Kinetic and dynamic interaction study of zolpidem with ketoconazole, itraconazole, and fluconazole

Background: Azole antifungal agents may impair hepatic clearance of drugs metabolized by cytochrome P450-3A isoforms. The imidazopyridine hypnotic agent zolpidem is metabolized in humans in part by P450-3A, as well as by a number of other cytochromes. Potential interactions of zolpidem with 3 commonly prescribed azole derivatives were evaluated in a controlled clinical study.

Methods: In a randomized, double-blind, 5-way, crossover, clinical pharmacokinetic-pharmacodynamic study, 12 volunteers received (A) zolpidem placebo plus azole placebo, (B) 5 mg zolpidem plus azole placebo (C) zolpidem plus ketoconazole, (D) zolpidem plus itraconazole, and (E) zolpidem plus fluconazole.

Results: Mean apparent oral clearance of zolpidem when given with placebo was 422 mL/min, and elimination half-life was 1.9 hours. Clearance was significantly reduced to 250 mL/min when zolpidem was given with ketoconazole, and half-life was prolonged to 2.4 hours. Coadministration of zolpidem with itraconazole or fluconazole also reduced clearance (320 and 338 mL/min), but differences compared to the zolpidem plus placebo treatment did not reach significance. Zolpidem-induced benzodiazepine agonist effects (increased electrocardiographic beta activity, digit-symbol substitution test impairment, and delayed recall) during the first 4 hours after dosage were enhanced by ketoconazole but not by itraconazole or fluconazole.

Conclusion: Coadministration of zolpidem with ketoconazole impairs zolpidem clearance and enhances its benzodiazepine-like agonist pharmacodynamic effects. Itraconazole and fluconazole had a small influence on zolpidem kinetics and dynamics. The findings are consistent with in vitro studies of differentially impaired zolpidem metabolism by azole derivatives. (Clin Pharmacol Ther 1998;64:661-71.)

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The imidazopyridine derivative zolpidem is extensively prescribed in clinical practice for the treatment of sleep disorders.[1-4] Its mechanism of action involves GABA-benzodiazepine receptor agonism, with relative selectivity for the Type 1 (BZ-1) class of central benzodiazepine receptors.[5] The elimination half-life of zolpidem is in the range of 1½ to 4 hours, and it therefore is classified as a short half-life hypnotic.[6,7] The initial metabolic transformation of zolpidem in humans involves hydroxylation at 3 different sites on the molecule.[6-8] These reactions are mediated mainly but not entirely by cytochrome P450-3A isoforms.[8,9] Available evidence suggests that P450-2C9 may make a significant contribution to metabolic clearance of zolpidem at therapeutically relevant substrate concentrations, with additional possible contributions of 1A2, 2D6, and 2C19.[9]

Pharmacokinetic drug interactions involving inhibition of cytochrome P450 activity have received considerable recent attention. Antifungal agents of the azole class are of importance as inhibitors of human cytochrome P450-3A isoforms.[10,11] Coadministration of 3A substrate drugs with azole derivatives such as ketoconazole, itraconazole, and fluconazole can result in impairment of clearance of such drugs which, in some cases, can be quantitatively large and clinically important. This is particularly true for high-extraction compounds given orally, since the azoles may inhibit both the gastrointestinal and hepatic components of presystemic extraction.[12,13] In the case of orally administered triazolam and midazolam (both relatively "pure" substrates for P450-3A[14-16]), their oral clearance may be impaired by 85% or more when given together with ketoconazole[16-19]; significant, although somewhat smaller, interactions are caused by itraconazole and fluconazole.[17,18,20-22]

Since zolpidem is metabolized partially, but not exclusively, by P450-3A isoforms, the possibility exists that azole antifungal agents have a lesser inhibiting effect on zolpidem clearance in vivo, resulting in a smaller clinical interaction, compared with their effect on triazolam or midazolam clearance. In studies of human liver microsomes in vitro, ketoconazole inhibition constants (K_i) in the low nanomolar range were observed for inhibition of midazolam or triazolam hydroxylation.[15,16] In this study we compared the susceptibility of zolpidem hydroxylation and triazolam hydroxylation to inhibition by ketoconazole in vitro. Possible interactions of zolpidem with ketoconazole, as well as with itraconazole and fluconazole, were tested in a controlled clinical pharmacokinetic-pharmacodynamic study.

METHODS

In vitro studies. Procedures for acquisition and storage of human liver tissue samples and for preparation of microsomal fractions have been described previously.[15,16,23,24] Zolpidem and its principal hydroxylated metabolite (termed the *M-3 metabolite*)[8] were provided by Synthélabo Recherche, Bagneux, France. Triazolam and its principal hydroxylated metabolites (α-hydroxytriazolam and 4-hydroxytriazolam)[16,25] were kindly provided by Pharmacia and Upjohn Co, Kalamazoo, Mich. Other chemical reagents and drug entities were purchased from commercial sources.

Zolpidem and triazolam were prepared in methanol solution. Aliquots were added to incubation tubes to produce a final concentration of 10 μmol/L zolpidem in one series, and 100 μmol/L triazolam in another series. For zolpidem, 10 μmol/L is below the concentration (K_m) corresponding to 50% of the maximum velocity of M-3 metabolite formation; for triazolam, 100 μmol/L is slightly above the K_m for α-hydroxytriazolam formation and below the K_m for 4-hydroxytriazolam formation. Ketoconazole in methanol solution was co-added to incubation tubes to yield final concentrations ranging from 0 to 2.5 μmol/L. The solvent was evaporated to dryness at 40°C under mild vacuum. To each tube was then added incubation buffer, appropriate cofactors, and an NADPH-regenerating system as described previously.[15,16,23,24] The mixture was heated to 37°C, and reactions initiated by addition of microsomal protein (up to 0.5 mg/mL). After 20 minutes at 37°C, reactions were stopped by cooling on ice and addition of acetonitrile. Internal standard was added, the mixtures centrifuged, and supernatants transferred to autosampling vials for HPLC analysis. All individual incubations were done in duplicate. Studies of zolpidem and triazolam each were performed using microsomal preparations from 4 human liver samples.

The HPLC mobile phase consisted of acetonitrile, methanol, and 50 μmol/L phosphate buffer. For analysis of the M-3 metabolite of zolpidem, mobile phase component proportions were 30:10:60, the analytical column was C_{18} Bondapak (30 cm × 3.9 mm), and the ultraviolet absorbance wavelength was 242 nm. For analysis of α-hydroxytriazolam and 4-hydroxytriazolam, mobile phase proportions were 22.5:10:67.5, the column was C_{18} NovaPak (15 cm × 3.9 mm), and ultraviolet absorbance was at 220 nm.

Design of clinical study. The protocol was reviewed and approved by the Human Investigation Review Committee serving Tufts University School of Medicine and New England Medical Center Hospital. Twelve healthy volunteers (8 men and 4 women), aged 20 to 40 years, participated after giving written informed consent. All were active ambulatory nonsmoking adults, with no evidence of medical disease and taking no other medications. Female subjects were not taking oral contraceptives and did not have contraceptive implants.

The study had a double-blind, randomized, 5-way crossover design, with at least 7 days elapsing between treatments. Medications were separately and identically packaged in opaque capsules and administered orally. The 5 treatments were as follows:

A. Zolpidem placebo plus azole placebo
B. Zolpidem (5 mg) plus azole placebo
C. Zolpidem (5 mg) plus ketoconazole, 200 mg twice daily
D. Zolpidem (5 mg) plus itraconazole, 100 mg twice daily
E. Zolpidem (5 mg) plus fluconazole, 100 mg twice daily

Table I. Summary of analytical methods for analysis of azole antifungal agents in plasma

	Ketoconazole	*Itraconazole/hydroxyitraconazole*	*Fluconazole*
Internal standard	Dextromethorphan	Clotrimazole	Phenacetin
Extraction solvent	Ethyl acetate/isoamyl alcohol (98.5:1.5)	Ethyl acetate	Ethyl acetate
Mobile phase composition	CH_3CN/CH_3OH/50 mmol/L $NH_4H_2PO_4$ (40:5:55)	CH_3CN/10 mmol/L KH_2PO_4 (50:50)	CH_3OH/10 mmol/L Na acetate (40:60)
Mobile phase flow rate	1.5 mL/min	1.5 mL/min	1.0 mL/min
Analytical column	C_{18} μBondaPak*(30 cm length, 10 μm particle size)	C_{18} NovaPak* (15 cm length, 5 μm particle size)	C_{18} μBondaPak* (30 cm length, 10 μm particle size)
Ultraviolet absorbance wavelength	220 nm	263 nm	261 nm
Lower limit of quantitation	0.05-0.1 μg/mL	0.03-0.05 μg/mL	0.1 μg/mL
Assay variance (%CV) within-day; between-day	<10%; <10%	<9%; <18%	<7%; <3%

CV, Coefficient of variance.
*Waters Associates, Milford, Mass.

Doses and dosage schedules for the azoles were chosen based on recommendations in approved labeling.

Procedures. At 8 AM on study day 1, subjects entered the outpatient Clinical Psychopharmacology Research Unit where they received the initial dose of azole (or placebo) and remained under observation for 30 minutes. Subjects took a second dose of azole (or placebo) at home at 4 PM on day 1. On the morning of day 2, after ingesting a standardized light breakfast with no caffeine-containing food or beverages and no grapefruit juice, they returned to the Research Unit at approximately 7:30 AM. They fasted until 12 noon, after which they resumed a normal diet (without grapefruit juice or caffeine-containing food or beverages). The third dose of azole (or placebo) was given at 8 AM, and the single dose of zolpidem or placebo was given at 9 AM. A final azole (or placebo) dose was given at 5 PM.

Venous blood samples were drawn from an indwelling cannula into heparinized tubes before zolpidem or placebo dosage and at the following postdosage times: ½, 1, 1½, 2, 2½, 3, 4, 5, 6, 8, and 24 hours. Samples were centrifuged, and the plasma separated and frozen until the time of assay.

The electroencephalogram (EEG) was recorded using a 6-electrode montage, with instrumentation and methodology described previously.[16,19,26-28] At 2 predosage times and during 8 hours after dosage 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 (Hz), then fast-Fourier transformed to determine activity over the 4.0 to 31.75 Hz spectrum, and in the "beta" (13.0 to 31.75 Hz) band.

Subjects' self-ratings of sedative effects and mood state were obtained on a series of 100-mm visual analog scales.[16,19,26-30] Ratings of sedation were also performed by trained observers, using the same rating instrument, without knowledge of the treatment condition. Self- and observer-ratings were obtained twice before medication administration and at postdosage times indicated above.

The digit symbol substitution test (DSST) was administered twice before dosing and at times corresponding to rating scales.[16,19,26-30] 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 using a word-list free recall procedure[16,19,26-31] that was administered at 1½ hours after zolpidem or placebo administration. Sixteen words, taken from 4 different categories, were read in random order in "shopping-list" fashion. Recall was tested immediately after presentation of each list. Subjects wrote down items immediately after lists were presented in random order. List presentation and recall were repeated a total of 6 times at 1½ hours after dosage. 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. In vitro rates of formation of metabolites of zolpidem or triazolam with coaddition of ketoconazole were expressed as a percentage ratio versus the control velocity with no inhibitor present. The relation of ketoconazole concentration to velocity

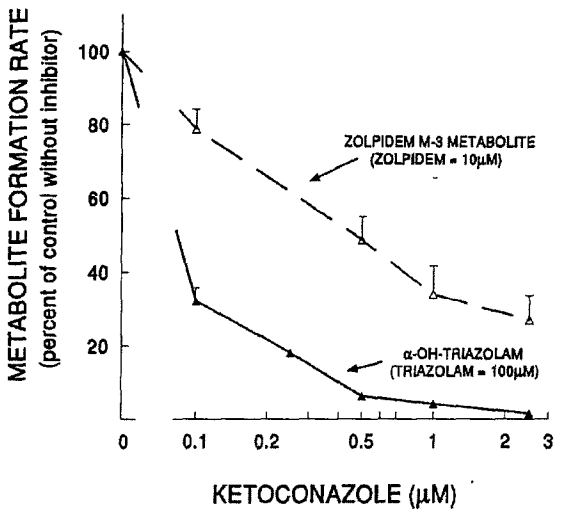

Figure 1. Rates of formation of the zolpidem M-3 hydroxylated metabolite from zolpidem (10 μmol/L) or of α-hydroxytriazolam (α-OH-triazolam) from triazolam (100 μmol/L) by human liver microsomes in vitro. Reaction velocities with coaddition of the inhibitor ketoconazole are expressed as a percentage ratio versus the control velocity with no ketoconazole present. Each *point* is the mean ± SE ratio from 4 separate human liver microsomal preparations. Mean 50% inhibitory concentrations (IC_{50}) for ketoconazole were 0.61 μmol/L versus the zolpidem M-3 metabolite and 0.053 μmol/L versus α-hydroxytriazolam. The IC_{50} value versus 4-α-hydroxytriazolam (data not shown) was 0.046 μmol/L.

ratio was analyzed by nonlinear regression to determine the ketoconazole concentration corresponding to a velocity ratio of 50% of the control value (IC_{50}).[19,32,33]

Plasma concentrations of zolpidem from the clinical study were determined by HPLC with fluorescence detection.[34] The sensitivity limit was 1 to 2 ng/mL, and the variance between replicate samples did not exceed 8%. The slope (beta) of the terminal log-linear phase of each zolpidem 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 versus time curve from time zero 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 beta, yielding the total area under the plasma concentration versus time curve (AUC). The peak plasma concentration and the time of peak concentration represented the rate of appearance of drug in systemic circulation. Apparent oral clearance was calculated as the administered dose divided by the total AUC.

Plasma concentrations of ketoconazole, itraconazole (and its metabolite hydroxyitraconazole), or fluconazole were determined by HPLC (Table I).

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

For each EEG recording session, the relative beta amplitudes (beta divided by total, expressed as percent) were calculated, and values from the left and right frontotemporal leads were averaged. The means of the relative beta amplitudes in the pre-dose recordings were used as baseline, and all post-dosage values were expressed as the increment or decrement over that treatment's mean pre-dose baseline value.

For each pharmacodynamic variable, the area under the 4-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. Also evaluated were pharmacodynamic effects at individual time points.

Statistical procedures included linear and nonlinear regression, ANOVA, the Student-Newman-Keuls procedure, and Dunnett's *t* test. One of the subjects did not ingest zolpidem as instructed during treatments D and E; accordingly mean values for treatments A, B, and C represent n = 12, and for treatments D and E, n = 11. For analysis of variance procedures, n = 11 was used throughout.

RESULTS

In vitro results. The mean ± SE (n = 4) IC_{50} value for ketoconazole versus formation of the M-3 metabolite of zolpidem was 0.61 ± 0.27 μmol/L (Figure 1). In contrast, the ketoconazole IC_{50} was 0.053 ± 0.009 μmol/L versus α-hydroxytriazolam formation, and 0.046 ± 0.007 μmol/L versus 4-hydroxytriazolam formation (Figure 1).

Clinical study: Plasma concentrations of azoles. Plasma levels of the 3 azole derivatives were consistent with those anticipated during administration of usual therapeutic doses[35] (Figure 2). As reported previously,[36,37] plasma concentrations of hydroxyitraconazole exceeded those of itraconazole. Since values of elimination half-life for itraconazole and fluconazole generally exceed 20 hours,[35] these 2 agents may not have actually reached steady state.

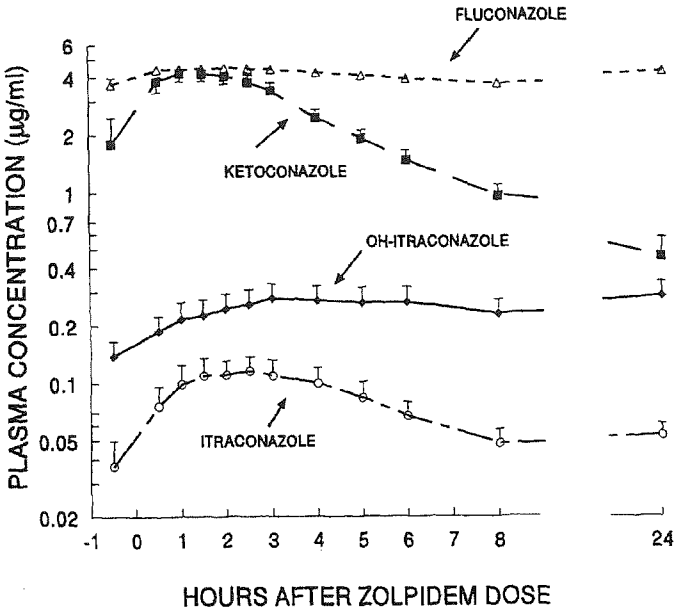

Figure 2. Mean ± SE plasma concentration of ketoconazole, itraconazole, hydroxyitraconazole, or fluconazole on study day 2. The third azole dose was given 1 hour before zolpidem.

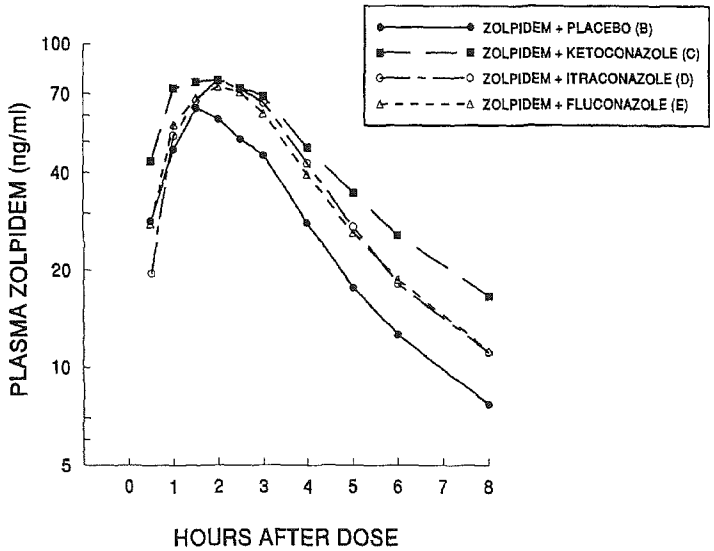

Figure 3. Mean plasma zolpidem concentrations following 5.0 mg zolpidem alone (treatment B) and with coadministration of ketoconazole, itraconazole, or fluconazole (treatments C, D, and E). See Table II for kinetic and statistical analysis.

Clinical pharmacokinetics of zolpidem. Coadministration of zolpidem with ketoconazole (treatment C) significantly prolonged zolpidem elimination half-life, increased total AUC, and decreased apparent oral clearance when compared to zolpidem plus placebo (treatment B; Figure 3 and Table II). Zolpidem AUC during treatment C was increased by a factor of 1.83 ± 0.24 (mean ± SE) compared to treatment B values, and clearance during treatment C was reduced to 64% ± 7% of treatment B values. However, the elimination half-life of zolpidem

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