5, 10, 15, 30 and 60 min after dosing. Plasma was separated by centrifugation and stored frozen (−20°C) until analysed.

Pharmacodynamic tests

Two pharmacodynamic tests were developed to measure the time to muscle relaxation. In Test a the rabbit was laid down with both hind legs to one side; the rabbit had to remain in this position after being firmly tipped and with a finger on the hip, without trying to stand up. In Test b the rabbit was placed on its abdomen with both hind legs resting on a 12 cm high vertical board, placed behind the rabbit; the rabbit had to remain in this position after being firmly tipped and with a finger on the hip. Testing was performed 2–3 times min^{-1} and every 10–15 s after the first sign of effect. Test a was generally performed before b. Beyond 5 min the frequency of testing was as low as possible to avoid fatigue of the animals.

Analysis

Plasma diazepam and desmethyldiazepam were analysed by HPLC after extraction at approximately pH 9·7. Extraction buffer solution (0·8 M sodium bicarbonate and 0·21 M sodium carbonate; 500 μL), internal standard working solution (1 μg mL^{-1} midazolam in methanol; 100 μL) and hexane-ethyl acetate (7:3; 4 mL) were added to the plasma (300 μL). The mixture was rotated at 30 rev min^{-1} for 15 min and centrifuged at 850 g for 5 min. A portion (3·5 mL) of the organic layer was evaporated to dryness at 55°C by means of a stream of nitrogen. The dry extract was dissolved in mobile phase (200 μL) and 50 μL thereof was injected into the HPLC system.

HPLC was performed with a Hitachi L6000 pump, a Hitachi L4000 UV detector (operated at 242 nm) connected to a Hitachi D2000 integrator (Merck) and a Rheodyne model 7125 injection valve (Cotati, CA). The 120 mm × 4·6 mm × 5 μm Spherisorb S% ODS1 column (Phase Separations, Clwyd, UK) and 30 mm × 4·0 mm Perisorb RP18 pre-column (Merck) were installed in a column oven (Microlab Aarhus, Aarhus, Denmark) at 55°C. The mobile phase was 41% acetonitrile in phosphate buffer (7 mM, pH 3·7); the flow-rate was 1·5 mL min^{-1}.

Calculation and statistics

The area-under-the-curve (AUC) from 0–30 min was calculated by the trapezoidal method. All values were dose- and weight-corrected. Statistical procedures were performed according to standard methodologies; Student's *t*-test was used for calculation of significance.

Results and Discussion

A comparison of pharmacokinetic parameters after single intranasal administration of diazepam in various formulations and after single intravenous injection is presented in Table 1. The pharmacodynamic responses and the onset times are presented in Table 2. Four formulations, glycofurol, tetraethyleneglycol, 30% glycofurol in tetraethyleneglycol and 15% tween 20 in poly(ethylene glycol) 200, enhanced absorption so effectively that the maximum plasma concentration (t_{max}) was achieved after 5 min. The pharmacodynamic response for these formulations was also very rapid, after 2·75, 3·33, 1·5 and 3·5 min, respectively, for Test a. This rapid absorption and onset time would be sufficient for successful treatment of medical emergencies such as epileptic seizures.

Poly(ethylene glycol) 200, a mixture of miglyol, poly(ethylene glycol) 200 and glycofurol and a mixture of 30% glycofurol in vegetable oil resulted in a t_{max} of approximately 10 min and onset times of approximately 4·5, 4·5 and 9·0 min, respectively. For other formulations t_{max} and onset-time were longer ($\geq$15 min and $\geq$8 min, respectively). The absorption rate from formulations containing considerable amounts of oils such as vegetable oil and miglyols was slow, in accordance with results obtained in man using cremophor EL (t_{max} = 1·4 h; Lau & Slattery 1989) and in rabbits using α-tocopherol emulsions (20% absorbed 20 min after administration; Nielsen 1996).

The bioavailabilities of the most promising excipients were 63·2, 42·9, 64·6 and 58·1%, respectively, for glycofurol, tetraethyleneglycol, poly(ethylene glycol) 200 and 30% glyco-furol in tetraethyleneglycol. These values were calculated for 0–30% min only, because the period after this is not relevant in acute treatment and because most of intranasally administered

Table 1. Comparison of pharmacokinetic parameters after intravenous and intranasal administration of diazepam (3 mg) in various formulations to rabbits.

Formulation	n	Dose (mg kg^{-1})	Maximum concn (ng mL^{-1})	Time to maximum concn (min)	Area under the plasma concn-time curve (ng mL^{-1} min^{-1})	F (%)
Intravenous	4	1·15	423	5	8107	-
Intranasal						
Poly(ethylene glycol) 200	4	0·92	227	10	4350	54
Glycofurol 75	2	0·91	241	5	5027	62
Tetraethyleneglycol	3	0·80	231	5	3949	49
30% Glycofurol in tetraethyleneglycol	6	0·79	246	5	4417	54
15% Tween 20 in poly(ethylene glycol) 200	3	0·78	203	5	3907	48
30% Glycofurol in vegetable oil	2	1·01	110	10	2565	32
30% Glycofurol in miglyol 840	2	0·93	116	30	2451	30
10% Ethanol in vegetable oil	2	0·96	57	15	2931	36
10% Ethanol in miglyol 840	2	1·02	54	60	820	10
Miglyol, poly(ethylene glycol) and glycofurol	3	0·86	138	10	2997	37
Pure diazepam (powder)	3	1·01	26	60	536	7

All calculated values are dose corrected.

Table 2. Comparison of the time until pharmacodynamic responses are induced after intravenous and intranasal administration of diazepam (3 mg) in various formulations to rabbits.

Formulation	n	Dose (mg kg^{-1})	Time to maximum concn (min)	Time to induced effect (min)	
				Test a	Test b
Intravenous	4	1·15	5	2·3	2·0
Intranasal					
Poly(ethylene glycol) 200	4	0·92	10	4·5	5·5
Glycofurol 75	2	0·91	5	2·8	2·3
Tetraethyleneglycol	3	0·80	5	3·3	7·0
30% Glycofurol in tetraethyleneglycol	6	0·79	5	1·5	1·8
15% Tween 20 in poly(ethylene glycol) 200	3	0·78	5	3·5	6·0
30% Glycofurol in vegetable oil	2	1·01	10	9·0	No effect
30% Glycofurol in miglyol 840	2	0·93	30	9·0	4·0
10% Ethanol in vegetable oil	2	0·96	15	8·0	6·5
10% Ethanol in miglyol 840	2	1·02	60	No effect	No effect
Miglyol, poly(ethylene glycol) and glycofurol	3	0·86	10	4·5	5·0
Pure diazepam (powder)	3	1·01	60	No effect	No effect

drug is cleared towards the nasopharynx and swallowed into the stomach approximately 30 min after administration. Swallowed drug might be absorbed from the GI tract as an oral formulation. The concentration–time curves for the above-mentioned formulations are shown in Fig. 1. The curves show there is no difference between the four most promising excipients. The concentration–time curve for desmethyldiazepam is not given because for this formulation the concentration was very low during the first 30 min. Although the surface of the nasal cavity in man is relatively smaller than in rabbits (Gizurarson 1990), the observed bioavailability is considered to be of clinical relevance.

The pharmacodynamic test was developed as a simple and fast method of screening new formulations or other benzodiazepines. The need to develop and perform plasma analysis might, therefore, be reduced. Correlations between t_{max} and

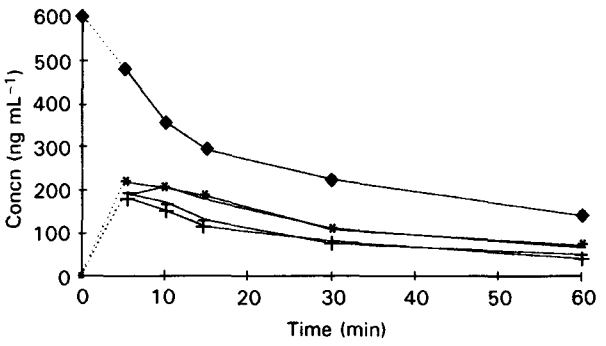

FIG. 1. Concentration–time profile for diazepam after intranasal and intravenous administration to rabbits. The diazepam was administered as: (◆) stesolid intravenous injection, (*) in glycofurol 75, (+) in tetraethyleneglycol, (△) in poly(ethylene glycol) 200, and (−) in a mixture of 30% glycofurol 75 in tetraethyleneglycol.

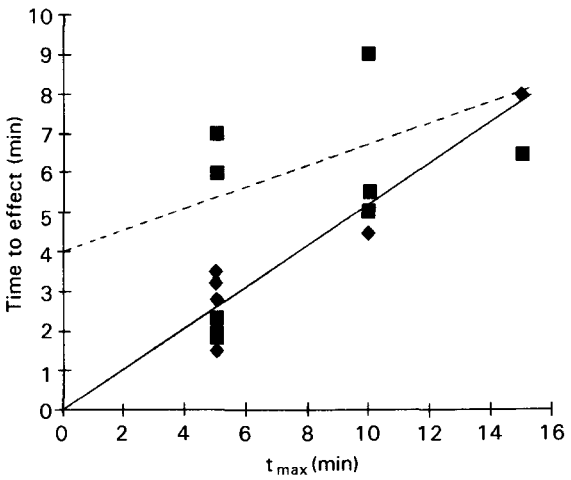

FIG. 2. Correlation between time to maximum plasma concentration and time to pharmacodynamic response for Test a (◆) and Test b (■). Only effects appearing within the first 15 min are included.

induction of pharmacodynamic response are shown in Fig. 2, from which it is apparent that the correlation for response times up to 15 min is relatively good for Test a ($r = 0{\cdot}82$), but not for Test b ($r = 0{\cdot}50$). Beyond 15 min the relationship is not linear. Therefore, Test a is preferred and a maximum response time of 10 min can be recommended. The observed fast response time might be a further indication of a possible direct 'pathway' from the nasal cavity to the brain, as described previously (Sakane et al 1991; Gizurarson et al 1997).

The data show that the nasal route might be an important site for emergency medication, such as the treatment of status epilepticus. The rate and extent of absorption and the rapid onset-time might be of clinical importance in the future.

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