Br. J. Pharmacol. (1991), **102**, 663–668

Pharmacokinetic-pharmacodynamic modelling of the EEG effects of midazolam in individual rats: influence of rate and route of administration

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1 The purpose of the present investigation was to quantify the concentration-pharmacological effect relationship of midazolam in individual rats by use of effect parameters derived from aperiodic EEG analysis. By varying the rate and route of administration the role of (inter)active metabolites and development of acute tolerance was evaluated.

2 The pharmacokinetics and pharmacodynamics of midazolam were determined after intravenous administration of 10 mg kg^{-1} during 5, 30 and 60 min and oral administration of 15 mg kg^{-1}. Following intravenous administration the pharmacokinetics were most adequately described by a bi-exponential equation. The values (mean ± s.e.mean, $n = 20$) of clearance, volume of distribution at steady-state and terminal half-life were 67 ± 2 ml min^{-1} kg^{-1}, 1.61 ± 0.07 l kg^{-1} and 27 ± 1 min, respectively. Following oral administration midazolam was rapidly absorbed with a systemic availability of 45 ± 9%.

3 The averaged amplitudes in the 11.5–30 Hz (beta) frequency band of the fronto-central lead on the left-hemisphere, as derived by aperiodic EEG analysis, was selected as a measure of the pharmacological effect of midazolam. By pharmacokinetic-pharmacodynamic modelling the individual concentration-EEG effect relationships of midazolam were derived, which were successfully quantified by the sigmoidal E_{max} model. No marked and systematic differences in pharmacodynamic parameters were found between the rates and routes of administration. The averaged pharmacodynamic parameters of midazolam obtained after combining the results of all rates and routes of administration were (mean ± s.e.mean, $n = 27$): Eo = 61 ± 3 μV s^{-1}, E_{max} = 85 ± 3 μV s^{-1}, EC_{50} = 40 ± 3 ng ml^{-1} and N = 0.84 ± 0.04.

4 The results of the present study show that the concentration-EEG effect relationship of midazolam can be characterized in individual animals using the amplitudes in the 11.5–30 (beta) frequency band as a measure of pharmacological response. Acute tolerance did not develop and (inter)active metabolites did not contribute to this effect parameter within the time span of the experiments.

Introduction

The availability of a quantitative measure of the drug response is a first prerequisite to study the kinetics of drug action *in vivo*. Such effect parameters are not readily available, which may explain the limited data on concentration-effect relationships of benzodiazepines, especially with regard to the effect of various (patho)physiological factors (Dingemanse *et al.*, 1988a). Quantitative EEG parameters have proven to be valuable measures of the central nervous system (CNS) effects of benzodiazepines (Fink *et al.*, 1976; Kurowski *et al.*, 1982; Laurian *et al.*, 1984; Saletu *et al.*, 1986) and recently, in man, the relationships between plasma concentrations and EEG effects of midazolam and diazepam have been quantified (Koopmans *et al.*, 1988; Greenblatt *et al.*, 1989; Breimer *et al.*, 1990). EEG parameters conform to most of the criteria of 'ideal' pharmacodynamic measures (Dingemanse *et al.*, 1988a): continuity, sensitivity, objectivity and reproducibility, allowing in principle the characterization of the complete concentration-effect relationship in individual subjects.

In kinetic-dynamic modelling it is important to establish the uniqueness of the derived concentration-effect relationship. Several potentially confounding pharmacokinetic factors, such as distribution to the site of action and formation of (inter)active metabolites, and pharmacodynamic factors, such as development of (acute) tolerance towards the measured effect, have to be considered (Dingemanse *et al.*, 1988a). The influences of these factors are difficult to assess when only one mode of drug administration is studied.

The purpose of the present investigation was to quantify the concentration-EEG effect relationship of midazolam in individual rats by use of effect parameters derived from aperiodic EEG analysis. By varying the rate and route of drug administration the role of (inter)active metabolites and development of acute tolerance was evaluated.

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Methods

Animals

Male SPF rats of Wistar descent (200–250 g) were used throughout the study. The animals were housed individually in plastic cages with a normal 12-h light-dark cycle and fed on laboratory chow (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands) and water *ad libitum*. From the night before the experiment onwards the animals were deprived of food but had free access to water.

Pharmacokinetic-pharmacodynamic experiments

To measure the EEG signals, EEG electrodes were implanted into the skull of the animals one week before the kinetic-dynamic experiment as described previously (Mandema & Danhof, 1990). Briefly, the electrodes were placed at the locations 11 mm anterior and ±2.5 mm lateral (F_l and F_r), 3 mm anterior and ±3.5 mm lateral (C_l and C_r) and 3 mm posterior and ±2.5 mm lateral (O_l and O_r) to lambda. A reference electrode was placed on lambda. Stainless steel screws were used as electrodes and connected to a miniature connector. The whole assembly was insulated and fixed to the skull with dental acrylic cement. Indwelling cannulae were implanted in the right jugular vein and right femoral artery under light ether anaesthesia one day before the experiment. During the experiment the animals were kept in a restraining cage.

Rats (27) were randomly allocated to four groups that received 10 mg kg^{-1} midazolam intravenously during 5, 30 or 60 min or 15 mg kg^{-1} of the drug orally. Midazolam was dissolved in 0.9% saline with the aid of an equimolar quantity of hydrochloric acid. A total volume of 0.75 ml was administered intravenously and 0.5 ml orally. To determine the pharmacokinetics of midazolam arterial blood samples of 100 μl, or 200 μl near the end of the experiment, were collected at fixed time intervals after drug administration over a period of 240 to 360 min, depending on the rate and route of administration. Heparinized blood samples were centrifuged and plasma was separated and stored at −35°C until the time of analysis. Bipolar EEG leads were continuously measured during the time course of the experiment using a System 50000 EEG recorder (Van Gogh BV, Amsterdam, The Netherlands). The low-pass filter was set at 75 Hz, the time constant at 0.3 s. The EEG recordings were started 10 to 15 min before drug administration. Two EEG leads (F_1–C_1 and C_1–O_1) were subjected to on-line aperiodic analysis for quantification, using the Lifescan EEG monitor (Neurometrics Inc., San Diego, U.S.A.). Aperiodic analysis was recently introduced as a new technique to quantify brain electrical activity (Gregory & Pettus, 1986). The aperiodic analysis algorithm calculates the amplitude and period of each EEG signal on a wave by wave basis. The analysed EEG data are then sampled into an IBM-AT computer and stored on magnetic diskette as the frequency in Hz, amplitude in μV, time of occurrence and artifact status of each wave. From these data the average amplitudes (μV s^{-1}) in the following frequency bands: 0.5–2.5 Hz (delta), 2.5–7.5 Hz (theta), 7.5–11.5 Hz (alpha) and 11.5–30 Hz (beta) were calculated and studied as descriptors of the drug effect. A total of 50 data points per animal were used as effect measures in the modelling procedure. The data points were calculated by averaging 1 min or more of consecutive EEG data. EEG effect measures were taken more often while the concentration of midazolam was changing rapidly, i.e. during the infusion and shortly thereafter.

Drug analysis

A sensitive assay was developed to measure midazolam concentrations in small plasma samples of 50 or 100 μl by high performance liquid chromatography (h.p.l.c.) with u.v. detection. A solution of 25 ng diazepam (internal standard) in 20 μl methanol was added to a 50 or 100 μl plasma sample which had been diluted with 0.5 ml 0.1 N NaOH solution. The mixture was extracted with 5 ml dichloromethane-pentane (1:1) for 30 s on a vortex mixer. After centrifugation the organic layer was separated and evaporated to dryness under reduced pressure. The residue was reconstituted with 150 μl of the mobile phase and 75 μl was injected into the chromatographic system. The chromatographic system consisted of a M-45 solvent pump (Waters, Milford, MA, USA), a Promis automatic sample injector (Kratos Analytical Instruments, Ramsey, USA), a Z-module containing a Radial-Pak C-18 column (Waters) and a spectroflow 757 UV detector (Kratos) set at 222 nm. The mobile phase consisted of a mixture of 0.01 M phosphate buffer (pH = 5.0) and acetonitrile (Rathburn) in a ratio 50/50 with a flow rate of 1.0 ml min^{-1}. Retention times were 4.4 and 5.0 min for diazepam and midazolam respectively. Data processing was performed using a Shimadzu C-R3A integrator. Coefficients of variation for identical samples were less than 5% (n = 5) with respect to intra- and inter-assay precision in the range 20–10000 ng ml^{-1}. The detection limit was approximately 10 ng ml^{-1} with a 100 μl sample.

Data analysis

The pharmacokinetics and pharmacodynamics of midazolam were quantified for the individual rats. The plasma concentration-time profiles of midazolam after intravenous infusion (eq. 1) and oral administration (eq. 2) were described by a poly-exponential equation:

$$C(t) = \sum_{i=1}^{n} A_i(e^{-\alpha_i(t-T)_+} - e^{-\alpha_i t}) \quad (1)$$

where $(t - T)_+$ is defined by:

$$(t - T)_+ \equiv \begin{cases} t - T \text{ for } t > T \\ 0 \text{ otherwise} \end{cases}$$

and

$$C(t) = \sum_{i=1}^{n} A_i(e^{-\alpha_i t}) \quad (2)$$

where C(t) is the plasma concentration of midazolam at time t, T is the infusion duration and A_i and α_i are respectively the coefficients and exponents of the equation. Different exponential models were investigated and the most suitable model was chosen according to standard criteria. Basic pharmacokinetic parameters as area under the curve (AUC), total clearance (Cl), volume of distribution at steady-state (Vdss) and terminal half-life ($t_{1/2}$, α_n) were calculated from the coefficients and exponents of the fitted functions according to standard procedures (Gibaldi & Perrier, 1982). The systemic availability (F) after oral administration was calculated from the averaged AUCs after oral and three rates of intravenous administration.

The sigmoidal E_{max} model was selected to describe the relationship between midazolam concentrations and EEG effect:

$$E(C) = Eo + \frac{E_{max} C^N}{EC_{50}{}^N + C^N} \quad (3)$$

where E is the observed EEG effect at plasma concentration C of midazolam, Eo is the baseline EEG value, E_{max} the maximal effect, EC_{50} the plasma concentration at half maximal effect and N is a constant expressing the sigmoidicity of the concentration-effect relationship. The pharmacokinetic model was used to calculate the concentrations of midazolam at the time points of effect measurement. The increase in average amplitude in the 11.5–30 Hz (beta) frequency band (F_1–C_1 lead) was used as measure of the pharmacological effect of midazolam.

The equations were fitted to the data by the nonlinear least squares regression programme Siphar (Siphar pharmacokinetic modelling software package, SIMED S.A., Creteil, France). The pharmacokinetic and pharmacodynamic parameters obtained after oral and three rates of intravenous administration were statistically compared by a parametric one-way analysis of variance (ANOVA) or a nonparametric Kruskal-Wallis test if more appropriate. The probability level was set at 5%.

Results

The characteristic EEG changes observed after administration of midazolam are shown in Figure 1 for the fronto-central lead on the left hemisphere (F_1–C_1). The upper trace shows the baseline EEG pattern (trace 1). As the concentration of midazolam increased, first a profound increase in high frequency (beta) activity was observed (trace 2), followed by an increase in EEG slow wave (delta) activity at higher concentrations of the drug (trace 3). The characteristic EEG changes observed in the raw EEG data are reflected in the changes in the calculated EEG parameters. The averaged time-effect profiles of the amplitudes in the 0.5–2.5 Hz and 11.5–30 Hz frequency range of all rats receiving 10 mg kg^{-1} of midazolam in 5 min are shown in Figure 2. During the infusion of midazolam there was a marked increase in the amplitudes in the 11.5–30 Hz frequency band. After the infusion was terminated, the effect returned gradually to pre-infusion values. The amplitudes in the 0.5–2.5 Hz frequency range were also increased after administration of midazolam, but this effect declined to baseline levels much more rapidly. A small increase was observed

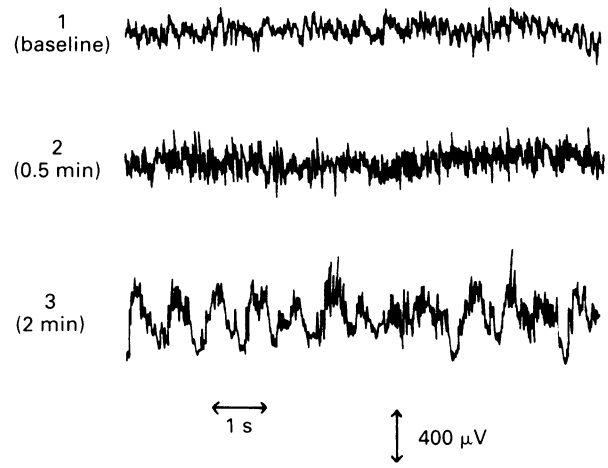

Figure 1 Characteristic EEG changes observed in the fronto-central lead on the left hemisphere (F_l–C_l) during intravenous administration of 10 mg kg^{-1} midazolam during 5 min. Trace 1 represents the baseline EEG pattern. As the concentration of midazolam increases first a substantial increase in high frequency (beta) activity is observed (trace 2; 0.5 min after start of the infusion), followed by an increase in EEG slow wave (delta) activity at higher concentrations of the drug (trace 3; 2 min after start of the infusion).

in the amplitudes in the 2.5–7.5 Hz frequency range and no consistent changes were observed in the amplitudes in the 7.5–11.5 frequency range.

Figure 3 shows the plasma concentration versus time profile of midazolam and the pharmacological effect versus time profile (change in amplitudes in the 11.5–30 Hz frequency band) in two individual rats after intravenous administration of 10 mg kg^{-1} during 5 min (Figure 3a) and 15 mg kg^{-1} orally

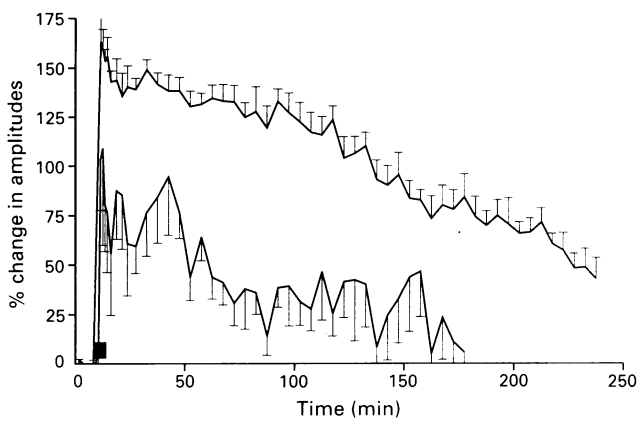

Figure 2 Percentage change over baseline value of the amplitudes in the 0.5–2.5 Hz (lower trace) and 11.5–30 Hz (upper trace) frequency bands. The curves represent the average increase (mean, with s.e.mean shown by vertical bars, $n = 8$) for all rats receiving 10 mg kg^{-1} midazolam intravenously during 5 min. The solid bar represents the infusion duration.

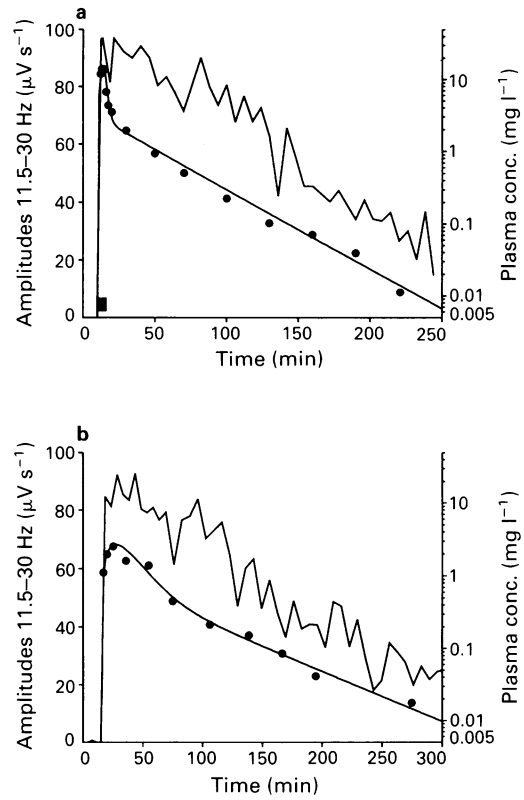

Figure 3 Midazolam plasma concentrations (●) and EEG effect (change in amplitudes in the 11.5–30 Hz frequency band) versus time profiles in two rats which had received 10 mg kg^{-1} intravenously during 5 min (a) and 15 mg kg^{-1} orally (b) respectively. The solid line fitted to the plasma concentrations represents the best fit according to the pharmacokinetic model.

(Figure 3b). After intravenous administration the concentration-time curves were most adequately described by a bi-exponential equation, after oral administration a bi- or tri-exponential equation best fitted the individual rat data. The pharmacokinetic parameters of midazolam following the different rates and routes of administration were summarized in Table 1. The averaged pharmacokinetic parameters calculated from all three rates of intravenous administration were (mean ± s.e.mean, $n = 20$): Cl = 67 ± 2 ml min^{-1} kg^{-1}, Vdss = 1.61 ± 0.07 l kg^{-1} and $t_{1/2}$, α_n = 27 ± 1 min. After oral administration midazolam was rapidly absorbed and peak concentrations were attained between 5 to 15 min. The systemic availability after oral administration was 45 ± 9%. No time delay (hysteresis) between plasma concentrations and EEG effect was observed and the two values were directly correlated to each other by the sigmoidal E_{max} model. Figure 4 shows the derived concentration-EEG effect relationship of the same two rats presented in Figure 3 after the pharmacokinetic-pharmacodynamic modelling procedure. The solid lines in this figure were fitted to the data using the sigmoidal E_{max} model. The pharmacodynamic parameters of

Table 1 Pharmacokinetic parameter estimates of midazolam after different rates and routes of administration

	10 mg kg^{-1} i.v. 5 min	10 mg kg^{-1} i.v. 30 min	10 mg kg^{-1} i.v. 60 min	15 mg kg^{-1} oral
Number of animals	8	6	6	7
Cl (ml min^{-1} kg^{-1})	60 ± 2[a]	71 ± 3	72 ± 5	169 ± 25
Vdss (l kg^{-1})	1.54 ± 0.10	1.42 ± 0.08	1.88 ± 0.10[b]	
$t_{1/2}$, α_n (min)	27 ± 1	24 ± 2	29 ± 2	
F (%)				45 ± 9

Values are means ± s.e.mean.
[a] Significantly different from i.v. 30 and 60 min ($P < 0.05$).
[b] Significantly different from i.v. 5 and 30 min ($P < 0.05$).

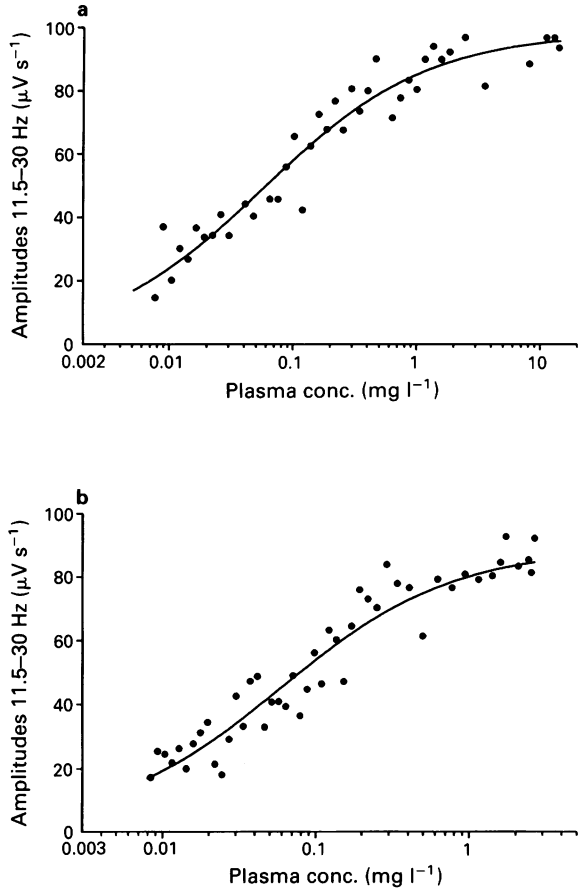

Figure 4 Concentration-EEG effect relationship of midazolam in two rats which had received 10 mg kg^{-1} intravenously during 5 min (a) and 15 mg kg^{-1} orally (b) respectively. The solid line represents the best fit to the actual data points (●) according to the sigmoidal E_{max} model.

midazolam following the different rates and routes of administration are summarized in Table 2. No statistically significant differences in the Eo, EC_{50} and N parameters were found between the different administrations. The E_{max} after intravenous infusion during 60 min and oral administration was found to be statistically significantly different from the values for 5 and 30 min infusion. No statistically significant differences in the pharmacodynamic parameters of midazolam were found between oral and the combined data after all intravenous administrations. The averaged pharmacodynamic parameters of midazolam calculated from all rates and routes of administration were (mean ± s.e.mean, $n = 27$): Eo = 61 ± 3 μV s^{-1}, E_{max} = 85 ± 3 μV s^{-1}, EC_{50} = 40 ± 3 ng ml^{-1} and N = 0.84 ± 0.04.

Discussion

The purpose of the present investigation was to quantify the concentration-EEG effect of relationships of midazolam in individual rats and to examine the influence of rate and route of administration on these relationships. Midazolam was selected as a model compound because of its water solubility, allowing intravenous administration in an aqueous solution, and its lack of a chiral centre.

The characteristic EEG changes of midazolam observed in the rat (Figures 1 and 2) correspond with EEG changes observed in man after benzodiazepine administration (Fink *et al.*, 1976; Kurowski *et al.*, 1982; Saletu *et al.*, 1986; Koopmans *et al.*, 1988). Initially at low benzodiazepine concentrations there is a profound increase in high frequency (beta) activity (11.5–30 Hz). At higher concentrations also, an increase in low frequency (delta) activity is observed (0.5–2.5 Hz). The increase of EEG beta-activity is most commonly used as a measure of the pharmacological effect of benzodiazepines. In the present study the amplitudes in the 11.5–30 Hz frequency range was preferred above the other parameters because it was stable at baseline and a profound effect, up to about 150% increase being observed with little spontaneous fluctuation during the duration of the experiment. Furthermore, in the individual animals the concentration-effect relationship could be described by the sigmoidal E_{max} model, which is preferred on theoretical grounds (Holford & Sheiner, 1982). The relatively low EC_{50} of 40 ng ml^{-1} shows the sensitivity of the EEG parameter for measurement of the CNS effects of benzodiazepines. Following intravenous administration midazolam plasma concentration-time data were most adequately described by a bi-exponential equation. Midazolam is rapidly eliminated from the body with an average elimination half-life of 27 ± 1 min, due to a large plasma clearance of 67 ± 2 ml min^{-1} kg^{-1}. The rapid disappearance of midazolam is another advantage of this drug, as it allows the determination of the complete concentration-effect relationship within a reasonable time span, thus minimizing influences due to diurnal variations.

Modelling concentration-effect relationships of CNS active drugs is often complicated due to an observed time lag between plasma concentrations and effect (hysteresis). In this study no delay between midazolam plasma concentrations and EEG effect was observed (Figure 4). After the relatively rapid intravenous administration of midazolam during 5 min, effects were maximal within 2 min after drug administration (Figure 3), indicating a rapid equilibration between plasma and effect site, which is to be expected considering the highly lipophilic character of the drug (Gerecke, 1983). This is consistent with the rapid equilibration of midazolam concentrations between plasma and cerebrospinal fluid (CSF) observed in the cat (Arendt *et al.*, 1983). Midazolam rapidly entered the CSF, with peak concentrations attained 3 min after dosage. After equilibrium was attained, disappearance of midazolam from the CSF and plasma occurred in parallel. In man a short delay was observed between plasma concentration of midazolam and EEG effects by Breimer *et al.* (1990), with an equilibration half-life ($t_{1/2}$, keo) of only 1.7 min. As no hysteresis was observed in this study, plasma concentrations were directly correlated to the EEG effects and successfully quantified using the sigmoidal E_{max} model (Figure 4).

In kinetic-dynamic modelling it is important to establish the uniqueness of the derived concentration-effect relationship, that is, the independence of this relationship of the pharmacokinetic profile of the drug. Several potentially confounding pharmacokinetic and pharmacodynamic factors

Table 2 Pharmacodynamic parameter estimates of midazolam after different rates and routes of administration

	10 mg kg^{-1} i.v. 5 min	10 mg kg^{-1} i.v. 30 min	10 mg kg^{-1} i.v. 60 min	15 mg kg^{-1} oral
Eo (μV s^{-1})	63 ± 2	75 ± 7	55 ± 5	53 ± 6
E_{max} (μV s^{-1})	92 ± 5	95 ± 5	73 ± 5[a]	78 ± 5[a]
EC_{50} (ng ml^{-1})	38 ± 4	37 ± 8	41 ± 7	44 ± 6
N	0.76 ± 0.08	0.91 ± 0.16	0.83 ± 0.07	0.87 ± 0.05

Values are means ± s.e.mean.
[a] Significantly different from i.v. 5 and 30 min ($P < 0.05$).

have to be considered, including the potential interfering role of (inter)active metabolites, development of acute tolerance and the role of counteracting homeostatic mechanisms (Dingemanse *et al.*, 1988a). These complicating factors are difficult to assess when only one mode of drug administration is studied, and Greenblatt *et al.* (1989) suggested that acute tolerance was likely to have contributed to the termination of the EEG effects of midazolam and diazepam in their study. To elucidate further the importance of these phenomena, the concentration-EEG effect relationship of midazolam was studied after various rates and routes of drug administration.

Acute tolerance is reported to develop towards several actions of benzodiazepines in man (MacLeod *et al.*, 1977; Ellinwood *et al.*, 1983; 1985) and animals (Yoong *et al.*, 1986; File *et al.*, 1989). It has been suggested that the rate of drug administration is an important determinant of the clinical effects of benzodiazepines and development of acute tolerance (Greenblatt *et al.*, 1977; Grundström *et al.*, 1978; Sellers, 1978; Ellinwood *et al.*, 1985). The concentration-EEG effect relationship of midazolam was studied at three rates of infusion. By varying the rate of drug administration the possible development of acute tolerance or role of counteracting homeostatic mechanisms can be elucidated. No marked or systematic differences were found in the pharmacokinetic and pharmacodynamic parameters of midazolam between the three rates of administration (Tables 1 and 2), indicating that acute tolerance towards the EEG effects of midazolam did not occur and that midazolam plasma concentrations and not the rate of drug administration determined the time-effect profile of this drug. Also the absence of 'clockwise' hysteresis in the plasma concentration versus effect profiles of the individual rats suggests that tolerance does not develop rapidly towards the EEG effects of midazolam. The E_{max} was slightly lower after the 60 min administration as compared to the 5 and 30 min (Table 2). This may in part have been caused by the lower baseline effect value in this group. The percentage increase over baseline did not differ between the three rates of administration. With regard to the pharmacokinetics, a significant lower clearance value was observed after the 5 min infusion (Table 1), which may be attributed to more pronounced cardiovascular effects of midazolam associated with higher drug concentrations attained during the rapid drug input (Reves *et al.*, 1985). Midazolam is a highly cleared drug in the rat, thus a reduction in liver blood flow would result in a reduction of the clearance. However, the reduction in clearance is small and not reflected in a change of the terminal half-life.

The possible contribution of metabolites to the pharmacological effect of the parent compound can be examined by studying the concentration-effect relationship under circumstances in which different amounts of the metabolites are formed (Holford & Sheiner, 1982; Dingemanse *et al.*, 1988b). In the present study the systemic availability after oral administration of 15 mg kg^{-1} was only 45% despite the rapid absorption of the drug (Table 2). This indicates the formation of a substantial amount of first-pass metabolites, which was to be expected on the basis of the intravenous data. However, no significant differences in the pharmacodynamic parameters of midazolam were found between oral and intravenous administrations, indicating that in the rat, metabolites of midazolam do not interfere with the effects of midazolam on the EEG. When the different groups were compared the E_{max} was slightly lower after oral administration as compared to 5 and 30 min intravenous administration (Table 2), which may be due to the lower baseline effect value in this group.

Interestingly, in man, metabolites of midazolam were found to contribute to the CNS effects of the drug (Crevoisier *et al.*, 1983). In man, midazolam is rapidly eliminated from the body by biotransformation to two metabolites, 1-hydroxy-midazolam and 4-hydroxy-midazolam, which are both pharmacologically active (Pieri *et al.*, 1981; Ziegler *et al.*, 1983). After oral, but not after intravenous, administration of midazolam, concentrations of 1-hydroxy-midazolam similar to those of the parent compound are observed, due to a substantial first pass metabolism (Heizmann *et al.*, 1983). These high concentrations of 1-hydroxy-midazolam were shown to contribute significantly to the pharmacological effect of the parent drug, which was reflected in a parallel shift to the left of the relationship between plasma concentrations of midazolam and reaction time observed after oral administration in comparison with intravenous dosage. The concentration-effect relationships were superimposable when the plasma concentrations of midazolam plus 1-hydroxy-midazolam were taken into account (Crevoisier *et al.*, 1983). This difference compared with the situation in rats can be explained by a different metabolic pathway of midazolam in the two species. It has been shown that in the rat midazolam is primarily metabolized by aromatic hydroxylation leading to the formation of a phenolic (4'-OH) derivative of midazolam, which is not formed in man (Woo *et al.*, 1981).

The results of the present study show that the concentration-EEG effect relationship of midazolam can be characterized in individual animals using the amplitudes in the 11.5–30 (beta) Hz frequency range as a measure of pharmacological response. It has been shown that acute tolerance did not develop and (inter)active metabolites did not contribute to the EEG effects of midazolam within the time span of the experiments. The EEG effect parameter (amplitudes in the 11.5–30 Hz frequency band) offers in principle the possibility of evaluating the effect of such factors as age, chronic drug use, disease states and drug interactions, on the pharmacokinetics and pharmacodynamics of benzodiazepines. However, the relationship between the benzodiazepine-induced EEG changes and their clinical, anxiolytic, anticonvulsant, muscle relaxant, sedative and hypnotic effects still remains to be established.

The authors wish to thank Professor Douwe D. Breimer for critically reading the manuscript, Josy Gubbens-Stibbe for her excellent help with the assays and Hoffmann-La Roche, Basel, Switzerland for their kind donation of midazolam. This study was supported by MEDIGON grant 900-521-106.

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(Received July 23, 1990
Revised November 8, 1990
Accepted November 9, 1990)