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In situ nasal absorption of midazolam in rats

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Abstract

Intranasal (i.n.) midazolam (MDZ) administrations may be used successfully for preoperative sedation, especially in young patients. However, clinicians have to use the commercial parenteral formulation, the low pH of which (3.3), necessary to solubilize MDZ (pK_a 6.1), is probably responsible for the signs of local irritation frequently reported. As a starting point to design a formulation suitable for the nasal route, MDZ nasal absorption was investigated in rats. The effects of the MDZ solution concentration (10–100 μg/ml), osmolality (from less than 10 mOsm/kg up to 450 mOsm/kg) and pH (3.3–7.4) were studied using an in situ perfusion technique. MDZ was determined by reversed-phase HPLC in the circulating solution and results were expressed in clearance terms. MDZ absorption was independent of its concentration. The pH of the solutions was the key-parameter and only a pH above 4 allowed significant absorption. These results were consistent with a passive diffusion absorption of MDZ and partly followed the pH partition theory. In conclusion, satisfactory MDZ absorption should be expected with a formulation at a pH suitable for the nasal route in human (5.5–6.5). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Intranasal administration; Midazolam; pH; Osmolality

1. Introduction

Midazolam (MDZ) is a short-acting benzodiazepine derivative frequently used for preanaesthetic sedation in children. Satisfactory results have been obtained after intravenous (Cole, 1982) or intramuscular administrations (Rita et al., 1985). Alternative routes for administration, such as rectal (Saint-Maurice et al., 1986), nasal (Wilton et al., 1988; Slover et al., 1990) and sublingual (Odou et al., 1998), have been proposed to solve the problem of young patients' stress induced by injections. Rectal and intranasal (i.n.) routes resulted in successful preoperative sedation. The latter is more rapidly effective (Slover et al., 1990), but signs of local irritation have been frequently reported (Rey et al., 1991; Zedie et al., 1996). With no formulation specifically available for the nasal route, clinicians have to use the parenteral formulation (Hypnovel, Roche). The low pH (3.3) of this formulation, required for adequate solubilization of the basic drug ($pK_a = 6.1$, Odou et al., 1998), is likely to have been responsible for the observed irritating

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effects. The aim of this study was to investigate formulation parameters, such as pH and osmolality, frequently shown to affect nasal absorption of drugs, in order to guide the formulator in the design of a novel formulation leading to satisfactory absorption and good tolerance by the i.n. route.

2. Materials and methods

2.1. Chemicals

MDZ base powder, Mw 325.77, was a gift from Roche (Basle, Switzerland) and MDZ solution for intravenous use (Hypnovel® 50mg/10ml), referred to as commercial MDZ formulation, was obtained commercially.

2.2. Animals

This work was done in accordance with the Principles of Laboratory Animal Care (NIH Publication no. 86-23, revised 1985). Male Sprague–Dawley rats (320 ± 30 g) obtained from Déprés Breeding Laboratories (St Doulchard, France) were housed in the animal breeding facilities of the laboratory (Authorization No. 0028) for 4–6 days before experiments. They were maintained in a light- (12-h light-dark cycle) and temperature-controlled environment, with food and water available ad libitum. Food was withdrawn 24 h before experiments.

2.3. In situ nasal perfusion experiments

2.3.1. Procedure

The absorption studies were carried out according to the in situ nasal perfusion technique of Hirai et al. (1981). Rats were anesthetized by intraperitoneal injections of sodium pentobarbital (60 mg/kg body weight). An incision was made in the neck of the rats laid on their backs and placed under a heating lamp to maintain body temperature. The trachea was cannulated with a polyethylene tube to aid breathing. Another tube was inserted through the esophagus into the posterior part of the nasal cavity. The nasopalatine duct was closed with an adhesive agent (cyanoacrylate glue) to prevent the drainage of the solution from the nasal cavity into the mouth. The tube inserted into the esophagus was connected to a reservoir of 50 ml drug solution under magnetic stirring and immersed in a water-bath at 37°C. The solution was circulated, by means of a peristaltic pump (Minipuls II, Gilson) from the reservoir through the nasal cavity and out of the nostrils back into the reservoir. Flow rate was set at 1 ml/min. Aliquots (200μl) were sampled every 5 min during 1 h and stored at -20°C until the MDZ assay. The pH of the circulating solutions was recorded at each sampling time with a pH-meter Knick Portamess 751 (Bioblock Scientific, Illkirch, France). Three rats were used for each condition tested.

2.3.2. Parameter tested

The effect of input rate, osmolality and pH on MDZ nasal absorption was assessed as follows.

2.3.2.1. Input rate. MDZ solutions, 10, 25, 50 or 100 μg/ml, were prepared by dilution of the commercial formulation with distilled water adjusted to pH 3.3 with 1 N hydrochloric acid. Osmolalities were determined to be less than 10 mOsm/kg using an Osmometer Automatic Roebling (Bioblock Scientific).

2.3.2.2. Osmolality. MDZ solutions, 50 μg/ml, were prepared in distilled water or in hypoosmotic (0.45% m/v sodium chloride), isoosmotic (0.9%) or hyperosmotic (1.35%) solutions. All the solutions were brought to pH 3.3 with 1 N hydrochloric acid. Respective osmolalities were determined to be 3, 142, 285 and 450 mOsm/kg.

2.3.2.3. pH. MDZ solutions, 50 μg/ml, were prepared in 0.01 M citrate buffer (pH 3.3), in 0.01 M acetate buffer (pH 5.5), or in 0.01 M phosphate buffer (pH 7.4). Solutions were brought to isoosmolality (285 mOsm/kg) with sodium chloride.

2.4. MDZ HPLC determination

The chromatographic system consisted of a pump LC-10AT Shimadzu, an autosampler

SP8780 Spectra-Physics and a spectrophotometer SpectroMonitor III MaxN® series. MDZ was analyzed by isocratic reversed-phase HPLC using a Nucleosil C18 Column 5 μm Interchrom, 150 × 4 mm (Interchim, Montluçon, France), a mobile phase made of 70:30 methanol: 10 mM potassium phosphate buffer, adjusted to pH 8 with 0.1N potassium hydroxide, at a 1 ml/min flow rate, and a 218 nm detection wavelength. Injection volume was 20 μl and MDZ concentration range for calibration was from 5 to 100 μg/ml. MDZ retention time was 5.5 min. Data were recorded and processed with an integrator SP4290 Spectra-Physics.

2.5. Data analysis

For comparison purposes, MDZ concentrations were expressed as the percentage of the initial concentration. The first-order rate constants of the decay of MDZ concentrations with time, k_{obs}, were estimated by linear regression analysis of the Log concentration versus time data, and used to calculate the nasal absorption clearance ($CL_{Nas,Abs}$), according to Eq. (1).

$$CL_{Nas,Abs} = k_{obs} \times V \quad (1)$$

where V corresponds to the volume of circulating solution (50 ml). Results are expressed as mean ± standard error (SE). Data were compared by the non-parametric Mann–Whitney test (for two groups) or the Kruskal–Wallis test followed by the Dunn's multiple comparison post test (for more than two groups), with the level of significance set at $P < 0.05$.

3. Results

In control experiments performed without animals, MDZ loss by adsorption onto or absorption into the tubes of the perfusion system was found to be insignificant at pH 3.3 and 5.4. At pH 7.4 (50 μg/ml MDZ concentration), however, MDZ concentration decreased by less than 10% after 1 h circulation of the solution in the tubes. Considered as negligible, this loss was not taken into account for data processing.

3.1. Effect of input rate

For all the input rates, a 15–20 min lag time was observed, followed by an apparent first order absorption process (Fig. 1). Input rates seemed to have no effect on the MDZ nasal absorption phase, which was confirmed by the non significant difference between clearance values (Table 1). The pH of the circulating solution rose from 3.3 at the beginning of the experiment to approximately 6.5 at the end (Fig. 2). Interestingly, absorption started at a pH value close to 4.

3.2. Effect of osmolality

Osmolalities from 142 to 450 mOsm/kg precluded MDZ absorption (Fig. 3). Only the very hypoosmotic solution (3 mOsm/kg) led to a significant absorption. The pH rose from 3.3 to 6.5 for the solution at 3 mOsm/kg (see Fig. 2, 50 μg/ml curve), and to 4.9, 3.7 and 3.9 for the solutions of osmolalities of 142, 285 and 450 mOsm/kg respectively.

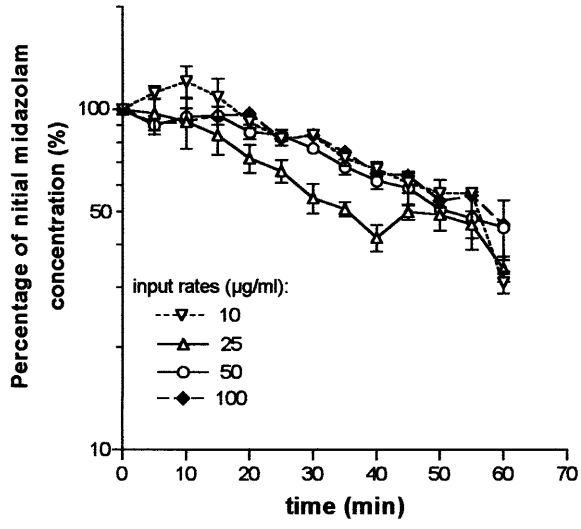

Fig. 1. Effect of the input rate on midazolam absorption. Conditions: unbuffered solutions, initial pH: 3.3, osmolality < 10 mOsm/kg ($n = 3$ rats per group).

Table 1
Effect of the initial input rate on midazolam nasal absorption clearance[a]

Initial input rate (μg/min)	$CL_{Nas,\ Abs}$ (ml/min)			
	Individual values			Mean[b] ± SE
10	1.07	1.02	1.02	1.04 ± 0.02
25	1.03	0.82	0.69	0.85 ± 0.10
50	1.08	1.10	0.91	1.03 ± 0.06
100	0.95	0.85	0.93	0.91 ± 0.03

[a] Conditions: unbuffered solutions, initial pH: 3.3, osmolality < 10 mOsm/kg.
[b] Not significantly different by the Kruskal–Wallis test.

3.3. Effect of pH

With solutions buffered at pH 5.5 or 7.4, MDZ absorption started without any lag time, whereas no absorption was observed at pH 3.3 (Fig. 4 and Table 2). Clearance values were not significantly different with the solutions at pH 5.5 or 7.4.

4. Discussion

The model of Hirai et al. (1981) is a useful tool to obtain accurate information about the basic parameters, such as pH and osmolality of formulations, that govern drug nasal absorption. It is the first time, to our knowledge, that a clearance term is applied to this model to characterize drug absorption. The apparent first order rate constant of absorption k_{obs} frequently used to describe the absorption process (Hirai et al., 1981) has been shown to be dependent on the volume of the circulating solution, since it relates the rate of elimination to the amount of circulating drug (Huang et al., 1985). As the volume of the circulating drug solution varies according to experimental conditions, k_{obs} cannot be used to characterize drug absorption. For example, in our experiments 50 ml were specifically chosen to obtain quantifiable decreases in MDZ concentration over a 1 h experimental run. In order to obtain a parameter independent of the volume of the circulating solution, the intrinsic absorption rate constant k_a, defined as the apparent rate constant k_{obs} multiplied by the volume of the perfusing solution, was introduced by Huang et al. (1985). In term of units (volume divided by time), k_a is actually a clearance term and corresponds to the volume of perfusing solution cleared from the nasal cavity per time unit due to nasal absorption. This term was therefore referred to as nasal absorption clearance $CL_{Nas,Abs}$ and was calculated according to Eq. (1).

Nasal MDZ absorption experiments performed with unbuffered solutions, at pH 3.3 and at a low osmolality (less than 10 mOsm/kg), showed that absorption occurred after a lag time of 15–20 min (Fig. 1). After this lag time, the log concentrations declined linearly with time, and clearances were independent of the initial input rates (Table 1). These results are in agreement with a passive

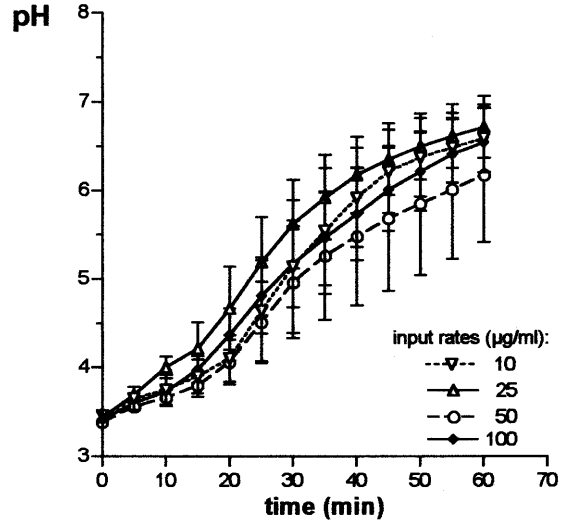

Fig. 2. Monitoring of the pH of the circulating solutions used in Fig. 1.

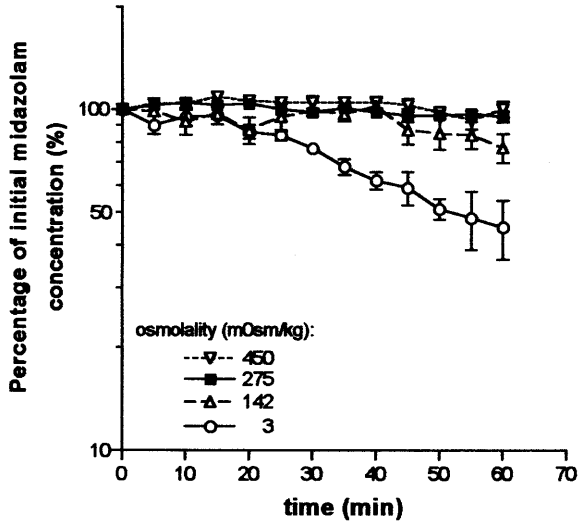

Fig. 3. Effect of osmolality on midazolam absorption. Conditions: unbuffered solutions, initial pH: 3.3, 50 μg/ml initial input rate ($n = 3$ rats per group).

diffusion entry of the drug through the nasal mucosa (Hirai et al., 1981). The observed lag time corresponded to the time needed for the pH to reach a value close to 4 (Fig. 2). Nasal secretions probably accounted for the change of pH. The effect of these secretions might depend on the

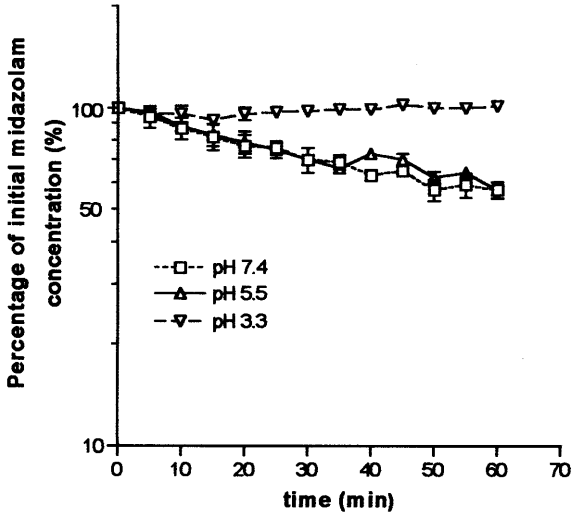

Fig. 4. Effect of the pH on midazolam absorption. Conditions: buffered solutions, isoosmolality, 50 μg/ml initial input rate ($n = 3$ rats per group).

Table 2
Effect of the pH on midazolam nasal absorption clearance[a]

pH	$CL_{Nas,\ Abs}$ (ml/min) Individual values			Mean[b] ± SE
7.4	0.50	0.50	0.45	0.48 ± 0.02
5.5	0.42	0.47	0.40	0.43 ± 0.02
3.3	n.d.[c]	n.d.	n.d.	–

[a] Conditions: buffered solutions, isoosmolality, 50 μg/ml initial input rate.
[b] Not statistically different by the Mann–Whitney test.
[c] Not determined (value close to 0).

osmotic pressure of the circulating solutions, as almost no change of pH, and consequently no absorption, was observed with solutions at pH 3.3 which had osmolalities of 142 mOsm/kg and above (see results for pH values and Fig. 3). Studies performed in buffered conditions confirmed pH as the main parameter for MDZ nasal absorption (Fig. 4 and Table 2). Absorption was significant and immediate at pH 5.5 or 7.4 leading to virtually equal clearance values, but no absorption occurred at pH 3.3. The nasal absorption of weak electrolytes has been shown to be governed, at least in part, by the ionization state of the diffusing molecules, in agreement with the pH partition theory (Huang et al., 1985). MDZ is a basic drug with a pK_a of 6.1 (Odou et al., 1998). Its absorption started at a pH around 4, i.e. when the drug existed to the extent of at least 1% in its unionized form. Surprisingly, absorption rate appeared rapidly to reach a maximum, as shown by the linearity of the absorption profiles (Fig. 1) despite further pH increase (Fig. 2). This observation is in agreement with the non-significantly different absorption profiles observed at pH 5.5 and 7.4 (Fig. 4 and Table 2), whereas the unionized form represented 20 and 95% respectively. Further experiments need to be carried out to elucidate this deviation from the theory.

Hypoosmolality seemed to play an additional role when pH conditions permitted absorption (above 4). Indeed, the clearance values obtained with the hypoosmotic unbuffered solution (Table 1) were twice the values obtained with solutions buffered at pH 5.5 or 7.4 which were isoosmotic

(Table 2). Hypoosmotic solutions which cause swelling were shown to alter the epithelium paracellular permeability (Pujara et al., 1995). Therefore, in addition to a passive diffusion through epithelial cells, a paracellular diffusion of MDZ could take place, at least in conditions where mucosa is swollen by hypoosmotic solutions. As such conditions may have deleterious effects on the mucosa, isoosmotic conditions should be retained.

In conclusion, MDZ diffusion through the nasal mucosa was highly dependent on the pH of the formulation, and was significant at pH above 4. Formulation of MDZ at pH appropriate for the nasal route in humans (around 6, Washington et al., 2000) should therefore lead to immediate and effective absorption. Satisfactory MDZ solubilization at this pH remains to be solved.

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