Pharmacokinetics and pharmacodynamics of midazolam administered as a concentrated intranasal spray. A study in healthy volunteers

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Aims To investigate the pharmacokinetic and pharmacodynamic profile of midazolam administered as a concentrated intranasal spray, compared with intravenous midazolam, in healthy adult subjects.

Methods Subjects were administered single doses of 5 mg midazolam intranasally and intravenously in a cross-over design with washout period of 1 week. The total plasma concentrations of midazolam and the metabolite 1-hydroxymidazolam after both intranasal and intravenous administration were described with a single pharmacokinetic model. β-band EEG activity was recorded and related to midazolam plasma concentrations using an exponential pharmacokinetic/pharmacodynamic model.

Results Administration of the intranasal spray led to some degree of temporary irritation in all six subjects, who nevertheless found intranasal administration acceptable and not painful. The mean (±s.d.) peak plasma concentration of midazolam of 71 (±25 ng ml^{-1}) was reached after 14 (±5 min). Mean bioavailability following intranasal administration was 0.83±0.19. After intravenous and intranasal administration, the pharmacokinetic estimates of midazolam were: mean volume of distribution at steady state 1.11±0.25 l kg^{-1}, mean systemic clearance 16.1±4.1 ml min^{-1} kg^{-1} and harmonic mean initial and terminal half lives 8.4±2.4 and 79±30 min, respectively. Formation of the 1-hydroxymetabolite after intranasal administration did not exceed that after intravenous administration.

Conclusions In this study in healthy volunteers a concentrated midazolam nasal spray was easily administered and well tolerated. No serious complications of the mode of administration or the drug itself were reported. Rapid uptake and high bioavailability were demonstrated. The potential of midazolam given via a nasal spray in the acute treatment of status epilepticus and other seizure disruptions should be evaluated.

Keywords: formulation, intranasal, intravenous, midazolam, modelling, pharmacokinetics pharmacodynamics, PK-PD

Introduction

The benzodiazepine diazepam is a standard treatment in the acute management of all types of seizures in both adults and children. However, it does have disadvantages including a short duration of action and a tendency to accumulate if repeated doses are given. For acute seizures diazepam must be applied either intravenously or rectally in order to reach an effective blood concentration as soon as possible. Non-professional carers may be reluctant to administer midazolam by these routes [1, 2].

The use of the water soluble benzodiazepine midazolam hydrochloride is well established as a premedicant, anxiolytic and anaesthetic induction agent [3]. Its safety and efficacy in the acute treatment of seizure exacerbations is well documented [4]. The efficacy of intranasal midazolam as premedication and sedative prompted its potential use in the management of acute seizures [1, 5–8]. The nasopharyngeal mucosal surface is relatively large

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and well vascularized, allowing for a rapid absorption of midazolam. Nasal absorption also avoids the high first-pass metabolism of midazolam after oral administration [9].

The dose of intranasal midazolam for treating seizure activity is based on body weight. It is 0.2 mg kg^{-1} for children, 5 mg for adults under 50 kg, and 10 mg of midazolam for adults weighing more than 50 kg. In most studies on intranasal midazolam, the undiluted, commercially available parenteral fluid containing 5 mg ml^{-1} midazolam has been used. This requires a relative large volume, 1–2 ml in adult patients, to be applied, which may account for the lacrimation, burning and general discomfort that is associated with intranasal midazolam [10, 11]. A significant amount of the fluid can be swallowed and absorbed from the gastrointestinal tract, which decreases bioavailability and therefore reduces efficacy [10]. Furthermore, treatment failure may occur due to poor technique in delivering an adequate volume of midazolam liquid [8].

These disadvantages might possibly be overcome by increasing the concentration of midazolam, thereby reducing the total volume of fluid to be delivered. In the present study we have investigated a concentrated intranasal formulation of midazolam. The aim was to compare the pharmacokinetic and EEG pharmacodynamic parameters after intranasal and intravenous administration of midazolam to healthy volunteers.

Methods

Midazolam formulations

The study medications were prepared by the Apotheek der Haarlemse Ziekenhuizen Hospital Pharmacy. Intravenous midazolam 5 mg ml^{-1} (pH 3.3) was formulated using midazolam hydrochloride (Spruyt Hillen, IJsselstein, The Netherlands). The intranasal midazolam formulation contained midazolam hydrochloride in a mixture of water and propylene glycol (pH 4). Benzyl alcohol 1% v/v was added as an antimicrobial preservative.

The intranasal device used (Spruyt Hillen) delivered an equivalent dose of 2.5 mg midazolam with each 90 μl spray. The stability of both formulations was investigated using a validated h.p.l.c. analytical method. During a 6 month test period at ambient temperature no significant changes in pH or midazolam concentration occurred. Kept in the dark, the solutions remained clear and colourless.

Subjects

The Ethics Review Board of the Leiden University Medical Centre approved the investigational protocol for this study. Six nonsmoking volunteers (two female, four male) provided informed consent and participated in the study. Their mean age was 40 ± 9 years (mean ± s.d., range: 27–47), and mean weight was 74 ± 5 kg (range: 66–80). None had a history of cardiac or neurological disease. They were not allowed to take any medication on a regular basis or benzodiazepines in the week before the study days. All subjects were healthy as assessed by medical history and physical examination, including ECG and blood pressure, and clinical chemical laboratory tests. The background EEG was normal.

Study design

The study was an open, crossover trial. On the two study days the subjects fasted and refrained from caffeine-containing beverages until 2 h after midazolam administration. Alcoholic beverages were avoided from the day prior to the study until completion of the study day. All subjects received a single dose of 5 mg midazolam either intravenously or intranasally, in random order with a 7 day washout period between treatments.

Intravenous midazolam was given as a bolus injection over 30 s into a peripheral vein of the lower arm. Intranasal midazolam was self-administered by one spray in each nostril. Subjects remained supine for the first 2 h of study. Blood samples were collected from a forearm vein in heparinized tubes. In addition to a pre-dose sample, blood was collected at 2, 5, 10, 15, 20, 30 and 60 min and 2, 3, 4, 5, 6, 7, and 8 h postdose. The blood samples were kept on ice for a maximum of 2 h until centrifuged. Plasma samples were stored frozen at -20° C until assayed.

Analysis of midazolam and 1-hydroxymidazolam

Plasma concentrations of both compounds were determined using a specific h.p.l.c. method with u.v. detection at 220 nm. Plasma aliquots of 1 ml were mixed with 40 ng of the internal standard chlordesmethyldiazepam (Hoffman-La Roche, Basel, Switzerland) and 0.5 ml sodium hydroxide (0.1 M). Following liquid-liquid extraction with 5 ml cyclohexane–dichloromethane (55–45 v/v), the organic layer was evaporated. The residue was dissolved in 250 μl water-acetonitrile (95–5 v/v) and 200 μl were injected onto the chromatograph. Separation was achieved on a custom-made column (15 × 0.46 cm) packed with Inertsil ODS-3 C-18 (Varian Chrompack, Houten, The Netherlands) with an isocratic mobile phase of 0.1 M phosphate buffer pH 7.0-acetonitrile (65–35 v/v).

Calibration curves were linear over the range of 0–400 ng ml^{-1} ($r^2 > 0.99$). Inter- and intra-day coefficients of variation were less than 7%. The limit of quantification for both compounds was 0.5 ng ml^{-1}.

EEG recordings

Ten silver-silver chloride electrodes were placed on the skull of the subjects at positions following the International 10–20 system of electrode placement. EEG was recorded using a 32 channel digital EEG (Vickers Medelec DG32) with a bandwidth of 0.5–50 Hz, gain 7 $\mu V\ mm^{-1}$, sample frequency 240 Hz. The EEG data were filtered, electrical noise reduced, digitized and stored on optical disks for later analysis. In addition, during acquisition the raw data were examined visually for artefacts.

Two-minute EEG-time epochs were analysed from 5 min before and 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 min after midazolam administration. During EEG recording, subjects closed their eyes. The EEG signals were quantified by use of fast Fourier transformation. The averaged amplitudes (square root of power (μV^2)) in the 14–40 Hz beta frequency band were calculated and used as the EEG effect measure.

Safety and tolerability assessment

After administration of midazolam, subjects were periodically questioned and monitored for any unusual symptoms. They were asked specifically to record any sensation (bitter taste, burning sensation, pain) experienced after intranasal administration.

Pharmacokinetic analysis

The time course of midazolam concentrations was described by a two-compartment model, whereas for 1-hydroxymidazolam, a one-compartment model was used. In the model development process, discrimination was based on visual inspection of the plasma profiles and the residuals, and on Akaike's information criterion [12]. The concentration data were weighted using iterative reweighting (WinNonlin version 3.1). The model consisted of the following differential equations, each describing the amounts of the parent compound midazolam (A_P) in the central and peripheral compartment denoted by the subscripts 1 and 2, respectively:

$$\frac{dA_{P1}}{dt} = k_A \cdot F \cdot D \cdot e^{-k_A \cdot t} - k_{10} \cdot A_{P1} - k_{12} \cdot A_{P1} + k_{21} \cdot A_{P2} - k_M \cdot A_{P1} \quad (1)$$

$$\frac{dA_{P2}}{dt} = k_{12} \cdot A_{P1} - k_{21} \cdot A_{P2} \quad (2)$$

in which F and D denote the bioavailability and dose, respectively. Absorption of midazolam from the nasal mucosa was described with a first order absorption-rate constant (k_A) and a lagtime (t_{lag}). k_A was reduced to zero for intravenous administration and when $t < t_{lag}$. k_M is the first-order rate constant for the conversion of midazolam to 1-hydroxymidazolam.

The amount of the active metabolite 1-hydroxymidazolam in the central compartment (A_M) was described by:

$$\frac{dA_M}{dt} = k_M \cdot A_{P1} - k_{10OH} \cdot A_M \quad (3)$$

here k_{10OH} denotes the elimination rate constant of 1-hydroxymidazolam.

Estimation of the volume of distribution of 1-hydroxymidazolam was not possible because inclusion of this parameter causes of the model. To solve this problem, the amounts of midazolam and of 1-hydroxymidazolam were related to the observed concentrations by dividing by the volume of distribution of midazolam (V_C). Previously, the volumes of distribution of midazolam and the metabolite were shown not to differ from each other [9]. Nevertheless, estimates of k_{10}, k_M and k_{10OH} would be affected if these volumes did differ. Therefore calculation of the clearance (CL) and half-life ($t_{1/2}$) of midazolam was based on the sum of k_{10} and k_M, and were calculated using standard equations [13]. Equations 1 and 2 cannot be solved algebraically to obtain explicit equations for the maximum plasma concentration of midazolam (C_{max}) and time to maximum concentration (t_{max}). Therefore, these parameters were determined manually from the curve fitted to the concentration-time data. The areas under the concentration curves (AUC) were calculated by the linear trapezoidal rule. Extrapolation to infinite time was achieved by dividing the last concentration by the terminal rate constant λ_β. The AUC values obtained from the midazolam curves after i.v. and i.n. administration were used to calculate absolute bioavailability (F_{AUC}) using the standard equation [13]:

$$F_{AUC} = \frac{AUC_{i.n.} \times D_{i.v.}}{AUC_{i.v.} \times D_{i.n.}} \quad (4)$$

Pharmacodynamics analysis

The EEG activity in the β-band (14–40 Hz) was related to the midazolam plasma concentration using an exponential model:

$$E(C) = E_0 + b^n \cdot C^n \quad (5)$$

in which E(C) is the observed effect at concentration C, E_0 is the baseline effect value, and b and n are parameters determining the curvature of the exponential function.

Statistical analysis

The Student's one tailed t-test with a 5% level of significance was used to test the difference of t_{lag} and F

from zero and one, respectively. The nonparametric Spearman correlation was calculated and tested for deviation from zero using GraphPad Instat version 2.05a software. All data are presented as the mean ± s.d., unless indicated otherwise.

Results

Safety and tolerability

No relevant changes were observed in blood pressure, heart rate or respiration after administration of midazolam. Within 2 min after intravenous administration, all subjects felt sedated, and two of them fell asleep. Intranasal administration led to some degree of nasal irritation in all subjects. Some subjects complained of teary eyes, a slightly bitter taste and a raw throat. The symptoms disappeared within 10 min in all but one subject, in whom intranasal irritation lasted for 25 min All subjects tolerated intranasal administration well and none found it to be painful.

Pharmacokinetics

The plasma concentrations of midazolam and 1-hydroxymidazolam were evaluated in all six volunteers using the composite model described above. An example of the curves fitted to the concentration data from one individual is given in Figure 1. The individual plasma concentrations after both intravenous and intranasal administration are shown in Figure 2. The pharmacokinetic parameters obtained using an integrated model for both midazolam and 1-hydroxymidazolam are summarized in Table 1. The model described the data adequately with the residuals being distributed satisfactorily and with a low correlation between the parameters. In two subjects, inclusion of the lagtime did not improve the fit to the data as determined by the Akaike criterion, and in these subjects the lagtime was constrained to 0 min.

Midazolam was rapidly absorbed after intranasal administration, with a mean peak concentration of 71 ± 25 ng ml^{-1} reached after 14 ± 5 min. The mean lagtime for the appearance of midazolam and its metabolite after intranasal administration was 0.87 ± 0.74 min, which was significantly different from 0 ($P = 0.02$). Mean bioavailability was high (0.83 ± 0.19), but was significantly different from 1.0 ($P = 0.04$; C.I. 0.68, 0.98). Mean F_{AUC}, calculated from noncompartmental analysis, was 0.80 ± 0.19 (Table 2). The mean ratio $AUC_{i.n.}/AUC_{i.v.}$ for 1-hydroxymidazolam, a measure for the relative amount of metabolite formed, was 0.79 ± 0.41. In addition, a moderate but significant correlation between this ratio and F_{AUC} was found ($r_s = 0.81$, $P = 0.01$). This indicates that there is no appreciable difference in the extent of metabolism after intranasal administration compared with intravenous administration.

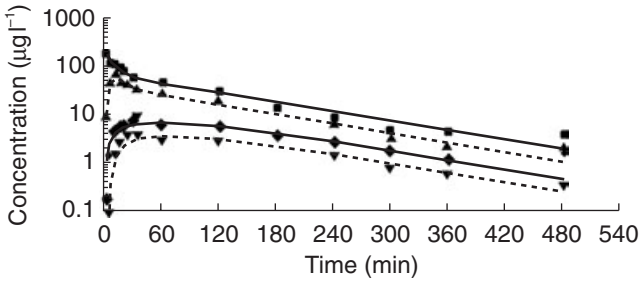

Figure 1 Fit of the composite model to the concentration *vs* time data for midazolam and 1-hydroxymidazolam in one volunteer. Solid lines indicate the time course of midazolam concentrations (■) and 1-hydroxymidazolam concentrations (◆) after intravenous administration. Dotted lines indicate the time course of midazolam concentrations (▲) and 1-hydroxymidazolam concentrations (▼) after intranasal administration.

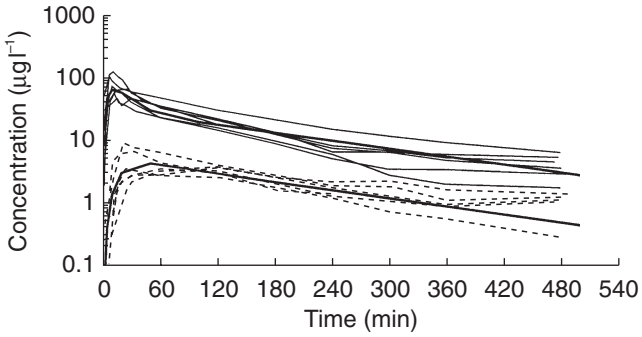

Figure 2 Individual plasma concentration *vs* time curves for midazolam (solid lines) and 1-hydroxymidazolam (broken lines) after intranasal administration of 5 mg midazolam. The bold curves represent the mean pharmacokinetic model fit to the data.

Pharmacodynamics

In one subject, no reliable EEG registration was obtained due to technical difficulties and the pharmacodynamic analysis was performed in the remaining five subjects. EEG activity *vs* midazolam plasma concentration data in individual subjects are shown in Figure 3. In all individuals the intraindividual basal EEG-activity after intravenous and intranasal administration was comparable. After intranasal administration, observable changes in EEG activity were observed in only one subject. Comparison of the pharmacodynamic parameter estimates after intravenous and intranasal administration would not be possible if the data were fitted to the model separately. Thus, the intravenous and intranasal EEG data of each subject were fitted to the pharmacodynamic model

Table 1 Pharmacokinetic measurements after intravenous and intranasal administration of midazolam obtained using a composite pharmacokinetic model (see text).

Parameter	*Estimate ± s.d.*
F	0.83 ± 0.19
k_A (min^{-1})	0.168 ± 0.053
t_{lag} (min)	0.87 ± 0.74
V_C ($l\ kg^{-1}$)	0.46 ± 0.14
V_{SS} ($l\ kg^{-1}$)	1.11 ± 0.25
CL ($l\ min^{-1}\ kg^{-1}$)	0.0161 ± 0.0041
$t_{1/2,\ \lambda_1}$ (min)★	8.4 ± 2.4
$t_{1/2,\ \lambda_z}$ (min)★	79 ± 30
k_M (min^{-1})	0.0036 ± 0.0015
C_{max} ($\mu g\ l^{-1}$)	71 ± 25
t_{max} (min)	14 ± 5

F: intranasal bioavailability, k_A: absorption rate constant, t_{lag}: lag-time, V_C: volume of the central compartment, V_{SS}: volume of distribution at steady state, CL: midazolam clearance, $t_{1/2,\ \lambda1}$: equilibration half-life, $t_{1/2,\ \lambda z}$: terminal half-life, k_M: formation rate constant of 1-hydroxymidazolam, C_{max}: maximum concentration after intranasal administration, t_{max}: time to maximum concentration after intranasal administration. Values are mean ± s.d. ★Half-lives are expressed as harmonic means.

Table 2 Estimates of the mean areas under the curve of the intravenous and intranasal formulations and mean bioavailability of the intranasal formulation from noncompartmental analysis for midazolam and 1-hydroxymidazolam. Values are mean ± s.d.

Parameter	*Midazolam*	*1-Hydroxymidazolam*
$AUC_{i.v.}$ ($mg\ l^{-1}\ min$)	13.0 ± 4.7	1.76 ± 0.61
$AUC_{i.n.}$ ($mg\ l^{-1}\ min$)	10.2 ± 3.3	1.27 ± 0.43
F_{AUC}	0.80 ± 0.19	0.79 ± 0.41

simultaneously with the average parameter estimates reported in Table 3. Since only small changes in EEG activity were observed, the model parameters could not be estimated reliably.

Discussion

An integrated pharmacokinetic model adequately described the concentration profiles of midazolam and its active metabolite 1-hydroxymidazolam after intravenous and intranasal administration. Using this model, the mean bioavailability of midazolam administered by nasal spray was estimated to be 0.83 ± 0.19, corresponding well with a mean F_{AUC} value of 0.80 ± 0.19 calculated from noncompartmental analysis.

The formation of 1-hydroxymidazolam was best described by a one-compartment model. Although it has been shown that 1-hydroxy-midazolam is distributed into two compartments in both man and rat [9, 14],

Table 3 Pharmacodynamic parameter estimates following intravenous and intranasal administration of 5 mg midazolam. The EEG data were fitted simultaneously to the model (equation 5) because the data obtained after intranasal administration did not allow separate estimation of the parameters. Values are mean ± s.d.

Parameter	*Estimate*
E_0 (μV^2)	0.044 ± 0.015
B	0.0027 ± 0.0027
n	2.3 ± 2.1

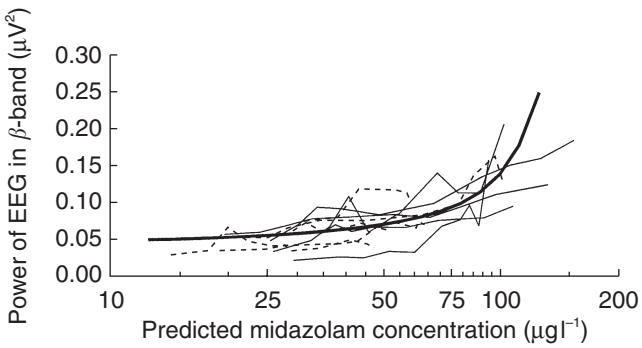

Figure 3 Individual EEG effect *vs* predicted plasma midazolam concentration after i.v. (solid lines) and i.n. (broken lines) administration of the drug. The bold curve represents the mean pharmacodynamic model fit.

distribution to a second compartment could not be identified in this data set due to the first order formation rate of the metabolite. In the case of the parent compound, distribution to a second compartment was characterized from the concentration-time data after intravenous administration.

In the composite model used, the formation rate of 1-hydroxymidazolam was assumed to be equal for the two routes of administration. Under this assumption, a good fit to both the midazolam and the 1-hydroxymidazolam data was obtained, indicating no difference in the extent of metabolism between the two routes. This was further confirmed by noncompartmental analysis. The mean ratio $AUC_{i.n.}/AUC_{i.v.}$ of 1-hydroxymidazolam was virtually identical to the mean value of $F_{AUC,}$ which rules out the possibility that intranasal metabolism by cytochrome P450 metabolizing enzymes on the nasal mucosa contributes substantially to the observed concentration time profiles. In addition, the similarity of these AUC values indicates that very little of the concentrated spray used in this study is swallowed. After oral administration to adults, midazolam has been reported to be subject to saturable first-pass metabolism, with reported bioavailabilities in the range of 0.24–0.5 [15, 16]. The bioavailability reported in the present study corresponds well with the value of 0.83 ± 0.15 reported in a study

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