Pharmacokinetic and electroencephalographic study of intravenous diazepam, midazolam, and placebo

Eleven healthy volunteers received a single intravenous dose of diazepam (0.15 mg/kg), midazolam (0.1 mg/kg), and placebo by 1-minute infusion in a double-blind, three-way crossover study. Plasma concentrations were measured during 24 hours after dosage, and the electroencephalographic (EEG) power spectrum was simultaneously computed by fast-Fourier transform to determine the percentage of total EEG amplitude occurring in the 13 to 30 Hz range. Both diazepam and midazolam had large volumes of distribution (1.2 and 2.3 L/kg, respectively), but diazepam's half-life was considerably longer (33 versus 2.8 hours) and its metabolic clearance lower (0.5 versus 11.0 ml/min kg) than those of midazolam. EEG changes were maximal at the end of the diazepam infusion and 5 to 10 minutes after midazolam infusion. Percent 13 to 30 Hz activity remained significantly above baseline until 5 hours for diazepam but only until 2 hours for midazolam. For both drugs, EEG effects were indistinguishable from baseline by 6 to 8 hours, suggesting that distribution contributes importantly to terminating pharmacodynamic action. The relationship of EEG change to plasma drug concentration indicated an apparent EC_{50} value of 269 ng/ml for diazepam as opposed to 35 ng/ml for midazolam. However, E_{max} values were similar for both drugs (+19.4% and +21.3%, respectively). (CLIN PHARMACOL THER 1989;45:356-65.)

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Understanding of the pharmacodynamic actions of benzodiazepines in relation to their plasma concentrations or pharmacokinetic properties requires quantitation of pharmacodynamic response. The primary actions of benzodiazepines are usually described as anxiolytic, sedative, and hypnotic,[1,2] for which replicable and objective quantitation is difficult, particularly in human beings. Among several approaches to objective quantitation of central effects of benzodiazepines,[3-5] the electroencephalographic (EEG) profile has received considerable attention.[6-9] Acute administration of benzodiazepines to awake human beings produces a shift in the EEG pattern, usually characterized as increased activity in the beta frequency region, ranging from 13 to 30 cycles/sec (Hz).[6-18] The present study used power spectrum analysis of the EEG[19,20] as a direct objective assessment of central effects of benzodiazepines. EEG changes were evaluated in relation to the plasma concentration and pharmacokinetic properties of diazepam and midazolam, two lipophilic benzodiazepine derivatives that differ widely in their rate of elimination and metabolic clearance.

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METHODS

Subjects. Eleven healthy volunteers aged 28 to 42 years participated after giving informed consent. Six subjects were men and five were women. All were healthy, active, ambulatory adults who had no history of medical disease and who were receiving no medications. Women were not taking oral contraceptives.

Procedure. Subjects participated in a single-dose, three-way crossover study, and at least 1 week elapsed between trials. The three treatment conditions were (1)

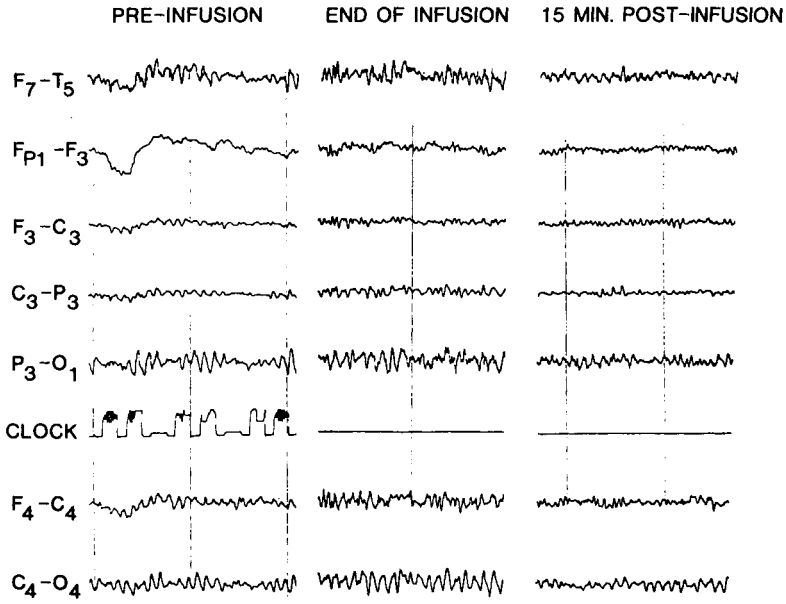

Fig. 1. Representative EEG tracing from a subject before drug administration, just after a 1-minute intravenous infusion of midazolam (0.1 mg/kg), and 15 minutes after the infusion. *Vertical lines* indicate 1-second segments. Note that the increase in higher frequency activity at the end of the infusion and 15 minutes later is most evident in the F_3-C_3 channel but can be seen in other channels as well.

placebo; (2) diazepam, 0.15 mg/kg intravenously; and (3) midazolam, 0.1 mg/kg intravenously. The sequence of the three trials was randomized. Study subjects and investigators responsible for EEG operation and interpretation (J. G. and B. L. E.) were unaware of the treatment condition. However, the physician who administered the medications (D. J. G.) was aware of the treatment condition.

Subjects were admitted to the study unit on the evening before each trial. They abstained from caffeine-containing foods and beverages beginning at midnight before medication administration and for the 8-hour duration of each EEG monitoring trial.

On the morning of each trial an intravenous cannula was inserted into a forearm or antecubital vein and kept patent by continuous infusion of physiologic saline solution. The appropriate medication (placebo, diazepam, or midazolam) was infused into a separate intravenous site over a period of 1 minute. Venous blood samples were drawn before drug or placebo administration, just at the end of the 1-minute infusion, and at the following postinfusion times: 5, 10, 15, 30, and 45 minutes and 1, 1½, 2, 2½, 3, 4, 5, 6, 8, 10, 12, 15, and 24 hours. Venous blood samples were centrifuged, and the plasma was separated and frozen until the time of assay.

Approximately 90 minutes before medication dosage on the morning of each study trial, gold-cup EEG electrodes were placed according to the International 10-20 system, with a nonstandard bipolar montage designed to allow observation of muscle and eye-blink artefacts and to provide two primary data sources (Fig. 1). Impedences were maintained below 5000 Ω. The EEG was recorded on an eight-channel FM-FM instrumentation recorder (Vetters Co., Rebersburg, Pa.) and simultaneously traced on paper with an eight-channel EEG instrument (Grass Instrument Co., Quincy, Mass.). The pass-band filter was set at 0.3 to 70 Hz. One channel was used for a digital clock signal (Ives Ambulatory Instrumentation, Montreal, Canada), so that sections of the paper EEG tracing could be inspected visually and matched to specific 64-second EEG segments recorded on the tape (Fig. 1). These EEG segments could in turn be matched to specific times before and after medication administration. Tracings were screened visually for muscle artefact and interference and played back through a Butterworth filter set to steeply attenuate activity above 37.5 Hz at 24 decibels per octave and amplify the signals from ±1.4 V (Inter-range Instrumentation Group standards) to ±5 V. This was for the purpose of matching to the analog-digital converter (Mountain Computer, Scotts Valley,

Table I. Pharmacokinetic results

	Mean ± SE (with range)	
	Diazepam	*Midazolam*
V_c (L/kg)	0.13 ± 0.03 (0.002-0.32)	0.73 ± 0.15* (0.39-1.88)*
V_{area} (L/kg)	1.24 ± 0.12 (0.64-1.95)	2.32 ± 0.17 (1.40-3.46)
Elimination $t_{1/2}$ (hr)	33 ± 5 (13-62)	2.79 ± 0.45 (1.6-6.5)
CL (ml/min/kg)	0.50 ± 0.06 (0.27-0.91)	11.0 ± 1.2 (5.1-16.4)

*Could be calculated for nine subjects after intravenous midazolam.

Calif.). The baseline setting was adjusted so that a flat EEG signal recorded on the tape, when played back through the system, yielded the smallest possible value on the analog-digital converter. This helped to minimize the direct current (0 Hz) signal contamination in the spectral analysis. The EEG power spectrum was computed by fast-Fourier transform with an Apple II computer and previously described software[21-26] adjusted for use in our laboratory.

After infusion of active drug or placebo, subjects were kept awake during EEG recording sessions by being asked to repeatedly count from 0 to 99 slowly by threes. This procedure was done to maintain wakefulness,[7] because preliminary analysis indicated that the characteristic result of benzodiazepine administration (increased EEG activity in the 13 to 30 Hz band) is lost during sleep.

Plasma concentrations and pharmacokinetic analysis. In the diazepam treatment condition, plasma concentrations of diazepam and its major pharmacologically active metabolite, desmethyldiazepam, in all plasma samples were determined by GC with electron capture detection.[27] Two other metabolites of diazepam (oxazepam and temazepam) did not appear in high enough concentrations to be quantitated. During the midazolam treatment condition, concentrations of midazolam were also determined by GC with electron capture detection.[28] Two metabolites of midazolam (1- and 4-hydroxymidazolam) can be quantitated by this method as well. However, chromatographic tracings indicated that the metabolites were present in low concentrations, if at all. Furthermore, both metabolites have considerably lower brain uptake, as well as lower benzodiazepine receptor affinity relative to the parent compound.[29] Therefore the midazolam metabolites were not quantitated specifically.

Plasma concentrations were then analyzed by iterative nonlinear least-squares regression techniques. Concentration-time points (excluding the concentration value just at the end of the infusion) were fitted to a linear sum of two or three exponential terms.[30] After appropriate correction for the infusion period, coefficients and exponents from the fitted function were used to determine central compartment volume (V_c), total volume of distribution by the area method (V_{area}), elimination half-life ($t_{1/2}$), and total clearance (CL).[30-32] In the case of diazepam, the duration of sampling after the single intravenous dose (24 hours) was not sufficiently long to provide a reliable trace of the terminal phase of drug elimination, which is critical to the calculation of $t_{1/2}$ and CL. Therefore, $t_{1/2}$ and CL values for diazepam are only tentative.

EEG analysis. For each subject, 64-second EEG segments recorded from the left frontocentral region (F_3-C_3, Fig. 1) were selected at times corresponding to blood sampling described above. The EEG segments were then analyzed as 32 2-second epochs by digitizing at 128 samples per second and then by performing the fast-Fourier transform to find the average power spectrum using the whole 64-second segment in 0.5 Hz steps from 0 to 64 Hz. The spectrum was then divided into individual frequency bands as follows: theta (4 to 7.5 Hz), alpha (8 to 12.5 Hz), sigma (13 to 14.5 Hz), and beta (15 to 30 Hz).

For each subject in each treatment condition the sigma band was first analyzed separately to ensure that brief sleep activity (stage I) did not occur during the sampled epochs. All EEG samples with an increase in sigma were reexamined, and when no sleep spindle activity was found the data were reanalyzed with sigma and beta bands combined. The percentage of total EEG amplitude (square root of power) falling in the 13 to 30 Hz (sigma plus beta) frequency range was then calculated at each sampling time. The percentages at each postmedication time point were then expressed as the increment or decrement over the baseline percentage measured in each subject before medication administration. For each postmedication point the statistical significance of that value in comparison with zero change was evaluated by the Student independent *t* test.

Relation of plasma levels and EEG changes. Plasma drug concentrations and EEG effects were evaluated by the following equation[33-35]:

$$E = E_{max}C^{\wedge}/(B + C^{\wedge}) \quad (1)$$

where E is the increase over predrug baseline in percentage of total EEG amplitude in the 13 to 30 Hz range and C is the plasma drug concentration at the corre-

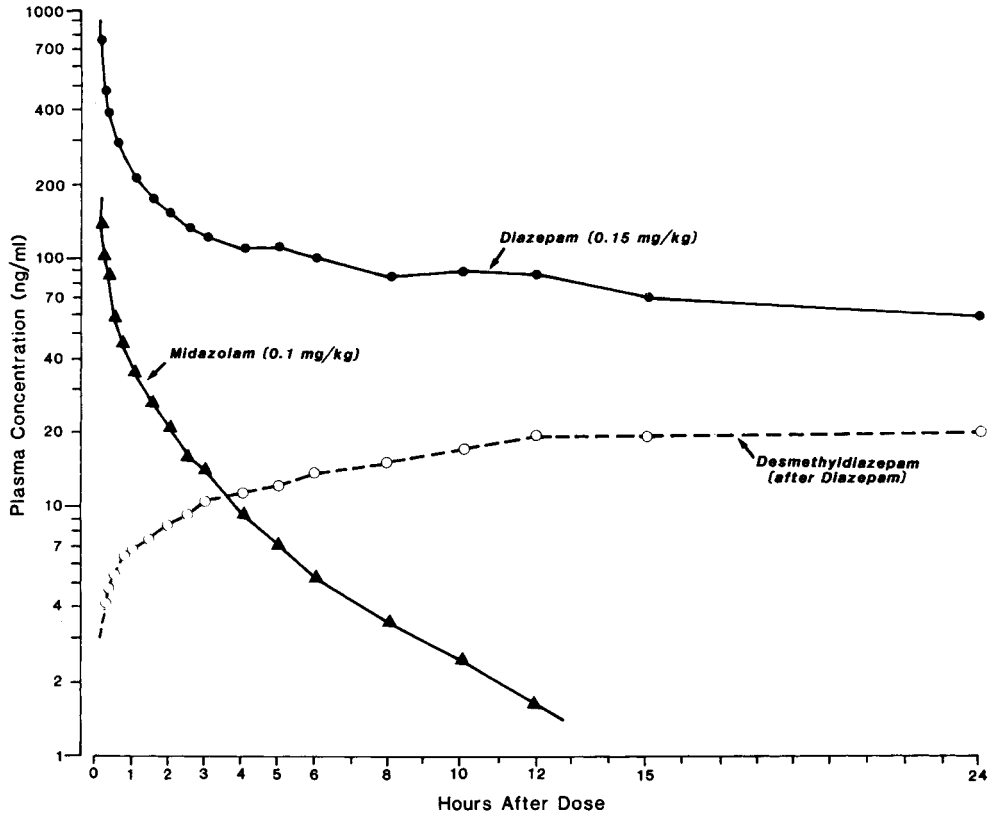

Fig. 2. Plasma concentrations of diazepam and its metabolite, desmethyldiazepam, after intravenous administration of diazepam (0.15 mg/kg) and plasma concentrations of midazolam after a 0.1 mg/kg intravenous dose of midazolam. Each point is the mean concentration for all subjects at the corresponding time. (Values immediately after the completion of the infusion are not shown.)

sponding time. Iterated parameters, determined by nonlinear least-squares regression techniques,[30] were: E_{max}, the projected "maximum" increase in EEG activity in this range; A, an exponent; and B, an entity with units of concentration that can be used to calculate the concentration associated with an increase in E corresponding to 50% of E_{max} (EC_{50}). No provision was made for an equilibration delay attributable to the time necessary for drug present in plasma to reach the active site in the central nervous system (CNS), inasmuch as experimental studies demonstrate rapid CNS entry and equilibration of midazolam and diazepam.[27,36,37]

This fitting procedure was applied to (1) individual data points for each subject in each treatment condition; (2) mean values of plasma drug concentration and EEG change across subjects at corresponding times for a given drug; and (3) aggregate data, consisting of all plasma drug concentrations and EEG changes for all subjects in a given treatment condition.

Desmethyldiazepam, the metabolite of diazepam that has pharmacologic activity similar to that of the parent compound,[29,38] appeared in plasma of all subjects who received diazepam. However, metabolite levels were in all cases considerably lower than those of the parent drug, making it unlikely that the presence of desmethyldiazepam influenced the concentration-response relationship. This was verified when equation 1 was applied to the sum of diazepam plus desmethyldiazepam concentrations for averaged data, yielding nearly identical parameter estimates as when diazepam concentrations alone were used (see below). Therefore plasma concentration versus EEG relationships were evaluated with diazepam alone.

RESULTS

Pharmacokinetics. After intravenous diazepam, plasma diazepam concentrations fell more than fivefold during the first 2 to 3 hours after administration. Thereafter the rate of disappearance from plasma slowed considerably. By 24 hours after administration, plasma levels were only slightly lower than at the 8-hour time point. Disappearance of diazepam was mirrored by for-

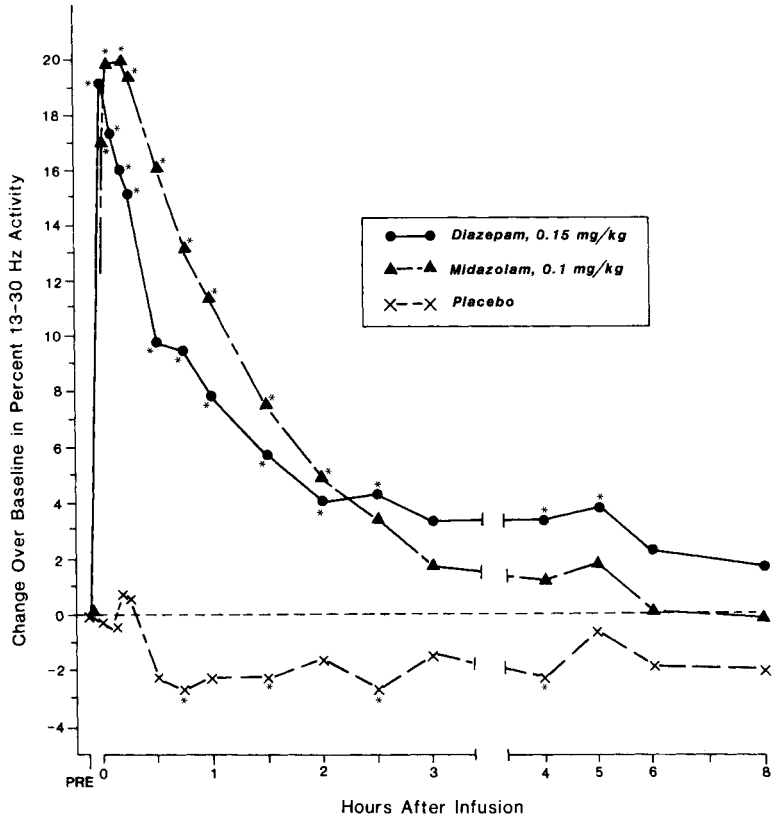

Fig. 3. Change over predrug baseline in the percentage of total EEG amplitude occurring in the 13 to 30 Hz frequency range. Each point is the mean for all subjects at the corresponding time. *Asterisks* indicate significant differences ($p < 0.05$) from zero change.

mation of its active metabolite, desmethyldiazepam (Fig. 2). Table I summarizes pharmacokinetic variables for diazepam. Despite the short sampling period, mean values of diazepam V_{area} (1.2 L/kg), elimination $t_{1/2}$ (33 hours), and CL (0.50 ml/min/kg), are similar to those reported in previous studies from this laboratory[39-43] and elsewhere.[44]

Mean plasma concentrations of midazolam likewise dropped more than fivefold during the first 2 to 3 hours after dosing (Fig. 2). Although midazolam also had a large pharmacokinetic V_{area} (mean 2.3 L/kg), its elimination $t_{1/2}$ was short (2.8 hours) and metabolic clearance was high (11 ml/min/kg). Again, these values are similar to those reported previously.[42,45-49]

EEG effects. Preinfusion values of the fraction of EEG activity in the 13 to 30 Hz range were highly replicable among the three trials. Mean (±SE) predrug control values during placebo, diazepam, and midazolam treatment conditions were: 46.2% ± 2.7%, 46.3% ± 1.3%, and 47.7% ± 2.1%, respectively. Overall differences were not significant ($F = 0.42$).

Placebo infusion produced no significant increase in 13 to 30 Hz EEG activity during the 8-hour monitoring period (Fig. 3). At several time points, fractional activity in this range actually was slightly decreased compared with baseline. In contrast, both diazepam and midazolam produced highly significant increases in fractional EEG activity in the 13 to 30 Hz range (Fig. 3). For diazepam, effects were maximal at the end of the infusion and then declined rapidly over time. The increase over baseline remained significant until the 5-hour postinfusion time point; at 6 and 8 hours, EEG changes were not significantly different from baseline. Midazolam produced maximal increases in 13 to 30 Hz activity that were similar in magnitude to those produced by diazepam; these occurred at 5 and 10 minutes after infusion. Thereafter, effects declined and by 2½ hours after administration were no longer significantly different from baseline (Fig. 3).

Relation of plasma concentrations and EEG changes. The relationship of mean plasma concentration to mean EEG change, based on mean values across

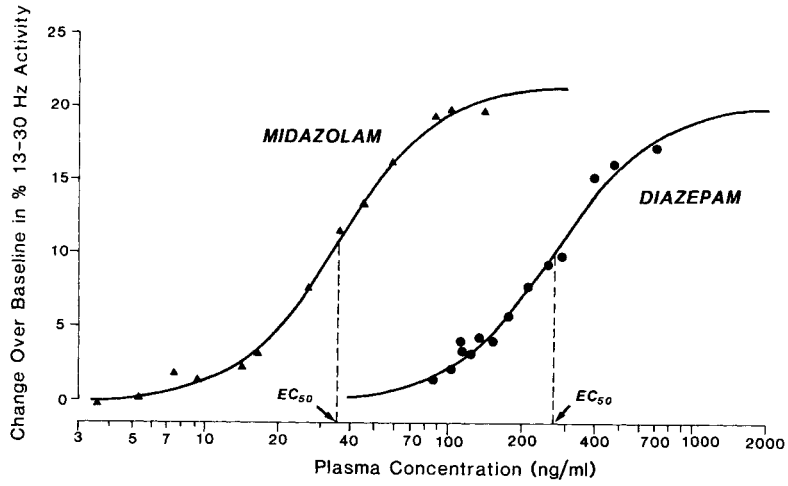

Fig. 4. Relation of plasma concentration of diazepam or midazolam to change over predose baseline in percentage of total EEG activity falling in the 13 to 30 Hz frequency range. Each point is the mean for all 11 subjects at the corresponding time. Lines represent functions consistent with equation 1, determined by nonlinear least-squares regression analysis. EC_{50} indicates the plasma concentration of either drug at which the change in EEG activity is 50% of the maximal change.

Table II. Parameters relating plasma concentrations to EEG effects based on equation 1

	E_{max} (%)	EC_{50} (ng/ml)	A (exponent)
Diazepam			
From mean data points (Fig. 4)	20.2	269	2.11
From aggregate data points	19.4	255	2.30
Midazolam			
From mean data points (Fig. 4)	21.4	35.2	2.19
From aggregate data points	21.3	32.0	2.38
From individual subjects (n = 10) (Fig. 5)	22.4 ± 2.4	40.0 ± 2.8	2.50 ± 0.42

subjects at corresponding times, was highly consistent with equation 1 (Fig. 4). E_{max} values for both drugs were similar (+20.2% for diazepam; +21.5% for midazolam), and for both drugs the exponent A in equation 1 was similar (2.11 for diazepam; 2.19 for midazolam). However, the apparent EC_{50} value for midazolam (35.2 ng/ml) was considerably smaller than that for diazepam (269 ng/ml) (Table II). Similar parameter estimates were obtained when all data points were fitted aggregately (Table II). Based on mean data, parameter estimates obtained when the sum of diazepam plus desmethyldiazepam plasma concentration was used (E_{max} = +19.8%; EC_{50} = 268 ng/ml; A = 2.34) were nearly identical to those generated with diazepam alone (Table II).

For midazolam, fitting of data points to equation 1 was possible in 10 of the 11 individual subjects (Fig. 5). Mean values of parameter estimates across the 10 subjects were similar to values produced by curve fitting of mean data or by fitting of aggregate data (Table II). In the case of diazepam, however, fitting of individual subjects' data to equation 1 was possible in only a few cases, in part because plasma diazepam concentrations in the "low" range (i.e., <70 ng/ml) were not adequately represented during the 8 hours of EEG monitoring (Fig. 2).

DISCUSSION

Measurement of the time course of clinical actions of benzodiazepines generally requires quantitation of such variables as sedative or anxiolytic effects, alteration in sleep profile, impairment of performance on tests of psychomotor or intellectual function, or alteration in information acquisition and recall. Methods are available to quantitate drug-related changes in such variables, but many measures nonetheless are at least

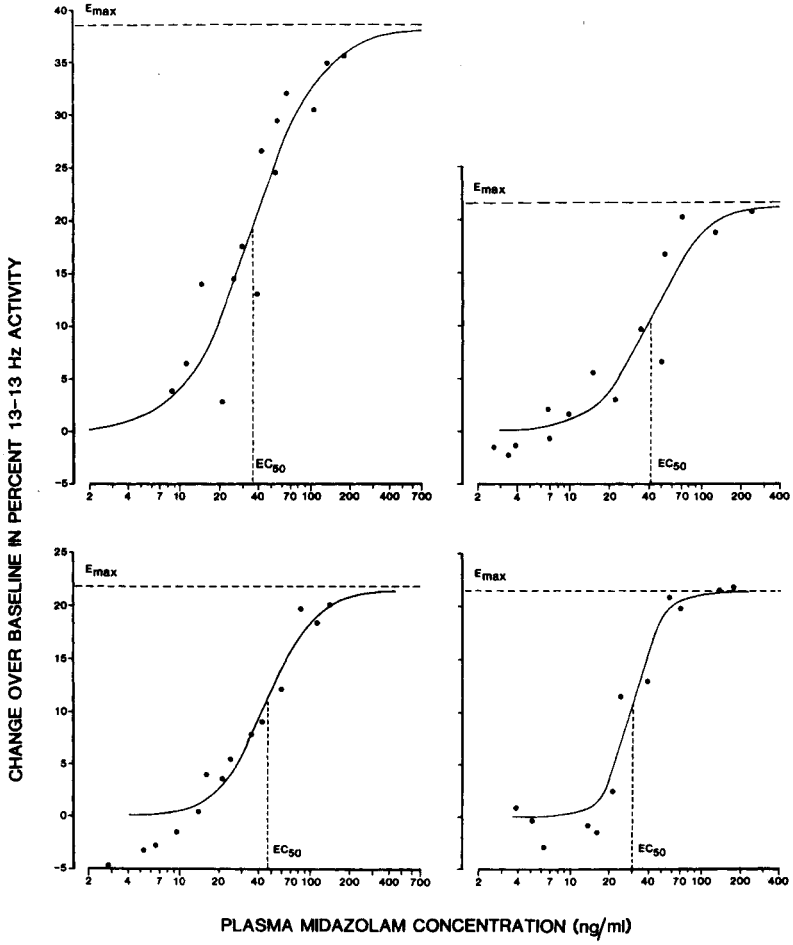

Fig. 5. Plasma concentrations and EEG changes after intravenous administration of midazolam in four individual subjects. *Solid lines* are the functions consistent with equation 1, determined by iterative nonlinear least-squares regression analysis. E_{max} represents the projected maximal EEG change; EC_{50} is the concentration at which the EEG change is 50% of the maximal value.

partly subjective. More objective biologic or physiologic measures of benzodiazepine action that have been used to elucidate pharmacokinetic-pharmacodynamic relationships include alterations in postural sway,[4] changes in saccadic eye movement velocity,[3,5] and alterations in hormonal responses.[5] Computerized quantitative analysis of the EEG likewise holds promise as an objective measure of central action of benzodiazepines[5-20] and of other classes of drugs.[50-53] Computerized quantitative analysis of the EEG was the approach used in the present study and was based on quantitation of increases of 13 to 30 Hz EEG activity attributable to acute benzodiazepine administration. The relatively small within- and between-subject variability in the fraction of total EEG amplitude within this frequency range in the baseline or drug-free state, together with the minimal EEG alterations in response to placebo, facilitated quantitation of drug-induced changes. Maintenance of wakefulness during the EEG assessment was crucial[7] because the increase in 13 to 30 Hz activity is lost if the patient is allowed to fall asleep. EEG activity in this range may actually reflect the response of the brain's "alerting system" to the pharmacologic sedative stimulus.[54-56] It is of interest that small but statistically significant reductions in 13 to 30 Hz activity were observed at several time points after placebo infusion, possibly reflecting individual variations in resting alertness levels.

The pharmacokinetic behavior of diazepam and midazolam after single intravenous doses in the present study are highly consistent with previous reports.[39-49] Both drugs are highly lipophilic benzodiazepines,[36]

characterized pharmacokinetically by large volumes of distribution, consistent with extensive tissue uptake.[29,57] Considering the extensive plasma protein binding of both of these drugs (1% to 2% unbound for diazepam; 3% to 4% unbound for midazolam),[39,45,58] the pharmacokinetic volumes of distribution based on total (free plus bound) drug considerably underestimate the extent of distribution of the unbound drug that is available for tissue distribution.[29,32,59] Despite similar distribution patterns, the two compounds differed greatly in their properties of elimination and clearance. Diazepam had a very long elimination $t_{1/2}$ in the postdistributive phase, consistent with the low metabolic clearance. On the other hand, midazolam had a very short $t_{1/2}$ and a high metabolic clearance that approached 50% of hepatic blood flow.

Both diazepam and midazolam produced EEG effects of rapid onset. Diazepam effects were maximal at the end of the 1-minute intravenous infusion, whereas those of midazolam were maximal, on the average, 5 to 10 minutes after the infusion. For both drugs the magnitude of the maximal EEG change was similar, with total EEG amplitude in the 13 to 30 Hz range and increasing from a mean of 47% in the baseline state to 65% to 70% immediately after drug administration. The EEG pattern reverted rapidly toward baseline over the next 3 to 4 hours. The duration of diazepam activity, based on a statistically significant difference over the predrug baseline values, was 5 to 6 hours; on the other hand, for midazolam the EEG was not significantly different from baseline by 2½ hours after administration. Neither drug had EEG effects significantly different from baseline 6 to 8 hours after administration. Thus despite the differing properties of elimination and clearance of diazepam and midazolam, the duration of central action based on objective EEG alterations was no greater than 6 hours. This emphasizes the importance of drug distribution in terminating the pharmacodynamic action of benzodiazepines after single intravenous doses. Although not quantitated in the present study, acute adaptation or tolerance is likely to have contributed to the termination of pharmacodynamic action of one or both of these drugs. That is, EEG effects became indistinguishable from baseline before plasma concentrations had fallen to zero, as reported in previous studies of midazolam.[16] More complete elucidation of this phenomenon would require study of the plasma concentration–EEG response relationship at various rates of infusion.

The relation of plasma concentration of either diazepam or midazolam to pharmacodynamic EEG activity was consistent with a mathematic function often used to characterize drug-receptor interaction phenomena.[33-35] In fact, qualitatively identical relationships have been observed in experimental studies that evaluate brain concentrations of benzodiazepines versus in vivo benzodiazepine receptor occupancy.[60] The predicted maximal EEG effects for both drugs in the present study were similar, whereas their EC_{50} values differed by approximately sevenfold, indicating "greater potency" of midazolam relative to diazepam at any absolute total plasma concentration. Consistent with this is the analogously greater affinity of midazolam for the benzodiazepine receptor in vitro.[29] Our EC_{50} values for midazolam are similar to those reported in previous pharmaco-EEG studies of oral midazolam.[16]

The relation of benzodiazepine-induced EEG alterations to their clinical sedative or anxiolytic effects is not clearly established. Nonetheless, the technique described herein may provide useful research methods to evaluate factors that can alter either pharmacokinetics or pharmacodynamics of benzodiazepines. Such factors include old age, hepatic or renal disease, cigarette smoking, chronic benzodiazepine administration, and coadministration of ethanol or other centrally acting compounds. The approach should allow estimation of the extent to which pharmacokinetic changes are the cause of altered pharmacodynamic responses. Midazolam, as opposed to diazepam, appears to be the most useful model compound in this context, inasmuch as a wide range of plasma concentrations, from essentially zero to well above the apparent EC_{50}, will be observed over less than a 24-hour period after a single intravenous dose. Furthermore, the short $t_{1/2}$ and high metabolic clearance of midazolam ensures that the same individual may be studied on more than one occasion with relatively short intervals between studies and with no residual drug present from prior studies.

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