PHARMACODYNAMICS AND DRUG ACTION

Age and gender effects on the pharmacokinetics and pharmacodynamics of triazolam, a cytochrome P450 3A substrate

Sixty-one healthy men and women, aged 20 to 75 years, received single 0.25-mg doses of triazolam, a cytochrome P450 (CYP) 3A substrate benzodiazepine, and placebo in a double-blind crossover study. Among women, age had no significant effect on area under the triazolam plasma concentration curve (AUC) (Spearman $r = 0.14$, $P = .44$) or clearance ($r = -0.09$, $P = .62$). Among men, AUC increased ($r = 0.43$, $P < .02$) and clearance declined ($r = -0.42$, $P < .02$) with increasing age. Gender differences in triazolam kinetics were not apparent. Compared with placebo, triazolam impaired digit-symbol substitution test performance, increased observer-rated sedation, impaired delayed recall of information learned at 1.5 hours after dosing, and increased electroencephalographic β amplitude. Among men, mean values of relative digit-symbol substitution test decrement ($P < .002$) and observer-rated sedation ($P < .05$) were significantly greater in elderly subjects compared with young subjects. Age-dependent differences among women reached significance for observer-rated sedation ($P < .02$). A combination of higher plasma levels and increased intrinsic sensitivity explained the greater pharmacodynamic effects of triazolam in elderly subjects. Although the findings are consistent with reduced clearance of triazolam in elderly men, individual variability was large and was not explained by identifiable demographic or environmental factors. (Clin Pharmacol Ther 2004;76:467-79.)

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The cytochrome P450 (CYP) 3A isoforms are of major importance in the biotransformation of many drugs used in psychopharmacology.[1-4] However, studies on the influence of age and gender on the activity of hepatic and gastrointestinal CYP3A isoforms, as well as the consequences of such differences in terms of pharmacodynamics and therapeutic drug response, have yielded inconsistent results.[5] This dilemma is exemplified by the existing database on the triazolobenzodiazepine triazolam, used as a hypnotic agent extensively in clinical practice. Triazolam is biotransformed exclusively by CYP3A isoforms in humans,[6-8] with both hepatic and gastrointestinal CYP3A contributing to net clearance after oral administration.[9] Some studies of age effects on triazolam kinetics have demonstrated significant decrements in weight-normalized clearance among healthy elderly subjects compared with young control subjects.[10,11] In other reports the effect of age was not statistically significant.[12,13] Kinetic-dynamic studies of triazolam suggest that enhanced benzodiazepine agonist effects of triazolam in elderly individuals

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Supported by grants AG-17880, MH-58435, DA-05258, DK-58435, DA-13834, DA-13209, AT-01381, and RR-00054 from the Department of Health and Human Services and by a grant in aid from The Upjohn Co (currently Pfizer, Inc), Kalamazoo, Mich.
Received for publication March 17, 2004; accepted July 23, 2004.

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0009-9236/$30.00

doi:10.1016/j.clpt.2004.07.009

Table I. Demographic characteristics and kinetic variables for triazolam

	Group (mean ± SE, range)			*Comparison of young and elderly groups (Kruskal-Wallis test)*
	Young	*Intermediate-aged*	*Elderly*	
Male subjects				
Demographic characteristics				
No.	10	11	9	
Age (y)	29 ± 1.6 (20–36)	47 ± 1.6 (40–56)	67 ± 1.5 (60–75)	$P < .001$
Weight (kg)	76.7 ± 3.8 (59–105)	77.3 ± 2.6 (66–98)	77.8 ± 1.9 (66–93)	$P = .93$
Height (cm)	178 ± 2 (168–185)	174 ± 3 (152–185)	167 ± 2 (162–183)	$P = .27$
Kinetic variables for triazolam				
C_{max} (ng/mL)	2.5 ± 0.2 (1.5–3.3)	2.4 ± 0.3 (1.4–4.0)	2.8 ± 0.4 (1.5–5.7)	$P = .74$
t_{max} (h after dose)	1.0 ± 0.2 (0.5–2.5)	1.0 ± 0.2 (0.5–2.0)	1.1 ± 0.2 (0.5–2.5)	$P = .48$
$t_{1/2}$ (h)	2.8 ± 0.3 (2.0–4.4)	3.1 ± 0.3 (2.3–5.7)	4.2 ± 0.5 (2.1–7.3)	$P = .05$
AUC (ng/mL · h)	9.8 ± 1.4 (6.0–20.8)	11.4 ± 1.7 (5.6–25.0)	17.2 ± 4.0 (4.1–44.3)	$P = .086$
Clearance (mL/min)	484 ± 48 (200–693)	437 ± 56 (166–748)	369 ± 93 (94–1018)	$P = .086$
Clearance (mL · min^{-1} · kg^{-1})	6.55 ± 0.82 (2.67–11.20)	5.67 ± 0.74 (2.09–10.75)	4.72 ± 1.07 (1.01–11.5)	$P = .14$
Female subjects				
Demographic characteristics				
No.	13	6	12	
Age (y)	29 ± 1.6 (20–36)	44 ± 1.9 (39–51)	68 ± 1.5 (61–75)	$P < .001$
Weight (kg)	65.0 ± 4.7 (48–109)	64.8 ± 2.0 (55–75)	56.4 ± 1.8 (46–64)	$P = .086$
Height (cm)	167 ± 2 (152–188)	164 ± 2 (157–168)	160 ± 2 (152–174)	$P < .05$
Kinetic variables for triazolam				
C_{max} (ng/mL)	2.7 ± 0.2 (1.6–3.7)	2.5 ± 0.3 (1.7–3.9)	3.2 ± 0.4 (1.8–5.6)	$P = .45$
t_{max} (h after dose)	1.3 ± 0.2 (0.5–2.5)	1.0 ± 0.2 (0.5–1.5)	1.0 ± 0.1 (0.5–1.5)	$P = .41$
$t_{1/2}$ (h)	2.6 ± 0.2 (1.6–4.3)	3.0 ± 0.3 (2.2–4.5)	2.6 ± 0.1 (2.0–3.9)	$P = .66$
AUC (ng/mL · h)	11.6 ± 1.6 (5.0–26.6)	11.4 ± 1.1 (6.3–13.0)	13.4 ± 2.0 (5.9–30.2)	$P = .45$
Clearance (ml/min)	445 ± 59 (157–838)	393 ± 56 (298–664)	382 ± 50 (138–708)	$P = .45$
Clearance (mL · min^{-1} · kg^{-1})	7.35 ± 1.14 (1.72–16.0)	5.97 ± 0.59 (4.99–8.86)	6.80 ± 0.92 (2.98–12.55)	$P = .83$

C_{max}, Peak plasma concentration; t_{max}, time to peak plasma concentration; $t_{1/2}$, elimination half-life; AUC, area under triazolam plasma concentration curve.

are at least partly explained by reduced clearance and increased plasma concentrations.[10] However, the possible influence of altered baseline function and performance values on age-dependent responses to active medication has also been emphasized.[10,13] Studies of other benzodiazepine agonists have demonstrated that age-dependent changes in intrinsic sensitivity, separate from or in addition to pharmacokinetic alterations, are likely to contribute to the observed clinical phenomena.[14a]

The possibility of gender-dependent differences in pharmacokinetics of CYP3A substrates is also discussed in the literature.[5,15-17] Studies of triazolam pharmacokinetics in healthy male and female volunteers generally demonstrate higher values of weight-normalized clearance in women compared with men.[11,12,18,19] This difference reached statistical significance in only one report.[19] For other CYP3A substrates, such as alprazolam and midazolam, inconsistent gender-dependent differences in clearance are similarly reported.[20] Some studies suggest that higher values of clearance in women may be attributable to increased enteric CYP3A.[21]

This study evaluated the effects of age and gender on the kinetics of the CYP3A substrate triazolam in a series of 61 healthy volunteers aged 20 to 75 years. The study included a cohort of individuals in the intermediate age range (39-56 years), which is a relatively unusual feature of pharmacogeriatric studies. Inclusion of this intermediate-aged group allowed us to address the hypothesis of whether age-dependent changes in pharmacokinetic or pharmacodynamic variables can be

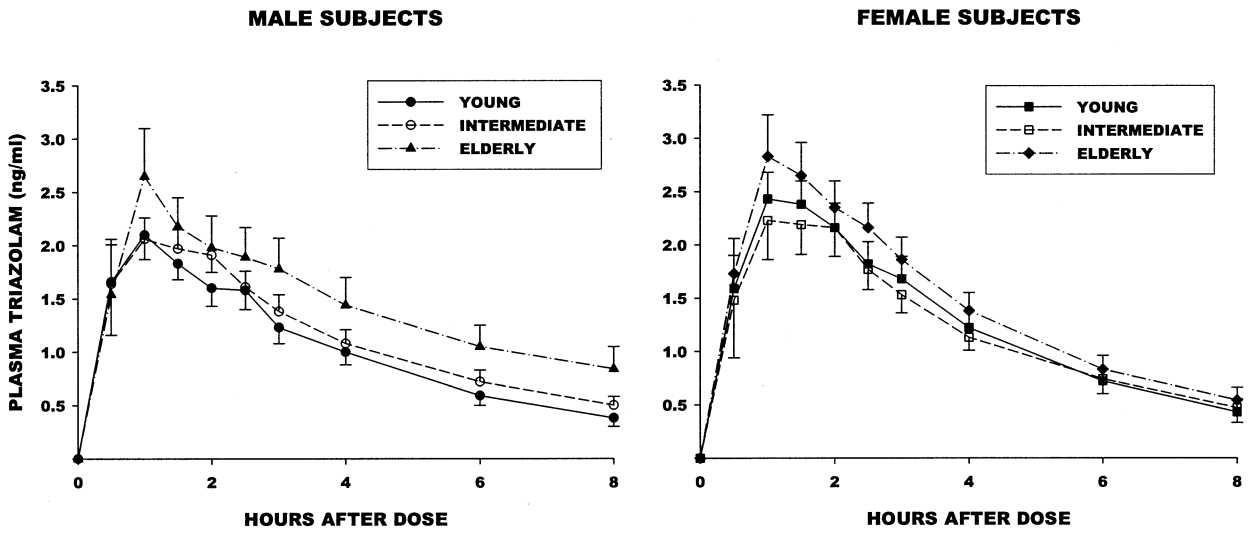

Fig 1. Mean (±SE) plasma triazolam concentrations at corresponding times for groups of young, intermediate-aged, and elderly men (*left*) and women (*right*).

understood as a function of age as a continuous variable. This study also included the electroencephalogram (EEG) as an objective measure of benzodiazepine agonist effects, by use of a typical therapeutic dose of triazolam (0.25 mg) in comparison to placebo.

METHODS

Subjects. The study protocol was reviewed and approved by the Human Investigation Review Committee, the institutional review board serving Tufts University School of Medicine and Tufts-New England Medical Center (Boston, Mass). After giving written informed consent, 61 male and female volunteers (58 white and 3 Hispanic), aged 20 to 75 years, participated in this study.

All study participants were healthy ambulatory adults who had no evidence of medical disease and were receiving no other medications. Young female subjects were not taking oral contraceptives and did not have contraceptive implants. Table I shows the characteristics of the study population.

Subjects participated in a 3-way crossover study. To allow volunteers to adapt to the study setting and procedures and to minimize the effects of practice, the first treatment in the sequence was a single-blind administration of placebo; data from this practice trial were not used in subsequent analyses. The next 2 treatments were double-blind and randomized in sequence. The 2 conditions were placebo and 0.25 mg triazolam. A minimum of 7 days and a maximum of 21 days elapsed between treatments. All medications were identically packaged.

On the morning of each study day, after ingesting a standardized light breakfast with no caffeine-containing food or beverages and no grapefruit juice, subjects arrived at the Clinical Research Unit at approximately 7:30 AM. They fasted until noon, after which they resumed a normal diet (again without grapefruit juice or caffeine-containing food or beverages). The single dose of triazolam or placebo was given with 240 mL of tap water at 9 AM.

Venous blood samples were drawn from an indwelling cannula into heparinized tubes before dosing and at the following postdose times: 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, 8, and 24 hours. Samples were centrifuged, and the plasma was separated and frozen until the time of assay.

The EEG was recorded via a 6-electrode montage, with instrumentation and methodology described previously.[17-19,22-25] At 2 predose times and during an 8-hour period after dosing at times corresponding to blood sampling, the EEG was quantified in 4-second epochs for as long as necessary to ensure at least 2 minutes of artifact-free recording. Data were digitized over the power spectrum from 4.0 to 31.75 cycles per second (in hertz) and were then fast Fourier–transformed to determine amplitude over the 4.0- to 31.75-Hz spectrum and in the β band (13.0-31.75 Hz).

Subjects' self-ratings of sedative effects and mood state were obtained with a series of 100-mm visual

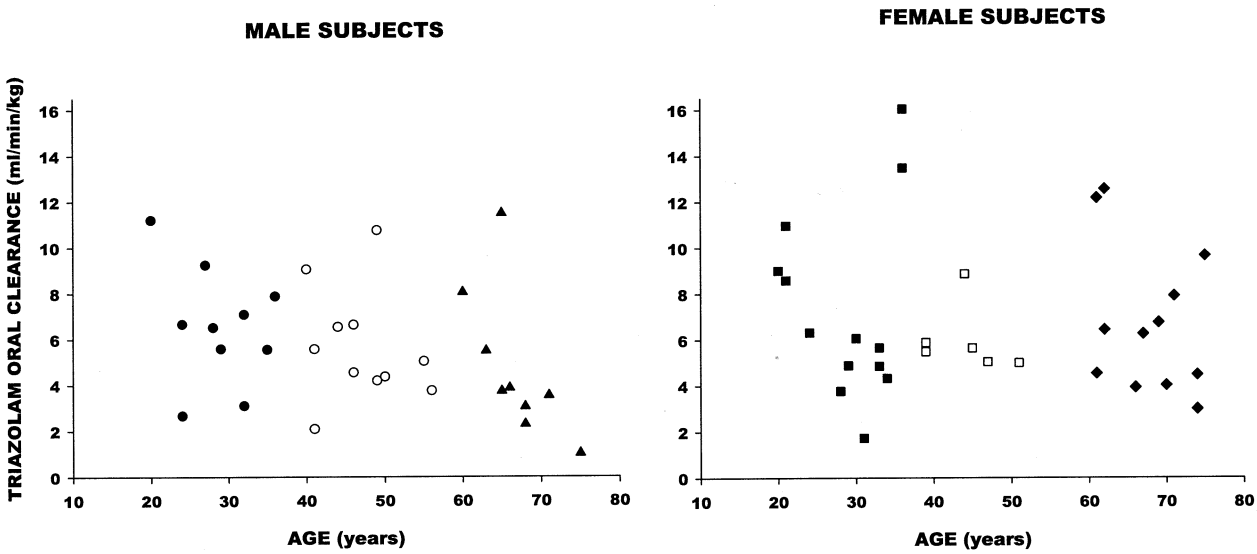

Fig 2. Relationship between age and triazolam oral clearance among men (*left*) and women (*right*). Among men, the decrement in clearance with age was significant (Spearman $r = -0.42$, $P < .02$). Among women, clearance was not significantly associated with age ($r = -0.09$, $P = .62$). *Symbols* represent age categories as indicated in Fig 1.

Table II. Spearman correlation analysis of age versus kinetic and dynamic variables

	Male subjects		*Female subjects*	
Variable	*r Value**	*Significance*	*r Value**	*Significance*
Pharmacokinetics				
C_{max} (ng/mL)	0.18	$P = .33$	0.18	$P = .33$
$t_{1/2}$ (h)	0.44	$P < .02$	0.16	$P = .40$
AUC (ng/mL · h)	0.43	$P < .02$	0.14	$P = .44$
Clearance (mL · min^{-1} · kg^{-1})	–0.42	$P < .02$	–0.09	$P = .62$
Predose pharmacodynamic values				
DSST score				
Placebo	–0.54	$P < .003$	–0.39	$P < .04$
Triazolam	–0.42	$P < .03$	–0.41	$P < .03$
% β EEG amplitude				
Placebo	0.31	$P = .10$	0.42	$P < .03$
Triazolam	0.27	$P = .16$	0.40	$P < .04$
8-h Pharmacodynamic effect				
AUC (triazolam-placebo difference)				
DSST % decrement	–0.32	$P = .08$	–0.12	$P = .53$
Self-rated sedation	0.05	$P = .79$	0.00	$P = 1.00$
Observer-rated sedation	0.26	$P < .20$	0.45	$P < .02$

DSST, Digit-symbol substitution test.
*Spearman correlation coefficient.

analog scales.[26] Ratings of sedation were also performed by trained observers, using the same rating instrument, who had no knowledge of the treatment condition. Self-ratings and observer ratings were obtained twice before medication administration and at postdose times indicated.

The digit-symbol substitution test (DSST) was administered twice before dosing and at times corre-

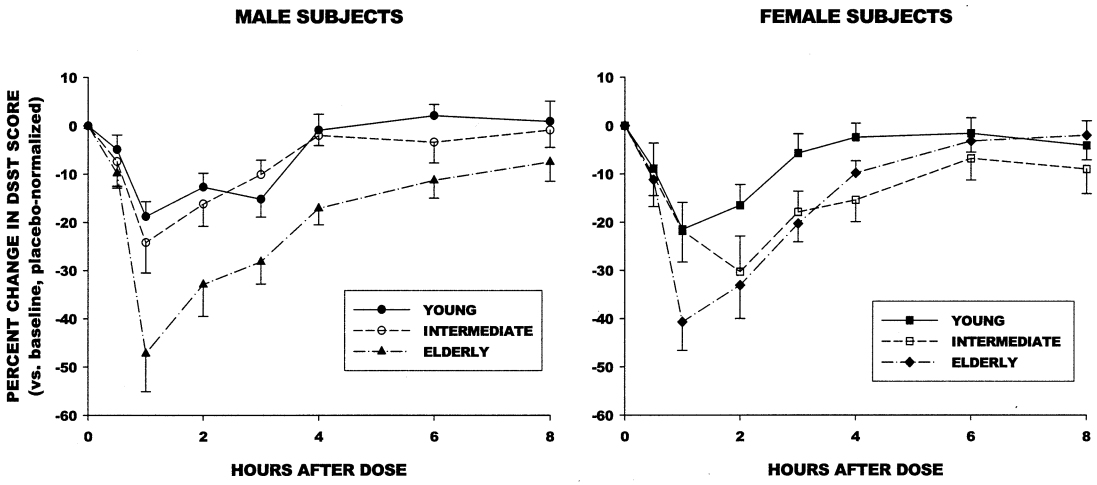

Fig 3. Mean (±SE) percentage changes over baseline in scores on digit-symbol substitution test (DSST) among groups of young, intermediate-aged, and elderly men (*left*) and women (*right*) after administration of triazolam. Changes are normalized for changes at corresponding times in the placebo treatment condition.

sponding to rating scales.[7,10,18,19,22-26] Subjects were asked to make as many correct symbol-for-digit substitutions as possible within a 2-minute period. Subjects completed equivalent DSST variants, with no individual taking the same test more than once.

Acquisition and recall of information were evaluated with the use of a word-list free-recall procedure that was administered at 1.5 hours after drug or placebo administration.[7,10,18,19,22-27] Sixteen words, taken from 4 different categories, were read in random order in "shopping-list" fashion. Subjects wrote down items immediately after lists were presented in random order. List presentation and recall were repeated a total of 6 times at 1.5 hours after dosing. At 24 hours after dosing, subjects were asked to remember as many words as possible from the previous day's list (delayed, or "free," recall). Thereafter the same lists were read in the same sequence in which they were presented on the previous day, to assess whether residual effects of drug administration on immediate recall were detectable.

Analysis of data. Plasma concentrations of triazolam were determined by gas chromatography with electron-capture detection,[7] with a sensitivity limit of 0.2 ng/mL for a 2-mL sample. The within-day variance for replicate samples did not exceed 7%. The between-day variances for 2 quality-control samples containing low and high concentrations of triazolam were 10.9% and 5.9%.

The slope (β) of the terminal log-linear phase of each triazolam plasma concentration versus time curve was determined by linear regression analysis. This slope was used to calculate the apparent elimination half-life. Area under the plasma concentration curve from time 0 until the last detectable concentration was determined by the linear trapezoidal method. To this area was added the residual area extrapolated to infinity, calculated as the final concentration divided by β, yielding the total area under the plasma concentration versus time curve (AUC). The residual area accounted for an average of 19% of the total AUC. The peak plasma concentration and time to peak concentration represented the rate of appearance of drug in the systemic circulation. Apparent oral clearance was calculated as the administered dose divided by the total AUC.

For self-ratings and observer ratings on visual analog scales, the 2 predose baseline ratings were averaged, and postdose scores were expressed as the increment or decrement relative to the mean predose value. Scores on the DSST were analyzed similarly. The word-list memory test was analyzed as the mean number of words remembered after 6 trials for immediate recall and as the mean absolute number of words correctly remembered for delayed recall.

For each EEG recording session, the relative β amplitudes (β divided by total, expressed as percent) were calculated, and values from the left and right fronto-temporal leads were averaged. The means of the relative β amplitudes in the predose recordings were used as baseline values, and all postdose values were ex-

Table III. Age and gender effects on predose baseline DSST scores and percent β EEG amplitude

	Group (mean ± SE)			*Comparison of young and elderly groups (Kruskal-Wallis test)*
	Young	*Intermediate-aged*	*Elderly*	
Male subjects				
No.	10	11	9	
Predose DSST score				
Placebo	65 ± 4	62 ± 5	46 ± 5	$P < .01$
Triazolam	65 ± 5	61 ± 4	50 ± 5	$P < .04$
Predose % β EEG amplitude				
Placebo	45 ± 2	47 ± 2	52 ± 3	$P < .03$
Triazolam	47 ± 2	47 ± 2	52 ± 3	$P = .086$
Female subjects				
No.	13	6	12	
Predose DSST score				
Placebo	74 ± 4	71 ± 5	58 ± 5	$P < .04$
Triazolam	74 ± 4	70 ± 5	57 ± 5	$P < .03$
Predose % β EEG amplitude				
Placebo	45 ± 2	53 ± 2	51 ± 2	$P < .05$
Triazolam	45 ± 2	51 ± 3	50 ± 1	$P < .04$

pressed as the increment or decrement over that treatment's mean predose baseline value.

For each pharmacodynamic variable, the area under the 8-hour plot of effect change score versus time was calculated to obtain a single integrated measure of pharmacodynamic action during the period of greatest drug effect. For statistical analysis of the influence of age, pharmacodynamic effect areas in the placebo treatment condition were subtracted from values in the triazolam treatment condition, thereby creating placebo-normalized effect areas. The ratio of 8-hour placebo-normalized pharmacodynamic effect area divided by area under the plasma concentration curve was used as a measure of drug sensitivity.

The relationship between pharmacokinetic or pharmacodynamic variables and age was evaluated by use of Spearman correlations. Mean values for young and elderly groups also were compared by use of the Kruskal-Wallis test on rank-transformed values. Relationships between plasma triazolam concentrations and pharmacodynamic variables were evaluated by linear and nonlinear regression analysis.

RESULTS

Pharmacokinetics. Among female subjects, there was no significant difference between young and elderly groups in any of the pharmacokinetic variables for triazolam (Table I and Fig 1). When age was evaluated as a continuous variable, it was not significantly correlated with AUC (Spearman $r = 0.14$) or clearance ($r = -0.09$) (Table II and Fig 2).

Among male subjects, the elimination half-life increased significantly with age. AUC values increased and clearance decreased with age. Differences in mean values of clearance between young and elderly groups just failed to reach statistical significance ($P = .086$). However, when age was evaluated as a continuous variable, AUC increased significantly with age ($r = 0.43$, $P < .02$) and clearance decreased with age ($r = -0.43$, $P < .02$) (Fig 2).

Pharmacodynamics. For all 6 study cohorts, triazolam and placebo treatments differed significantly in pharmacodynamic effect areas for observer-rated sedation, self-rated sedation, decrements in DSST performance, and increases in β EEG amplitude. Among women, triazolam and placebo also differed in self-ratings of fatigue, thinking slowed down, and feeling "spacey."

A significant effect of age was observed in predose baseline DSST scores, with lower baseline values in elderly subjects (Tables II and III). For subsequent analyses, changes over baseline in DSST scores were normalized as a percentage change relative to the baseline value.[10] Predose baseline values of percent β EEG amplitude also changed with age to a quantitatively

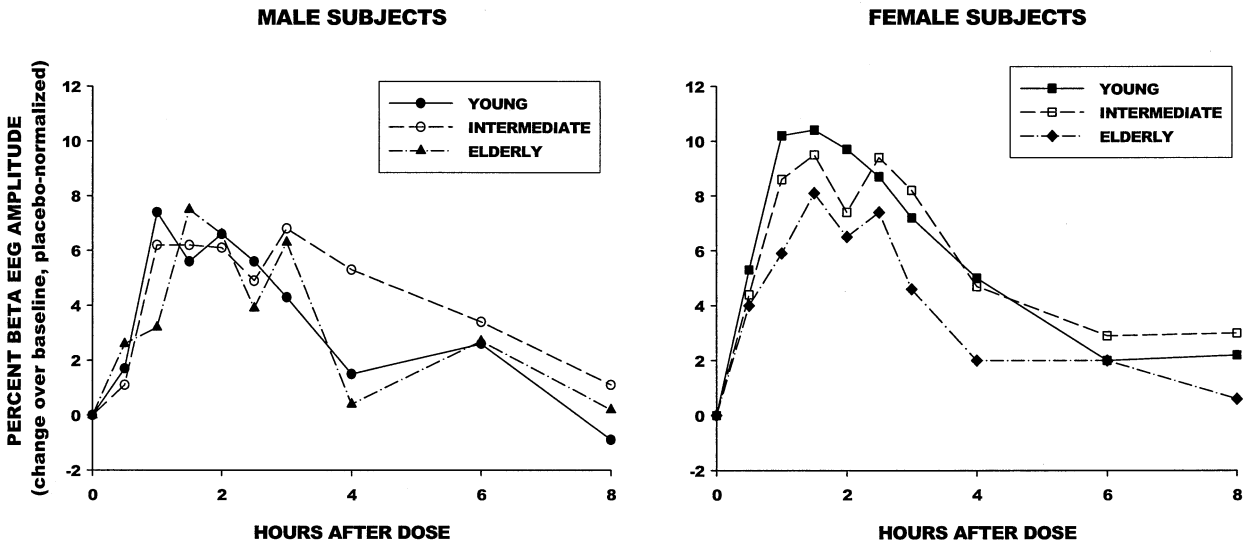

Fig 4. Mean (±SE) changes over baseline in relative electroencephalographic (EEG) amplitude in β frequency range among groups of young, intermediate-aged, and elderly men (*left*) and women (*right*) after administration of triazolam. Changes are normalized for changes at corresponding times in the placebo treatment condition. SEs for individual points, omitted for clarity, are available on request from the authors.

Table IV. Eight-hour pharmacodynamic effect areas (triazolam-placebo differences)

	Group (mean ± SE)			*Comparison of young and elderly groups (Kruskal-Wallis test)*
	Young	*Intermediate-aged*	*Elderly*	
Male subjects				
No.	10	11	9	
% β EEG amplitude	24.0 ± 9.0	30.1 ± 3.9	22.7 ± 6.6	$P = .33$
DSST % decrement	41 ± 14	59 ± 22	157 ± 25	$P < .002$
Self-rated sedation	32 ± 30	26 ± 14	14 ± 24	$P = .85$
Observer-rated sedation	33 ± 10	61 ± 20	87 ± 24	$P < .05$
Female subjects				
No.	13	6	12	
% β EEG amplitude	40.7 ± 10.3	40.1 ± 5.3	24.9 ± 9.1	$P = .17$
DSST % decrement	54 ± 23	128 ± 32	113 ± 22	$P = .10$
Self-rated sedation	61 ± 28	46 ± 16	49 ± 21	$P = .84$
Observer-rated sedation	47 ± 13	13 ± 8	102 ± 16	$P < .02$

small extent (Table III); postdose changes were expressed as absolute differences from baseline.

Among male subjects, placebo-normalized pharmacodynamic effect areas increased significantly with age for DSST decrement ($P = .08$) and observer-rated sedation (Table II and Fig 3); young and elderly groups differed significantly in mean DSST decrement and mean increase in observer-rated sedation (Table IV), with much larger changes in the elderly group. There was no significant influence of age on effect areas for β EEG amplitude (Fig 4 and Table IV) or for self-rated sedation.

Among female subjects, effect area for observer-rated sedation increased significantly with age (Table II), and young and elderly groups differed significantly in mean 8-hour effect areas for observer-rated sedation (Table IV). DSST decrements also became greater with age, although the difference did not reach statistical

Table V. Effect of age, gender, and triazolam on information requisition and recall

	Immediate recall (1.5 h after dosage)	*Free (delayed) recall (24 h after dosage)*
Male subjects		
Young (n = 10)		
Placebo	12.7 ± 1.0	10.0 ± 1.4
Triazolam	9.9 ± 1.0	2.0 ± 0.9
Intermediate-aged (n = 11)		
Placebo	12.8 ± 0.9	10.3 ± 1.2
Triazolam	9.0 ± 0.8	1.8 ± 0.6
Elderly (n = 9)		
Placebo	10.8 ± 1.3	7.4 ± 2.0
Triazolam	6.9 ± 1.5	1.0 ± 0.6
Female subjects		
Young (n = 13)		
Placebo	14.5 ± 0.4	10.8 ± 1.1
Triazolam	10.7 ± 0.9	4.3 ± 1.2
Intermediate-aged (n = 6)		
Placebo	14.5 ± 0.8	12.2 ± 1.7
Triazolam	11.2 ± 2.1	1.0 ± 0.7
Elderly (n = 12)		
Placebo	13.1 ± 0.7	10.0 ± 1.3
Triazolam	7.8 ± 1.1	1.7 ± 0.9

Values shown are mean (±SE) number of words recalled (out of 16 total).

significance. Effect areas for β EEG amplitude and self-rated sedation did not increase with age.

Information acquisition and recall. At 1.5 hours after dosing in the placebo treatment condition, the number of words recalled immediately after 6 presentations of the 16-word list declined with age in male and female subject groups (Table V), although the effect of age was not statistically significant. Immediate recall in all cohorts was significantly lower ($P < .01$) after triazolam than after placebo, indicating impairment of information acquisition and/or immediate recall attributable to triazolam. Again, a decrement with age was observed, but the effect was not statistically significant.

In all subject groups the number of words recalled at 24 hours after dosing (free, or delayed, recall) in the placebo condition was significantly lower ($P < .01$) than with immediate recall, indicating some degree of incomplete information storage attributable to the passage of time. The impairment of delayed recall was dramatically increased in the triazolam condition. In all cohorts, free recall after triazolam was significantly less ($P < .01$) than after placebo, even though initial acquisition and immediate recall were also impaired by triazolam. It is of interest that the number of words recalled by female subjects was consistently greater than the number recalled by male subjects of corresponding age. However, a statistical analysis of these differences was not undertaken.

Relearning of the 16-item word list at 24 hours after dosing (after the free-recall procedure) indicated no significant difference between triazolam or placebo (data not shown).

Kinetic-dynamic relationships. Table VI shows the ratio of 8-hour area under the placebo-normalized pharmacodynamic effect curve divided by total area under the plasma concentration curve. For DSST decrements, ratios increased with age among men, with a significant difference between young and elderly groups. Among female subjects, elderly subjects had significantly greater ratios for observer-rated sedation than young subjects. Ratios did not increase with age for percent β EEG amplitude or self-rated sedation.

Within each cohort, mean plasma concentrations at individual time points were significantly related to placebo-normalized percent DSST decrements at corresponding times (Fig 5). The best-fitting function was of the following form: $y = Bx^A$, where x is plasma concentration, y is DSST decrement, B is a coefficient having units of concentration, and A is an exponent. The values of B and A from the function of best fit can be used to calculate the plasma concentration corresponding to a 30% decrement in DSST score (EC_{30}). Mean values of EC_{30} decreased with age in both male and female subjects (Fig 6), consistent with an increase in drug sensitivity associated with age. However, a formal statistical analysis of EC_{30} values was not undertaken.

DISCUSSION

The triazolobenzodiazepine triazolam is biotransformed exclusively by CYP3A isoforms in humans.[6-8] Previous studies of intravenous and oral triazolam have indicated an absolute bioavailability of approximately 45% to 50%, despite estimated hepatic extraction ratios on the order of 0.2.[28-30] Thus presystemic extraction of oral triazolam is accounted for mainly by enteric metabolism,[9] given a lack of evidence supporting efflux transport by P-glycoprotein.[31] This indicates that factors influencing the clearance of orally administered triazolam act at least in part through modulation of expression or function of enteric CYP3A.

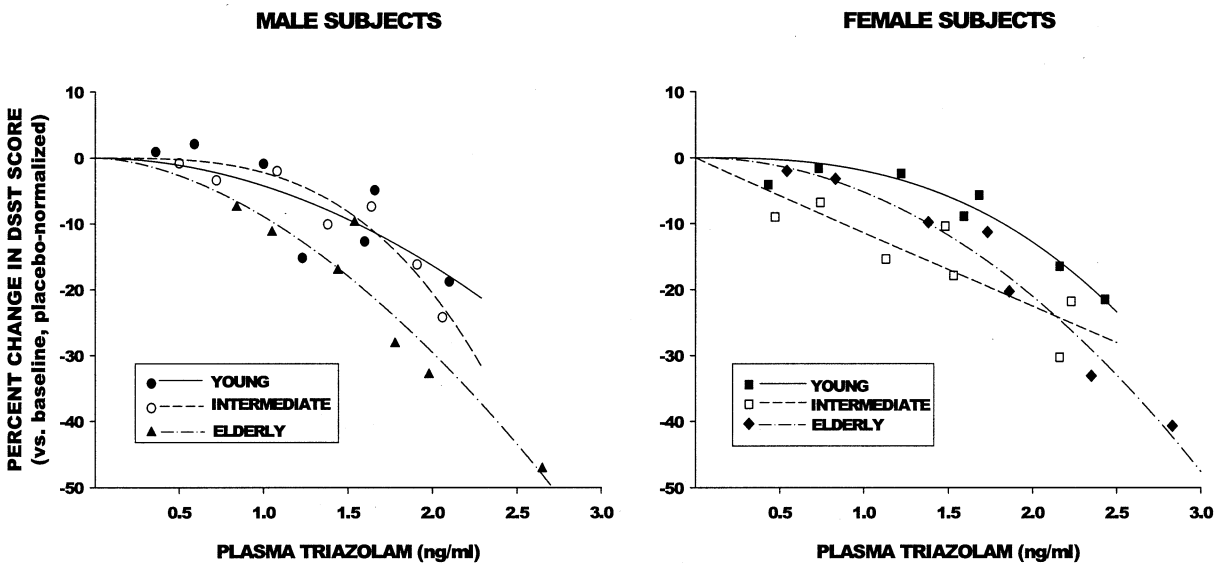

Fig 5. Relationship between mean plasma triazolam concentrations (*x-axis*) and mean placebo-normalized percentage changes over baseline in DSST scores at corresponding times (*y-axis*) among elderly men (*left*) and women (*right*). Function lines, determined by nonlinear regression analysis, are of the following form: $y = Bx^A$, as described in the text.

Age and gender effects on the clearance of triazolam and of other CYP3A substrate benzodiazepines are not clearly or consistently established. In the case of triazolam, 2 studies demonstrated a substantial decrement in clearance among healthy elderly individuals compared with young control subjects.[10,11] In a third study, a clearance decrement was evident in elderly men but did not reach significance.[13] A fourth study showed no significant changes in clearance between elderly men or women compared with young subjects of the same gender.[14,14a] Among studies comparing triazolam kinetics between groups of young men and women, only one report indicated significantly higher weight-normalized clearance in women compared with men.[18] Two other studies showed a nonsignificant trend toward higher clearance in women,[11,12] and 1 study showed no effect of gender.[10] The existing literature on midazolam and alprazolam, 2 other benzodiazepines with CYP3A-dependent clearance, indicates a similar pattern. Several reports have demonstrated age-related decrements in clearance of these drugs,[32-37] whereas others have not.[38-40] Likewise, higher clearances in women compared with men have been described in some studies[21,41] but not in others.[32,34,35,42,43]

This study demonstrated no evidence of a meaningful effect of gender on triazolam clearance among subjects aged less than 40 years. Weight-normalized clearance in young women averaged 12% higher than in young men. The effects of age, however, differed between men and women. Among women, there was a small increase in AUC and reduction in clearance with

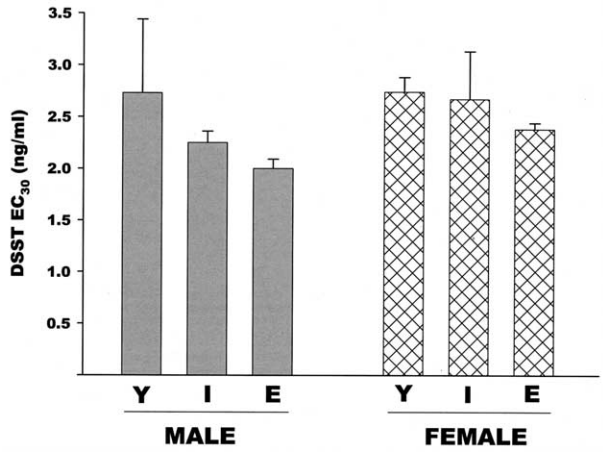

Fig 6. Mean (± asymptotic SE of parameter estimate) EC_{30} values, representing triazolam concentration producing a 30% decrement in DSST score based on analysis shown in Fig 5. (A formal statistical analysis of EC_{30} values was not undertaken.) Age ranges were as follows: young subjects (Y), 20 to 36 years; intermediate-aged subjects (I), 39 to 56 years; and elderly subjects (E), 60 to 75 years.

Table VI. Ratio of 8-hour pharmacodynamic effect area (triazolam-placebo differences) divided by total area under plasma concentration curve

	Group (mean ± SE)			*Comparison of young and elderly groups (Kruskal-Wallis test)*
	Young	*Intermediate-aged*	*Elderly*	
Male subjects				
No.	10	11	9	
% β EEG amplitude	2.7 ± 1.0	3.0 ± 0.4	2.0 ± 0.8	$P = .37$
DSST % decrement	–5.4 ± 2.0	–5.1 ± 2.4	–11.4 ± 2.0	$P < .05$
Self-rated sedation	4.5 ± 4.1	3.1 ± 1.7	0.7 ± 1.6	$P = .56$
Observer-rated sedation	3.6 ± 1.1	6.3 ± 2.0	5.6 ± 1.7	$P = .42$
Female subjects				
No.	13	6	12	
% β EEG amplitude	4.7 ± 1.2	4.1 ± 1.3	2.6 ± 0.9	$P = .20$
DSST % decrement	–5.9 ± 1.9	–10.5 ± 2.1	–8.6 ± 1.9	$P = .36$
Self-rated sedation	7.9 ± 3.5	4.6 ± 1.9	5.6 ± 2.7	$P = .75$
Observer-rated sedation	5.0 ± 1.5	1.4 ± 0.8	8.7 ± 1.7	$P = .05$

age; these changes did not approach statistical significance. Among male subjects, however, mean AUC values averaged 75% higher in elderly subjects compared with young subjects and clearance averaged 28% lower in the elderly group. These differences approached, but did not attain, statistical significance ($P = .086$). When age was evaluated as a continuous variable, the overall correlations between age and AUC, as well as between age and clearance, were significant ($P < .02$). Taken together, the findings are consistent with many but not all previous studies on age-related changes in the clearance of CYP3A substrate drugs, indicating decrements in clearance in elderly subjects that may be more marked in men than in women.[5]

Elderly individuals had greater decrements in DSST performance than young control subjects after administration of triazolam. The age-related difference was quantitatively greater among men than among women and was statistically significant for male subjects based on a direct comparison of young and elderly groups. Conclusions were the same whether decrements were analyzed as absolute changes or as fractional (percent) changes. Ratings of sedation by trained observers (blinded to the treatment condition) also indicated significantly greater increases in sedation in elderly subjects compared with young subject groups. Exposure-response relationships, evaluated by use of the ratio of effect AUC divided by plasma AUC as well as concentration-effect patterns at individual time points, suggested that a combination of increased intrinsic sensitivity together with higher plasma levels accounted for the quantitatively greater benzodiazepine agonist effects in elderly subjects. Prior clinical and experimental studies have likewise demonstrated the contribution of either kinetic or dynamic factors (or both) in explaining benzodiazepine sensitivity in elderly subjects.[10,14,14a,33,39,40,44] The role of altered intrinsic baseline performance in elderly subjects has been discussed as a potential confounding factor in evaluating age-related differences in response to benzodiazepine agonists.[10,13] A generally accepted approach to accounting for such baseline differences has not been established. In this study we observed significantly lower predose DSST scores in elderly subjects compared with young subjects. We normalized for these differences by expressing postdose changes from baseline as a fraction (percent) of the predose baseline value itself. Nonetheless, the conclusions were identical if absolute rather than relative changes from baseline were used in the analysis.

It is of interest that enhanced performance-impairing and observer-rated sedative effects of triazolam in elderly subjects were not reflected in the older subjects' self-ratings of sedation. The elderly individuals either were not aware of the intensity of benzodiazepine agonist effects or did not report such effects in a manner congruent with that of younger subjects. In any case, the possibility that older individuals are not fully aware of the extent of benzodiazepine-induced sedation and performance impairment continues to be a concern associated with the treatment of older persons.

The quantitative EEG is a validated and extensively used objective measure of the time course and intensity of benzodiazepine agonist action in humans.[45,46] The method has been applied to evaluate the pharmacody-

namic consequences of administering equivalent doses of different benzodiazepines,[22,24,47] the effects of complex administration schemes,[25] or the consequences of pharmacokinetic drug interactions.[7,22,23,48,49] However, this approach is not fully validated as a method for investigating altered sensitivity in elderly subjects. Although the EEG clearly distinguished triazolam from placebo in each of the 6 study cohorts, the EEG did not reflect increased pharmacodynamic effects in elderly groups, as was observed for the DSST or observer-rated sedation. This could be related in part to the small but significantly higher baseline relative β EEG amplitude values in the elderly individuals. In any case, the possible value of the quantitative EEG in pharmacodynamic studies of aging is not established by our study and will require further investigation.

The effects of triazolam on information acquisition and recall are consistent with the recognized syndrome of benzodiazepine-induced anterograde amnesia.[7,10,18,24,27,50-52] In all 6 study cohorts, initial acquisition and immediate recall of information presented at 1.5 hours after dosing were modestly but significantly impaired by triazolam compared with placebo. However, triazolam generally impaired storage of the acquired information, assessed as free recall (delayed recall) of the same information at 24 hours after dosing. The findings emphasize the highly significant and clinically important impairment of consolidation and storage of information acquired while benzodiazepine agonist effects are present. The effect may occur with all benzodiazepine agonists and is dependent on dose and plasma concentration.

The mechanisms of impaired clearance of CYP3A substrate benzodiazepines in elderly subjects, as well as the reason for differences in research results among study sites, remain to be established. One study of human liver microsomes indicated reduced expression and function of CYP3A isoforms in liver samples from elderly men, reflected in reduced intrinsic clearance of triazolam in vitro.[53] Similar findings have been reported in some in vitro studies[54] but not others.[55,56] Rodent studies indicate reduced expression of CYP3A isoforms in aging male mice and rats.[57,58] It is of interest that hepatic CYP3A but not enteric CYP3A is influenced by age in the rat model[57] and is associated with down-regulation of CYP3A messenger ribonucleic acid expression (Warrington JS, et al, unpublished data, June 2003). In any case, the factors that ultimately account for individual variability in CYP3A expression, whether associated with age or with other characteristics such as gender or ethnicity, remain to be identified and are an active topic of investigation.

Dr Wright was an employee of The Upjohn Co at the time this study was conducted. The other authors do not have potential conflicts of interest.

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