PHARMACOKINETICS AND DRUG DISPOSITION

The erythromycin breath test predicts the clearance of midazolam

Midazolam, a commonly used sedative and amnestic medication, has recently been shown to be largely metabolized in the liver by a cytochrome P450, termed CYP3A4. There is at least a tenfold intersubject variability in the liver content and catalytic activity of CYP3A4, which may in part account for the known interpatient differences in the kinetics of midazolam. To test this hypothesis, we determined the intravenous midazolam kinetics of 20 medically stable, hospitalized patients, whose hepatic CYP3A4 activities were determined with use of the [^{14}C-*N*-methyl]erythromycin breath test. During the kinetic study, we also performed psychometric testing designed to quantitate the level of sedation and amnesia. We found a significant positive correlation between the erythromycin breath test results and weight adjusted clearance (in milliliters per minute per kilogram) of both total midazolam ($r = 0.52$; $p = 0.03$) and unbound midazolam ($r = 0.61$; $p < 0.01$). The relatively low dose of midazolam used (0.0145 mg/kg) produced significant but transient sedation and memory impairment in some of the patients. We conclude that interpatient differences in liver CYP3A4 activity in part account for the variations in midazolam kinetics. Our observations account for reported drug interactions involving midazolam and suggest that patients with low CYP3A4 activity may be most susceptible to prolonged amnestic effects occasionally produced by this short-acting benzodiazepine. (Clin Pharmacol Ther 1995;57:16-24.)

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Midazolam is a short-acting benzodiazepine that is administered intravenously to provide sedation and anterograde amnesia. It is routinely used before brief medical and surgical procedures. There are significant interpatient differences in both the depth and duration of the amnestic effects after a standard dose of midazolam. This makes it necessary to closely monitor all patients for several hours after treatment with the drug. In addition, most physicians advise their patients not to drive a vehicle on the day they receive midazolam and, as a result, patients must generally be accompanied by someone to drive them home after outpatient procedures.

Midazolam is extensively metabolized, with less than 0.03% being excreted unchanged in the urine.[1] 1′-Hydroxymidazolam is the major midazolam metabolite and accounts for 98% of metabolites recovered in urine, with 45% to 57% of the dose of midazolam being recovered as 1′-hydroxymidazolam metabolites in the urine.[2] Kronbach et al.[3] have shown that cy-

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tochromes within the CYP3A* family are the major liver enzymes involved in the metabolism of midazolam. Members of the CYP3A subfamily have also been shown to catalyze the major pathways for the metabolism of many commonly used drugs, including erythromycin,[5] cyclosporine,[6,7] lidocaine,[8] tamoxifen,[9] vindesine,[10] and dihydropyridine calcium channel blockers.[11,12] The intrinsic clearance for CYP3A-dependent metabolic pathways varies at least tenfold among patients.[13,14]

The major CYP3A enzyme present in human liver is CYP3A4.[15] We have found that the in vivo catalytic activity of hepatic CYP3A4 can be conveniently and noninvasively estimated as the rate of $^{14}CO_2$ exhaled after an intravenous test dose of [^{14}C-*N*-methyl-]erythromycin. This test is based on the observation that CYP3A4 exclusively catalyzes the *N*-demethylation of erythromycin in liver microsomes[16] and that the carbon atom in the resulting formaldehyde should largely appear in the breath as CO_2.[17] Several lines of evidence strongly support the idea that this erythromycin breath test (ERMBT) specifically measures CYP3A4. First, ERMBT results significantly rise or fall in patients after they are given either inducers or inhibitors, respectively, of CYP3A4.[16] In addition, we have found that in renal transplant patients, the ERMBT correlates with the apparent oral clearance of cyclosporine, a specific substrate for CYP3A4.[18] Finally, in patients with severe liver disease who underwent liver biopsy after receiving the ERMBT, we found that the results of the breath test correlated significantly with the liver microsomal concentration of CYP3A4.[19] In this same study, the ERMBT results did not correlate at all with the microsomal concentration of five other major liver P450s.

We speculated that interpatient differences in the pharmacokinetics of midazolam may in part reflect heterogeneity in CYP3A4 activity. To test this hypothesis, we administered the ERMBT to measure CYP3A4 catalytic activity and determined the pharmacokinetics of intravenous midazolam in 20 patients.

METHODS

Population. Written informed consent was obtained from 20 subjects recruited from the adult inpatient service at the University of Michigan Medical Center. To be included in the study, patients had to be medically stable and could not be taking any medications known or suspected to influence the catalytic activity of CYP3A4. Patients were excluded if they had any history of an allergy to erythromycin or midazolam. In addition, patients under the age of 18 years or women with childbearing potential were excluded to eliminate any possible risks associated with the use of carbon 14. This study was approved by the Institutional Review Board at the University of Michigan.

ERMBT. The ERMBT was administered as previously described.[16] Patients were given an intravenous dose of 0.074 mmol (0.0543 mg, 3 μCi) of [^{14}C-*N*-methyl]erythromycin. At timed intervals after the injection, patients were asked to exhale through a tube creating bubbles in a hyamine solution designed to trap exactly 2 mmol carbon dioxide. The vials were capped and the amount of exhaled ^{14}C was determined by scintillation counting. Breath test results were expressed as the percent of administered ^{14}C that was exhaled in the first hour after the injection of erythromycin.

Midazolam pharmacokinetics. As soon as patients had completed the ERMBT and the baseline psychometric tests (see below), they were given a single 0.0145 mg/kg intravenous dose of midazolam. Venous blood samples were drawn from the arm opposite the injection site just before the injection and at 5, 15, and 30 minutes and at 1, 2, 3, 4, and 5 hours after the injection. The blood samples were kept on ice until the plasma was separated by centrifugation. The plasma samples were frozen and kept at −20° C pending analysis for midazolam and 1′-hydroxymidazolam.

Analysis of midazolam and metabolites. Midazolam, 1′-hydroxymidazolam, diazepam, and temazepam standards were provided by Dr. Bill Garland of Roche Laboratories (Nutley, N.J.). Quantitation of midazolam and 1′-hydroxymidazolam in plasma was performed by gas chromatography–selective ion mass spectrometry. For measurement of midazolam and 1′-hydroxymidazolam in plasma and midazolam in urine, samples (1 ml) were thawed and spiked with internal standards: 20 ng diazepam and 10 ng temazepam. The pH was adjusted to ~11 with the addition of 50 mmol/L sodium bicarbonate and the samples were extracted with two 3 ml volumes of toluene/isoamyl alcohol (20:1). After extraction, solvent was removed under nitrogen and the remaining solid was dissolved in 50 μl of derivatizing reagent, 20% *N*-(tert butyldimethylsilyl)-*N*-methyltrifluoroacetamide (Regis

*The cytochromes P450 are a gene superfamily that has been divided into families that share greater than 40% amino acid sequence homology and subfamilies that share greater than 70% amino acid homology. In this article, we refer to specific P450s according to the recently recommended "CYP" nomenclature.[4] The term "CYP3A" has been referred to as "P450IIIA" in our previous publications.

Table I. Midazolam pharmacokinetic parameters

	Men (n = 13)	*Women (n = 4)*	*All subjects (n = 17)*
Body weight (kg)	84.1 ± 14.9	58.5 ± 3.1	78.1 ± 17.1
Midazolam concentration at 15 minutes (ng/ml)	26.6 ± 8.2	28.0 ± 11.1	25.7 ± 8.7
CL (L/min)	0.45 ± 0.17	0.24 ± 0.09	0.40 ± 0.18
CL (ml/min/kg)	5.4 ± 1.9	4.1 ± 1.4	5.1 ± 1.9
f_u (%)	2.1 ± 0.9	2.5 ± 1.1	2.2 ± 0.9
CL/f_u (L/min)	23.8 ± 13.2	11.4 ± 6.6	20.9 ± 13.0
CL/f_u (ml/min/kg)	288.2 ± 158.0	192.9 ± 109.4	265.8 ± 150.7
V_{ss} (L)	59.5 ± 23.1	71.5 ± 15.7	62.3 ± 21.7
$t_{1/2}$ (min)	127 ± 58	294 ± 116	166.4 ± 101.6

Data are mean values ± SD.
CL, Clearance; f_u, fraction drug unbound; V_{ss}, steady-state volume of distribution; $t_{1/2}$, half-life.

Table II. Correlation coefficients for ERMBT: midazolam clearance comparisons

	CL		*CL/f_u*		*1′-Hydroxymidazolam/midazolam plasma ratio (30 min)*
	L/min	*ml/min/kg*	*L/min*	*ml/min/kg*	
All subjects (*n* = 17)	*r* = 0.39 *p* = 0.13	*r* = 0.53 *p* = 0.03	*r* = 0.54 *p* = 0.03	*r* = 0.63 *p* = 0.007	*r* = 0.60 *p* = 0.012
Male only (*n* = 13)	*r* = 0.59 *p* = 0.03	*r* = 0.70 *p* = 0.008	*r* = 0.77 *p* = 0.002	*r* = 0.83 *p* = 0.0005	*r* = 0.71 *p* = 0.007

Chemical Co., Morton Grove, Ill.) in acetonitrile, and heated at 70° C for 2 hours. Derivatized samples were then transferred to autoinjector vials (100 μl inserts), and the vials were sealed. Coupled gas chromatography/mass spectrometry (GC/MS) analysis was performed on a Hewlett-Packard model 5970B MSD mass spectrometer interfaced to a Hewlett-Packard model 5890A gas chromatograph (Hewlett-Packard, Wilmington, Del.) equipped with an autoinjector and a DB-17 fused capillary column (30 m × 0.32 mm internal diameter, 25 micron film thickness; J&W Scientific, Ventura, Calif.). With use of helium carrier gas at a head pressure of 10 psi, the sample (2 μl) was injected in the splitless mode (injector temperature of 260° C). The initial column temperature of 160° C was held for 1 minute, followed by a 5° C/min ramp to 280° C and a final rapid ramp to 300° C, which was held for 5 minutes. The retention times and base peak ions individually monitored (dwell, 400 msec) during the analysis were diazepam (19.5 minutes; *m/z* 284), midazolam (21.6 minutes; *m/z* 310), temazepam (23.6 minutes; *m/z* 357), and 1′-hydroxymidazolam (25.7 minutes; *m/z* 398). Concentrations of 1′-hydroxymidazolam and midazolam were quantitated in the samples by comparing the peak area ratios to their respective standard curves (area ratio versus concentration) prepared from additions of known concentrations of midazolam and 1′-hydroxymidazolam to blank plasma. The interday coefficient of variation was 12.1% for midazolam (at 15 ng/ml) and 12.5% for 1′-hydroxymidazolam (at 3 ng/ml).

The binding of midazolam to plasma proteins was determined by equilibrium dialysis in all 20 patients. Blank plasma samples (1 ml) were spiked with 10 ng midazolam and dialyzed across a semipermeable membrane (Spectra Por* 2, 12,000 to 14,000 molecular weight cutoff; Spectrum Medical Industries Inc., Los Angeles, Calif.) against a 67 mmol/L sodium phosphate buffer (1 ml), pH 7.4. Four hours of dialysis (with shaking) at 37° C was sufficient to establish an equilibrium with unbound midazolam. Both plasma and dialysate buffer were analyzed for midazolam concentration as described above. The free fraction in plasma (f_u) was calculated as the ratio of midazolam concentration in buffer/plasma.

Midazolam plasma concentration–time data were fitted to a linear, two-compartment model by use of a least-squares, polyexponential curve-fitting program (RSTRIP, version 5.0, Micromath, Inc., 1989). Estimates of the zero and first statistical moments (AUC and AUMC) and elimination half-life ($t_{1/2}$) were obtained. Systemic clearance (CL) and steady-state dis-

tribution volume (V_{ss}) were calculated by standard pharmacokinetic methods.[20] The clearance of unbound midazolam (CL/f_u) was calculated as the ratio of systemic clearance to midazolam plasma free fraction.

Psychometric testing. To monitor the effects of midazolam over time, each patient was given two simple psychometric tests just before receiving midazolam and then 15 and 60 minutes after the injection of the drug. The first test, the State-Trait Anxiety Inventory (STAI), consisted of a standardized self-report of consciously perceived feelings of tension, apprehension, nervousness, worry, and heightened activation of the autonomic nervous system.[21] This test has been shown to be a reliable and sensitive measure of changes in the anxiety state. This allowed us to measure subtle changes in patients' subjective feelings of anxiety over time in response to the test dose of midazolam.

The second test, the Verbal Selective Reminding Task,[22] consisted of reading a series of 12 words aloud at 2-second intervals to patients and then asking them to recall as many of the words as possible. The subjects were then reminded only of the words they failed to remember and again asked to recall as many words as possible. This process was repeated for a total of 3 cycles. The pattern of recalled objects was then analyzed to generate results for long-term storage and consistent long-term recall. A decrease in the score on these tests indicates a deterioration in a patient's memory. These tests have been shown to be accurate and independent measures of memory storage and retrieval, respectively.[22]

Statistical analysis. Statistical analysis of the data was done with use of the program StatView 4.02 (Abacus Concepts Inc., Berkeley, Calif.). Correlations between the ERMBT and midazolam kinetics were determined by linear regression. Comparisons of means of the psychometric data were determined by use of two tailed t tests.

RESULTS

Twenty patients were enrolled in this study. Each patient received the ERMBT and baseline psychometric testing and then immediately received 0.0145 mg/kg midazolam intravenously. Venous blood samples were obtained at intervals as described in the Methods section and the psychometric tests were repeated 15 and 60 minutes after the midazolam injection. At the completion of the study, it was discovered that one patient had been receiving rifampin, a known potent inducer of CYP3A4,[16,23] for several days before and during the study. Because this patient did not

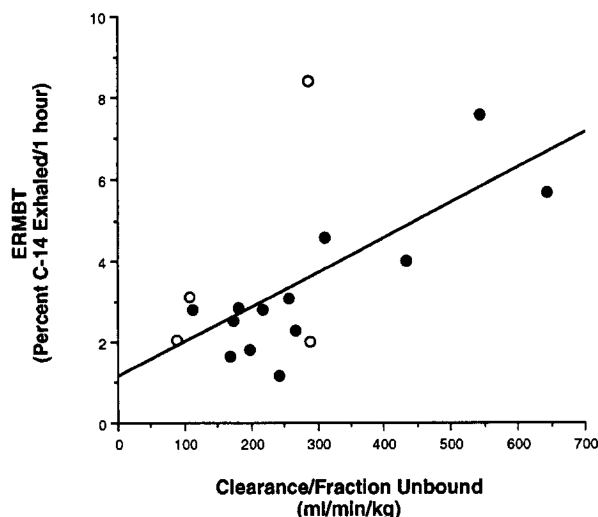

Fig. 1. Comparison of the erythromycin breath test (ERMBT) with the unbound midazolam clearance ($r = 0.63$, $p < 0.007$). *Solid circles,* men; *open circles,* women.

meet our entrance criteria for the study, the data obtained from this patient were excluded from our analysis. No other patients were receiving any known inducers or inhibitors of CYP3A4 activity. At the time of analysis of plasma samples, two patients were found to have diazepam and its metabolite temazepam in their baseline blood samples, which prevented the quantitation of midazolam. The data obtained from these two patients were also excluded from our analysis.

Table I shows the pharmacokinetic parameters of midazolam determined from the GC/MS analysis of plasma samples in the 17 evaluable patients. Estimates of the kinetic parameters for intravenous midazolam disposition in this study are in agreement with those reported in the literature.[1] Midazolam plasma clearance was, on average, approximately 27% of hepatic blood flow and 50% of hepatic plasma flow (estimated as 1.5 and 0.8 L/min, respectively).

The correlations between the ERMBT results and the calculated values for midazolam clearance are summarized in Table II. When all 17 subjects were considered there was no significant correlation observed between the ERMBT result and the total plasma clearance of midazolam. There was a weak but significant correlation obtained for a similar comparison with the plasma clearance of unbound midazolam ($r = 0.54$; $p = 0.03$). When the midazolam clearance was expressed as a function of the subject's body weight (in milliliters per minute per kilogram), there was an improvement in the correlations between

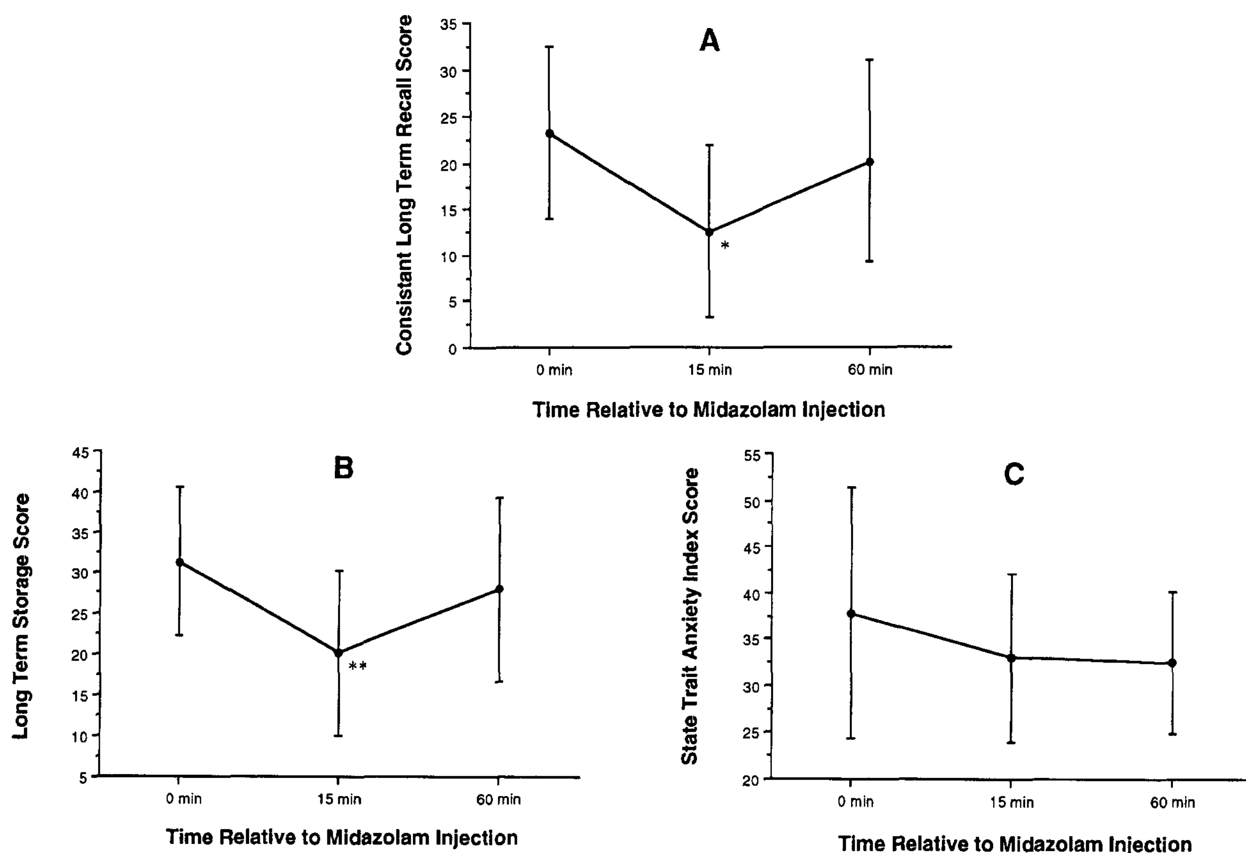

Fig. 2. Results of psychometric tests before and after intravenous midazolam dose. Consistent long-term recall (**A**) and long-term storage (**B**) are measures of memory, whereas state trait anxiety index (**C**) reflects anxiety levels. *Points* represent the mean of all patients, and *bars* show the standard error about the mean. The consistent long-term recall (**A**) and long-term storage (**B**) at 15 minutes after midazolam injection were significantly reduced compared with baseline values (* $p = 0.0004$; ** $p = 0.0001$; two-tailed paired t test).

ERMBT result and the total midazolam clearance ($r = 0.53$; $p = 0.03$) and between the ERMBT result and clearance of unbound midazolam ($r = 0.63$; $p < 0.007$; Table II and Fig. 1). In addition to the comparisons with midazolam clearance, the plasma concentration ratio of 1′-hydroxymidazolam to midazolam, measured at 30 minutes after administration of the midazolam dose, correlated significantly with the ERMBT result ($r = 0.60$; $p = 0.012$).

When data from the 13 male subjects were analyzed separately, there was a substantial improvement in the correlations between the ERMBT results and the total and unbound midazolam clearance, as well as between the ERMBT results and plasma 1′-hydroxymidazolam/midazolam concentration ratio (Table II). For either all 17 subjects or the 13 male subjects, there was no significant correlation between the ERMBT results and any of the other midazolam pharmacokinetic parameters, including peak midazolam concentration (C_{max}), V_{ss}, and terminal $t_{1/2}$.

The dose of midazolam administered to these patients produced marked changes in the mean consistent long-term recall and the long-term storage scores (Fig. 2, *A* and *B*). The mean value for both memory test scores decreased significantly 15 minutes after injection and had returned to near baseline when rechecked 1 hour after injection (Fig. 2, *A* and *B*). The state anxiety measurement (STAI) was also reduced, although not significantly, at 15 minutes after the midazolam dose and remained below the baseline 1 hour after the test dose (Fig. 2, *C*).

The extent of memory impairment observed in the patients 15 minutes after the midazolam dose did not correlate with either the total or free plasma midazo-

lam levels observed at that time (data not shown). There was a significant correlation between the ERMBT result and the percent improvement in the consistent long-term recall and long-term storage from 15 minutes to 1 hour time interval ($r = 0.56$, $p = 0.024$ and $r = 0.55$, $p = 0.022$, respectively; data not shown). However, the statistical significance of both of these comparisons was lost when data from a single outlying patient were excluded from this analysis. There were no significant correlations between the observed rate of improvement in memory function and the clearance of total or unbound midazolam or the estimated terminal $t_{1/2}$ of midazolam.

DISCUSSION

We found a significant correlation between the ERMBT results and the plasma clearance of both total and unbound midazolam in the patients studied (Fig. 1 and Table II). This supports our hypothesis that variability in the catalytic activity of CYP3A4 accounts, in part, for the interpatient differences in the pharmacokinetics of midazolam. The observed positive correlations between the ERMBT results and midazolam elimination are also consistent with the recent finding of a significant correlation between hepatic CYP3A4 levels and in vivo midazolam clearance in liver transplant patients.[24]

It is noteworthy that normalization of both total and unbound midazolam clearance for individual body weight resulted in an improvement in the correlations with the ERMBT results. This reflects the fact that the ERMBT does not measure erythromycin clearance per se, but is more accurately a measure of the amount of $^{14}CO_2$ derived from erythromycin demethylation in the first 60 minutes after intravenous injection. The pharmacokinetic relationship between a $^{14}CO_2$ breath test and systemic clearance can be described mathematically [see Lane and Parashos[25] for detailed derivation]. The fraction of an intravenously administered ^{14}C parent drug exhaled in the breath as $^{14}CO_2$ is represented in the following equation, assuming a one-compartment model and first-order kinetics:

$$\frac{CO_2\,(T)}{Dose_p} = fm\left[1 + \frac{CL_{sys,p}/V_p}{CL_c/V_{form} - CL_{sys,p}/V_p} \cdot (e^{-(CL_c/V_{form})T}) - \frac{CL_c/V_{form}}{CL_c/V_{form} - CL_{sys,p}/V_p}(e^{-(CL_{sys,p}/V_p)T})\right] \quad (1)$$

In this equation, $Dose_p$ is the dose of parent drug (erythromycin in this case), fm is the fraction of parent drug metabolized by demethylation to formaldehyde, $CL_{sys,p}$ is the systemic clearance of the total parent drug by all pathways, V_p is the volume of distribution of the parent drug in liters, CL_c is the clearance of formaldehyde to CO_2, V_{form} is the volume of distribution of formaldehyde, and T is time. Because the demethylation of erythromycin is assumed to be the rate-limiting step in the production of radiolabeled CO_2 in the breath, then CL_c/V_{form} (the formaldehyde elimination rate constant) must be significantly larger than $CL_{sys,p}/V_p$ (the erythromycin elimination rate constant). In this case the expression:

$$\frac{CL_c/V_{form}}{CL_c/V_{form} - CL_{sys,p}/V_p}$$

in equation 1 will approximate a value of 1. If we assume that the $t_{1/2}$ of formaldehyde is on the order of a few minutes for the collection period of 1 to 2 hours, the term $e^{-(CL_c/V_{form})T}$ will approach zero and the contribution from the first exponential term in equation 1 will be negligible. Therefore equation 1 will simplify to the following:

$$\frac{CO_2\,(T)}{Dose_p} = fm\left[1 - e^{-(CL_{sys,p}/V_p)T}\right] \quad (2)$$

Thus one can see that, for the ERMBT, the fraction of the dose excreted as $^{14}CO_2$ during 1 hour is related to the systemic clearance of erythromycin by a factor of $1/V_p$. The dependence on the V_p has also been reported for carbon dioxide breath test results based on other substrates.[26] Dependence on V_p would explain why the ERMBT result correlated best with the weight-adjusted clearance of midazolam in the current study and cyclosporine in our previous study.[18] This could also explain why in previous studies the ERMBT result predicted trough blood levels of the CYP3A4 substrates cyclosporine,[27] OG 37-325 (cyclosporin G),[28] and tacrolimus (FK506)[29] because blood level at a given point in time after dosing is dependent on both systemic clearance and volume of distribution.

It should be noted that $CL_{sys,p}$ and hence the ERMBT may vary with changes in the fraction unbound of erythromycin. Because there is very little variation in the fraction unbound in normal subjects,[30] this is unlikely to have significantly affected the ERMBT in our relatively healthy patients. However, this may become important in patients with severe liver disease or other conditions that affect the protein binding of erythromycin.

The dependence of the ERMBT result on volume of distribution makes intuitive sense as well as being mathematically shown by equation 2. Because an

identical dose of erythromycin is administered to each subject (0.074 mmol), differences in volume of distribution between subjects will affect the initial blood level after the intravenous injection. The initial rate of erythromycin demethylation (and hence the ERMBT result) will tend to be lower in those subjects with a lower plasma concentration of erythromycin. For example, consider two hypothetical subjects who have identical rates of erythromycin clearance but whose body weights differ twofold. Because the volume of distribution between subjects should largely be a function of body weight, the heavier patient will have approximately twice the volume of distribution for erythromycin and the initial blood level after the intravenous injection will be half that of the lighter patient. The net rate of erythromycin demethylation (ERMBT result) will therefore be half as great in the heavier subject than in the lighter subject even though they have the same erythromycin clearance.

As an additional way to test our conclusion that the ERMBT is related to clearance by the volume of distribution, we calculated a systemic clearance from the ERMBT result using equation 2. V_p for erythromycin was determined from the patients' weights and the reported values of volume of distribution of 0.66 L/kg for men and 0.75 L/kg for women.[30] With the calculated V_p and making the assumption that fm = 1, we were able to use the ERMBT result to solve equation 2 for $CL_{sys,p}$. The resulting calculated erythromycin clearance correlated significantly better with either total or unbound midazolam clearance expressed as liters per minute ($r = 0.564$, $p = 0.018$ and $r = 0.655$, $p = 0.004$, respectively; data not shown) than did the ERMBT result.

We found that the correlations between the ERMBT and midazolam kinetics improved when men were considered separately (Table II). We have previously found that women have a higher mean breath test result than men.[16] The number of women included in this study was too small to say whether gender-related differences might have affected the correlations between the ERMBT result and midazolam clearance.

The pharmacokinetics of midazolam did not appear to account for its acute effects on memory. Fifteen minutes after the midazolam was administered, there was significant interindividual variation in the measured blood levels of midazolam (Table I) and in the degree of memory impairment as measured by our tests (Fig. 2, *A* and *B*). However, there were no significant correlations between midazolam blood level (total or unbound) and the degree of memory impairment as measured by our tests. This is consistent with observations by others[31,32] and suggests that factors other than blood levels, such as brain density of γ-aminobutyric acid receptors, are more important determinants of the acute response to midazolam.

It has been shown that changes in encephalographic activity after the infusion of midazolam correlate with the plasma concentrations of the drug.[33] We therefore expected to find that the rate of memory recovery in an individual would correlate with the serum $t_{1/2}$ of midazolam. Our failure to find such a correlation probably does not reflect an insensitivity of our psychometric tests because we generally found substantial changes in memory ability attributable to the drug. Furthermore, the measured decrease in memory ability did not appear to be a function of changes in the anxiety state. This is because the anxiety state remained reduced compared with baseline at 1 hour (Fig. 2, *C*), whereas the memory test results had returned to baseline. The most likely reason for our inability to detect a correlation between the rate of improvement in memory test results and midazolam kinetic parameters was our choice of midazolam dose and the timing of the memory tests. With the relatively low dose of midazolam used in these studies, the measured memory effects were gone in most patients by the time they were retested at 1 hour. We were therefore unable to discriminate between patients on the basis of their actual rates of recovery.

There are several potential clinical implications of our observations. First, it has been reported that patients receiving treatment with erythromycin have a prolonged clearance of midazolam and a prolonged sedative effect from standard doses of midazolam.[34] Erythromycin is known to be a selective inhibitor of CYP3A4,[23] and inhibition of CYP3A4 therefore provides a likely explanation for the observed drug interaction. It seems probable that other potent inhibitors of CYP3A4,[23] such as the imidazole antifungal drugs (i.e., ketoconazole and miconazole) and troleandomycin, would also prolong the effects of midazolam. Caution should therefore be used when administering midazolam to patients receiving these drugs.

Our observations also suggest that treatment with medications known to increase (induce) CYP3A4 activity, such as rifampin and certain antiepileptic drugs, should significantly increase the clearance of midazolam. We had assumed that including patients in our study who were receiving CYP3A4 inducers would have dramatically improved the correlation observed between the ERMBT and midazolam clearance. However, it is interesting to note that the one patient tested

who was receiving an inducer of CYP3A4 (rifampin), and was excluded from our analysis, had very high CYP3A4 activity as measured by the ERMBT (11.25% of administered carbon 14 exhaled in 1 hour; data not shown) but did not have the highest clearance of unbound midazolam (212 ml/min/kg; data not shown). This may suggest that, at least in some patients, induced levels of CYP3A4 activity may no longer be rate limiting in the elimination of midazolam. Midazolam is considered to be a medium extraction drug, and therefore liver blood flow may become rate limiting when CYP3A4 activity becomes very high.

Midazolam 1′-hydroxylation can also be catalyzed by CYP3A5,[35] an enzyme that is detectable in the liver of only 20% to 30% of adults.[36] CYP3A5 does not readily catalyze the *N*-demethylation of erythromycin[37] and therefore would not be measured by the ERMBT. It is statistically likely that several of the patients in this study expressed CYP3A5, which may have decreased the correlation between the ERMBT results and midazolam clearance. However, because CYP3A5 "expressors" would be expected to have a higher midazolam clearance than predicted by the ERMBT, the presence of CYP3A5 alone can not account for the female outlier shown in Fig. 1 or the patient discussed above who had received a CYP3A4 inducer.

Our observations with midazolam may also be relevant to the structurally similar medication triazolam, a commonly used sleeping pill. Triazolam is a substrate for CYP3A4,[3] and at least some patients have been reported to have prolonged anterograde amnesia after taking this drug. A testable hypothesis is that these individuals have low CYP3A4 activity.

In summary, we have shown that in stable hospitalized patients, midazolam clearance correlates with liver CYP3A4 activity as measured by the ERMBT. This suggests that the catalytic activity of CYP3A4 is generally rate limiting in the in vivo elimination of midazolam. It would therefore seem reasonable to avoid coadministration of midazolam with drugs known to be potent inhibitors of CYP3A4. In addition, it seems likely that people who have low CYP3A4 activity (in the absence of drug inhibitors) may be at increased risk for prolonged amnestic effects after treatment with midazolam. Our data raises the possibility that convenient tests of in vivo CYP3A4 activity, such as the ERMBT, may be useful in identifying patients likely to have prolonged memory impairment after treatment with midazolam and structurally related sedatives.

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