

Two Intranasal Administration Techniques Give Two Different Pharmacokinetic Results

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Summary

Minor changes in the administration technique used for intranasal instillation of clonazepam, have been found to influence the results significantly. A simple study was performed, where rabbits received 0.5 mg clonazepam intranasally. One group received the drug while fixed in a sitting position, where the other group was fixed in a supine position. The results show that both techniques were able to provide a rapid absorption with a t_{\max} around 3–4 min. The C_{\max} and AUC, however, were very different. The C_{\max} was found to be 40 ng/ml and 86 ng/ml, respectively, and the AUC was found to be 891 and 2249 (ng/ml/min), respectively, for the sitting and the supine position. The relative bioavailability for *sitting/supine* was found to be 38%. These results show that the administration technique is very important and should not be underestimated.

Introduction

Intranasal administration of diazepam to rabbits has been shown to provide a rapid absorption with t_{\max} around 5 min. The results are reproducible and the onset time for the effect was found to be varying within 1.5–4.5 min (Bechgaard *et al.*, 1997). The success of each experiment depends highly on which techniques and animal species are used and how one masters the techniques (Gizurarson, 1993). Animal models influence the results so much that good results may be lost due to a wrongly chosen technique or animal species. The influence of techniques and models should therefore never be underestimated, even when simple studies are performed such as estimating the absolute bioavailability. In all experiments it is important to know the anatomy and the physiology of the animal

model chosen and to understand its limitations and how it may influence the results.

The rabbit is a common animal model for intranasal drug delivery studies due to the size of the nasal cavity; also blood samples may be collected frequently and easily (Gizurarson, 1990). The rabbit nasal cavity has a similar surface area to the human nasal cavity or 60 cm² and 160 cm² for rabbit and human, respectively.

The aim of the study was to investigate how two administration techniques, used to instill drug into the nasal cavity of rabbits, may influence the pharmacokinetics of clonazepam. Two techniques are compared, the administration to rabbits in either sitting or supine position during the application.

Material and Methods

Clonazepam was kindly provided by Roche A/S (Hvidovre, Denmark), glycofurolosum 75 (GF) was from Hoffman-la Roche (Basle, Switzerland), desmethyldiazepam from Ferrosan A/S (Søborg, Denmark) and highly purified tetraethyleneglycol (4EG) was purchased from Fluka Chemie AG (Buchs, Switzerland). All other chemicals were of

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analytical grade.

The clonazepam was dissolved in a solution containing 5% GF in 4EG, by the means of an ultrasonic bath (5 mg/ml). The formulation was prepared fresh prior the study.

Sixteen New Zealand White rabbits, weighing about 3 kg (Novo Nordisk A/S, Bagsværd, Denmark), were divided into two groups. Each rabbit received 50 µl into each nostril, using an Eppendorph pipette.

The first group was manually fixed in a supine position during and for about 1 min after the administration.

The second group was fixed in a sitting position during the administration.

Blood samples were collected by venepuncture of the marginal ear vein prior to dosing and at 5, 10, 20, 30 and 60 min. after dosing. Plasma was separated by centrifugation and stored at -20°C until analysed on HPLC.

The plasma concentration of clonazepam was analysed by the HPLC method described by Bechgaard *et al.* (1997) and calculated on the basis of the peak height, relative to an internal desmethyl-diazepam standard.

Standard statistical methods (*t*-test) were used throughout. All pharmacokinetic values were corrected, relative to the dose/weight relationship. The area under the plasma concentration curve (AUC) was calculated, using the trapezoidal method.

Results and Discussion

The results from the experiments are shown in Table 1 and Fig. 1. The tables show that both techniques were equally rapid, relative to the rate of absorption (t_{\max} was 2.75 to 4.00 min), but the amount absorbed was greatly affected by the techniques. The relative bioavailability (F_{rel} (*sitting/supine*)) was only 37.7% and the C_{\max} was found to be 36.9 ± 20.3 ng/ml and 85.9 ± 21.8 ng/ml for the sitting and supine position, respectively. The results were significant ($p < 0.05$) and show that the right choice of technique has much more influence than expected.

Various factors may affect the systemic absorption of the drugs from the nasal cavity such as the anatomy, the mucociliary clearance and the epithelial barrier in different regions of the nasal cavity. Intranasal administration of a dye showed that the distribution inside the nose was not the same for these two techniques (unpublished results), but it has been described that the location of the drug inside the nasal cavity is of importance (Johansson *et al.*, 1991). Necropsy of rabbits receiving instillation of a dye in a sitting position, show that the dye is solely found on the septum and the nasal floor. Rabbits, however, fixed in a supine position, had the dye distributed all over the middle nasal conchae as well as the septum and the nasal floor. The conches are the most vascularised area inside the nasal cavity, which may explain the outcome of the results. Other factors such as the viscosity, the vol-

Table 1.

Kinetic parameters for clonazepam in rabbits after single 0.5 mg intranasal dose, using two techniques (n=8).

	Sitting position	Supine position	% difference (supine/sitting)
Dose (µg/kg)	165 ± 10	172 ± 11	4
C_{\max} (ng/ml)	37 ± 20	86 ± 22	233
t_{\max} (min)	4,0 ± 4,6	2,8 ± 1,4	69
k_{abs} (ng/min)	15 ± 12	38 ± 17	250
k_e (ug/min)	11 ± 6	15 ± 4	137
$t_{0,5}$ (min)	62 ± 20	45 ± 9	73
AUC (ng/ml/min)	891 ± 523	2249 ± 320	253

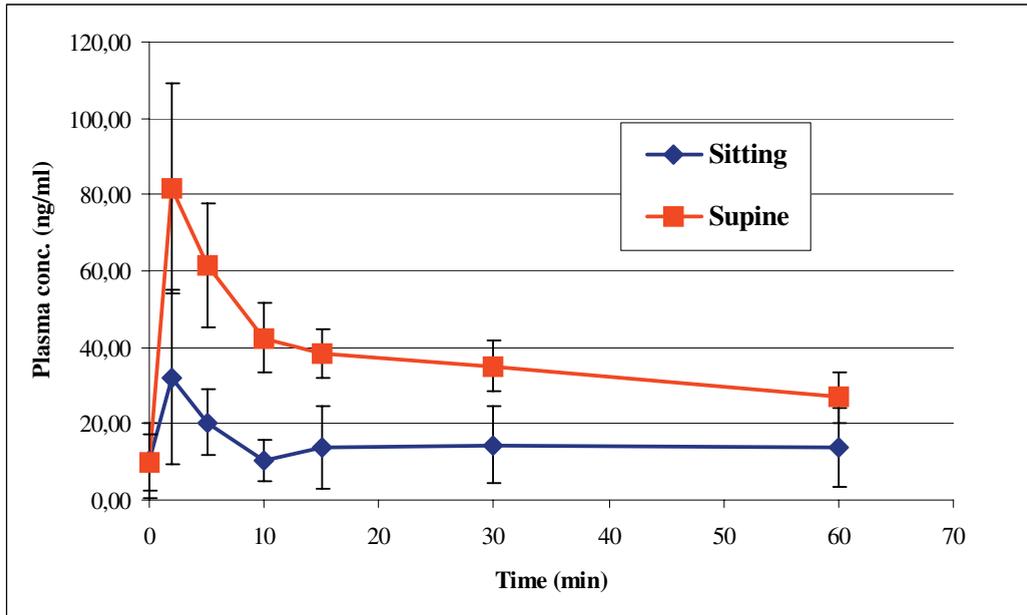


Figure 1

Plasma concentrations after intranasal administration of 50 μ l clonazepam solution (5 mg/ml) into each nostril. ■— supine position; ◆— sitting position.

ume of the vehicle, the bend from the nostrils into the nasal cavity, the droplet size and the spray characteristics may also affect where and how far into the nasal cavity the drug may travel (*Harris et al., 1998*) and may also affect the results. It is also important to keep the volume adequate so the drug is inside the nose and not lost in the interior or to the nasopharynx: too many work has been published where up to 100 μ L have been administered “intranasally” to mice, without checking the total nasal volume of the animal (which is only 22 μ L). In some animal species, it is almost impossible to administer the formulations intranasally, unless the animal is kept in a supine position, such as in mice (*Egino et al., 1999*). Due to the narrow nostrils, the small volume administered and the risk of failure during administration, it is important to keep the animal in a supine position and allow the animal to assist in receiving the formulation by using each inhalation to deliver the drug into the nose.

A study in humans, using 100-750 μ L in one nostril, administered either as drops in a supine position or by spraying (over 2-3 sec with steady pressure and gently inhalation by the volunteer) in the sitting position supports these findings (*Aoki & Crawley, 1976*). Two formulations were tested, 3 or 30% HSA, labelled with ^{99m}Tc (the 30% solution could not be sprayed). Although the results could not be translated into recommendations for intranasal dosing, there was an indication that droplets administered in a supine position resulted in good distribution for all volumes tested. Surprisingly, in this study, all spray resulted in poor distribution, which may indicate that the technique was not optimal. For spraying, the authors used a Jencon Repette-injector designed to produce fine spray (droplet size not indicated), where the tip was held horizontally, resting just inside the nasal orifice, which may indicate that the majority of the spray did not reach the cavity of the nose.

In conclusion, it has been shown that the technique used for instilling a drug into the nasal cavity has more influence on the outcome than expected. A small and simple change in the administration technique may result in higher bioavailability. A factor of 2.5 fold increase in bioavailability was observed in this study.

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