Pharmacokinetic properties of zolpidem in elderly and young adults: possible modulation by testosterone in men

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Aims The influence of ageing on the pharmacokinetics of zolpidem, an extensively prescribed hypnotic medication, was evaluated in healthy human volunteers.

Methods A series of 16 elderly (age: 61–85 years) and 24 young (age: 22–42 years) volunteers received single 5 mg oral doses of zolpidem tartrate. Serum zolpidem concentrations were determined by HPLC with fluorescence detection in samples drawn during 8 h after dosage. The effect of testosterone on zolpidem biotransformation was evaluated *in vitro* using human liver microsomes. Possible induction of CYP3A protein expression and function was studied in cultured human hepatocytes.

Results Among men, apparent oral clearance of zolpidem was decreased in elderly compared to young subjects (3.8 *vs* 11.0 ml min^{-1} kg^{-1}, $P < 0.01$), C_{max} was increased (93 *vs* 40 ng ml^{-1}, $P < 0.01$), and half-life increased (2.7 *vs* 1.5 h, $P < 0.03$). Among women, zolpidem oral clearance was decreased in the elderly (3.0 *vs* 5.8 ml min^{-1} kg^{-1}, $P < 0.02$), C_{max} increased (108 *vs* 60 ng ml^{-1}, $P < 0.001$), with no difference in $t_{1/2}$ (2.3 *vs* 2.4 h). Among male subjects, free serum testosterone concentrations were lower in the elderly (10.5 *vs* 19.0 pg ml^{-1}, $P < 0.01$), and were significantly correlated with zolpidem clearance ($r^2 = 0.46$, $P < 0.001$). Multiple regression analysis indicated a greater relative contribution of serum testosterone than age to the oral clearance of zolpidem among men. In human liver microsomes, co-incubation of zolpidem (10 μM) with varying concentrations of testosterone produced activation of biotransformation of zolpidem to its principal hydroxylated metabolite. Maximum activation was achieved at equimolar concentrations of testosterone (10 μM). However, testosterone did not induce immunoactive CYP3A4 expression or catalytic function in cultured human hepatocytes.

Conclusions The increased C_{max} and lower oral clearance of zolpidem in the elderly are consistent with recommendations of lower clinical doses of zolpidem in the elderly. Our clinical and *in vitro* data both suggest that reduced free serum testosterone may have a modulatory role in age-dependent changes in zolpidem pharmacokinetics in men.

Keywords: ageing, pharmacokinetics, testosterone, zolpidem

Introduction

The proportion of the elderly in the US and Europe is increasing and is expected to increase further within the next few decades [1]. Sleep disorders requiring the use of hypnotics are also quite common in elderly individuals [2, 3], as is multiple drug use with the attendant possibility of drug interactions, adverse drug reactions, cognitive impairment, and injuries [4–8]. As such, a better understanding of the pharmacokinetics of commonly used drugs in the elderly is of considerable importance. Older persons appear to be more susceptible to adverse drug reactions compared to patients of younger age. This is particularly important with respect to drugs that influence the central nervous system. Drug action and response in the elderly may differ from that in the younger population as a result of changes in disease characteristics associated with old age, age-related intrin-

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sic changes in drug sensitivity [9], or pharmacokinetic alterations related to the ageing process [9–15].

Zolpidem is a nonbenzodiazepine imidazopyridine hypnotic, which is a full agonist for the benzodiazepine (BZ) component of the $GABA_A$ receptor complex, and has relative selectivity for the BZ-1 (omega-1) subtype and lower affinity for the other receptor subtypes [16–20]. Since its introduction to clinical use in the United States in the early nineties, zolpidem has become one of the most commonly prescribed hypnotics in the elderly population [18]. Because of its short elimination-half-life, zolpidem appears to have a low likelihood of producing residual sedative effects, with the probability of a reduced risk of falls and fractures in the elderly. Zolpidem also is reported to have a lower risk of causing rebound insomnia upon discontinuation [21].

In vitro studies using human liver microsomes and heterologously expressed individual human cytochromes P450 (CYPs) have demonstrated that zolpidem is biotransformed to three pharmacologically inactive hydroxylated metabolites [22, 23] by a series of CYP enzymes including CYP3A4, CYP2C9, CYP1A2, CYP2D6 and CYP2C19, in decreasing order of importance [22]. CYP3A4 is the principal enzyme responsible for zolpidem metabolism, accounting for approximately 60% of net CYP-mediated hepatic clearance. Three distinct hydroxylated metabolites result from zolpidem biotransformation. One of these, termed the M-3 metabolite, accounts for 83% of net metabolite formation, and 58% of net M-3 formation is attributed to CYP3A4. Absolute bioavailability of orally administered zolpidem in healthy young male volunteers averages approximately 70%, suggesting that presystemic extraction is not extensive [24]. The effect of age and gender on zolpidem bioavailability is not established. There is no available evidence to suggest that zolpidem is a substrate for transport by enteric P-glycoprotein or other transporter systems. Zolpidem itself is not a significant inhibitor of human CYP isoforms [25].

Age-related changes in the pharmacokinetic properties of zolpidem are described in summary reports and in labelling information [16, 26]. Because many (but not all) clinical studies of CYP3A substrates show reduced clearance in the elderly [11–15], age-related changes in zolpidem kinetics require further investigation. Among other factors, the contributory importance of age-dependent changes in testosterone homeostasis in elderly men has also not been evaluated. Although testosterone is mainly a substrate for CYP3A4, some *in vitro* studies suggest that it is also a modulator of CYP3A4 activity. During simultaneous incubation of some CYP3A4 substrates with testosterone, substrate clearance was activated, possibly through conformational changes in the enzymatic active site [27–35].

This study was carried out to assess the pharmacokinetic properties of zolpidem in normal elderly compared with young adult subjects and to assess the possible modulatory role of free testosterone in elderly and young males. We also evaluated the effect of testosterone on zolpidem biotransformation using human liver microsomes *in vitro*, and on CYP3A expression and activity in cultured human hepatocytes.

Methods

Subjects

The study protocol was reviewed by the local Institutional Review Board and informed consent was obtained from all subjects. Forty Caucasian volunteers participated in the study. They were divided into two groups of those aged 21–42 years ($n = 24$), and those aged 66–85 years ($n = 16$) (Table 1). All subjects were healthy, ambulatory and medically stable. Among the elderly, two subjects were on digoxin, one on aspirin, and one each on perindopril and phenprocoumon**.** None of these drugs is known to induce or inhibit CYP3A4. Eight of the 16 young female subjects were taking oral contraceptive

Table 1 Demographic characteristics (mean (± SD)) of the subject population and the relevant laboratory values.

	Young male	*Elderly male*	*Kruskal–Wallis test*	*Young female*	*Elderly female*	*Kruskal–Wallis test*
Demographics						
Number	8	8		16	8	
Age (years)	23.4 (±5.5)	73.1 (±8.5)	$P < 0.001$	27.8 (±5.3)	74.3 (±5.9)	$P < 0.001$
Weight (kg)	76.4 (±5.9)	74.0 (±4.0)	NS	66.3 (±6.6)	72.1 (±11.0)	NS
Height (m)	1.84 (±0.11)	1.72 (±0.04)	$P < 0.05$	1.73 (±0.07)	1.61 (±0.05)	$P < 0.001$
Body mass index (kg m^{-2})	22.6 (±1.8)	25.0 (±0.9)	$P < 0.01$	22.3 (±2.3)	27.9 (±4.0)	$P < 0.001$
Laboratory values						
Serum albumin (g 100 ml^{-1})	6.4 (±0.2)	5.8 (±0.5)	$P < 0.05$	6.0 (±0.3)	6.0 (±0.03)	NS
Serum creatinine (mg 100 ml^{-1})	0.9 (±0.1)	1.1 (±0.3)	$P < 0.1$	0.9 (±0.1)	0.9 (±0.2)	NS
Free testosterone (pg ml^{-1})	19.0 (±6.4)	10.5 (±3.7)	$P < 0.01$	1.8 (±3.5)	1.4 (±1.0)	NS
Growth hormone (ng ml^{-1})	0.53 (±0.66)	0.38 (±0.29)	NS	2.83 (±4.56)	0.8 (±0.6)	$P < 0.1$

preparations; a previous study demonstrated that oral contraceptives have no significant influence on zolpidem clearance [36].

Procedure

Subjects fasted overnight prior to the study. After a baseline blood sample was obtained (time 0 h), 5 mg of zolpidem tartrate (containing 4.2 mg of zolpidem base) was given with water by mouth. Subjects had a small breakfast (excluding grapefruit juice) 2 h after the oral dose. Venous blood samples were drawn at time 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 8 h after zolpidem dosage. The serum was separated by centrifugation and samples were frozen at −80 °C until assayed.

Analysis of samples

Zolpidem concentrations in serum were determined by high-performance liquid chromatography (HPLC) using fluorescence detection, as described in detail previously [37]. After addition of an internal standard, samples were extracted with hexane. The organic extract was separated, evaporated to dryness, and reconstituted for HPLC analysis. The HPLC system used in the analysis was from Waters Associates (Milford, MA). The instruments and reagents included a reverse-phase C-18 microBondapak column (30 cm by 3.9 mm), a mobile phase consisting of acetonitrile : 50 mM potassium dihydrogen phosphate (50 : 50) at pH 7.4 and a flow rate of 1.8 ml min^{-1}. The effluent from the column was monitored by a Waters (Milford, MA) fluorescence spectrophotometer at excitation and emission wavelengths of 254 and 390 nm, respectively, with the system operating at room temperature. The assay sensitivity limit was 1 ng ml^{-1}, and the within- and between-day coefficients of variance did not exceed 10%. Across 24 analytical runs, the coefficient of variation (CV) for a quality control sample analysed with each set of unknowns was 9.2%. The between day CV in the slope of the calibration curve was 10.6%.

Free testosterone concentrations in single serum samples were determined by standard radioimmunoassay (RIA) and human growth hormone by the double antibody RIA technique.

The slope (λ_z) of the terminal log-linear phase of each zolpidem serum concentration-time curve was determined by linear regression analysis, and was then used to calculate the apparent elimination half-life. The area under the serum concentration-time curve from time 0 h to the last concentration was determined by the linear trapezoidal method. The residual area extrapolated to infinity (the final concentration divided by λ_z) was then added to give the total area under the serum concentration-time curve (AUC). Apparent oral clearance was calculated as the dose of zolpidem base divided by the total AUC. The peak plasma concentration (C_{max}) and the time to achieve C_{max} (t_{max}), representing the rate of drug appearance in the systemic circulation, were determined for each subject.

The results were analysed by both linear and nonlinear regression. Since the distributions under study were vulnerable to the effects of outlying values and heterogeneous variance, nonparametric Kruskal–Wallis tests were used to evaluate statistical differences between mean values.

In vitro *study of human liver microsomes*

Liver samples from four individual human donors with no known liver disease were provided by the International Institute for the Advancement of Medicine, Exton, PA, the Liver Tissue Procurement and Distribution System, University of Minnesota, Minneapolis, MN, or the National Disease Research Interchange, Philadelphia, PA. Acquisition of de-indentified tissue samples from these sources was reviewed by the Human Investigation Review Committee (the Institutional Review Board) serving Tufts University School of Medicine and Tufts-New England Medical Center, and designated as exempt. All samples were of the CYP2D6 and CYP2C19 extensive metabolizer phenotype based on prior *in vitro* studies.

Human liver microsomes were prepared by ultracentrifugation, and microsomal pellets were suspended in 0.1 M potassium phosphate buffer containing 20% glycerol and stored at −80 °C until use [22]. Samples of zolpidem and its metabolites were provided by Synthélabo Recherche, Bagneux, France. Other chemical reagents and drug entities were purchased from commercial sources or kindly provided by their manufacturers.

Zolpidem (10 μM) and testosterone (0–50 μM) were added to the incubation tubes in a small volume of organic solvent, which was evaporated to dryness. Phosphate buffer 50 mM, 5 mM Mg^{++}, 0.5 mM $NADP^{+}$, and an isocitrate/isocitric dehydrogenase regenerating system were then added and the mixture was brought to 37 °C. Reactions were initiated by addition of microsomal protein (0.25 mg ml^{-1}). After 20 min of incubation, reactions were stopped by addition of acetonitrile and cooling on ice. Propranolol (5 μg) was added as the internal standard. The samples were centrifuged, and the supernatant was transferred to an autosampling vial for HPLC analysis.

The HPLC system consisted of a Waters C-18 micro-BondaPak column (30 cm length, 10 μ particle size) kept at room temperature, and detection was by UV at 242 nm. The mobile phase was 50 mM KH_2PO_4 : acetonitrile : methanol (60 : 30 : 10). Assay of incubations containing zolpidem, microsomes and cofac-

tors yielded a major chromatographic peak corresponding to the principal hydroxylated metabolite (designated as M3) [22]. Other metabolite peaks either were not large enough for quantitation, or co-eluted with a metabolite of testosterone. M3 metabolite formation rates with coaddition of testosterone were expressed as ratios versus the reaction velocity in the control condition without testosterone.

In vitro *study of cultured human hepatocytes*

Fresh liver tissue was obtained from a single human donor. The hepatocytes were isolated and cultured in 24-well plates with plating media for a 24 h attachment period at 37 °C in an atmosphere of 5% CO_2, and then were cultured with incubation media [38–40]. Testosterone 10 μM or rifampicin 20 μM in 0.5% or vehicle control (0.5% DMSO) in DMSO, were added and incubated with hepatocytes for 48 h at 37 °C in an atmosphere of 5% CO_2.

The probe substrate for CYP3A4 activity was triazolam 250 μM prepared in media containing 0.5% methanol. Cells were incubated with substrate for 1.5 h at 37 °C in an atmosphere of 5% CO_2. Reactions were stopped by the addition of 200 μl of acetonitrile. Plates containing media were frozen at −80 °C until HPLC analysis.

The media was thawed and the internal standard phenacetin (50 μl of 4.5 mg 100 ml^{-1} methanol solution) was added directly to the plate wells and mixed by pipetting. Media containing internal standard was transferred directly to autosampling vials for HPLC analysis of α-hydroxytriazolam. The HPLC mobile phase consisted of 710 ml of 10 mM potassium phosphate buffer, 200 ml acetonitrile, and 100 ml methanol. A reverse phase Nova-Pak C18 column (Waters Associates, Milford, MA) was used with a flow rate of 1.5 ml min^{-1} and a detection wavelength of 220 nm [53, 54]. Peak height ratios of α-hydroxytriazolam and internal standard phenacetin were determined.

For the analysis of CYP3A4 immunoactive protein, the media was aspirated from the cells, and the latter were lysed by addition of a lysis buffer. The contents of each well were sonicated, centrifuged, and stored at −80 °C. CYP3A4 immunoactive protein was quantitated by Western blot analysis as described previously [55–57].

Results

Clinical study

Demographic features of the subject groups and pharmacokinetic parameters for zolpidem are shown in Tables 1 and 2. Overall, apparent oral clearance values ranged from 66 to 1595 ml min^{-1} and the overall mean (± SD) value was 411 (± 345) ml min^{-1}.

Among men, zolpidem oral clearance (with and without normalization for body weight) was significantly lower in the elderly, AUC and C_{max} were significantly higher, and elimination half-life was longer (Table 2, Figure 1). Among women, C_{max} and AUC were also significantly higher in the elderly group, and zolpidem oral clearance was lower in the elderly group (Table 2, Figure 1). Elimination half-life was unchanged between young and elderly women.

Among male subjects, mean free serum testosterone concentration was significantly lower in the elderly compared with the young subjects (10.5 and 19.0 pg ml^{-1}, respectively, $P < 0.01$). Free testosterone concentration was significantly correlated with log-transformed zolpi-

Table 2 Pharmacokinetic parameters (mean (± SD)).

	Young male	*Elderly male*	*Kruskal–Wallis test*	*Young female*	*Elderly female*	*Kruskal–Wallis test*	*Difference in mean value between young and elderly* *Men*	*Women*
C_{max} (ng ml^{-1})	40 (± 16) [26, 53]	93 (± 45) [56, 131]	$P < 0.01$	60 (± 19) [49, 70]	108 (± 30) [83, 133]	$P < 0.001$	54 [17, 90]	48 [27, 69]
t_{max} (h)	0.8 (± 0.3) [0.6, 1.2]	1.1 (± 0.4) [0.7, 1.4]	NS	1.2 (± 0.4) [0.9, 1.4]	0.8 (± 0.7) [0.2, 1.4]	NS	0.25 [−0.12, 0.62]	−0.34 [−0.83, 0.14
Total AUC (ng ml^{-1} h)	110 (± 68) [54, 167]	400 (± 326) [127, 672]	$P < 0.01$	249 (± 133) [178, 320]	398 (± 189) [241, 557]	$P < 0.05$	289 [37, 542]	150 [12, 287]
Clearance								
(ml min^{-1})	820 (± 445) [448, 1193]	276 (± 179) [126, 425]	$P < 0.01$	376 (± 271) [232, 520]	209 (± 122) [107, 311]	$P < 0.05$	−545 [−909, −181]	−167 [−377, 44]
(ml min^{-1} kg^{-1})	11.0 (± 6.4) [5.6, 16.3]	3.8 (± 2.5) [1.6, 5.9]	$P < 0.01$	5.8 (± 4.8) [3.3, 8.4]	3.0 (± 1.9) [1.4, 4.5]	$P < 0.02$	−7.2 [−12.4, −2.0]	−2.9 [−6.5, 0.8]
Half-life (h)	1.5 (± 0.5) [1.1, 1.8]	2.7 (± 1.2) [1.7, 3.6]	$P < 0.03$	2.4 (± 0.9) [1.9, 3.9]	2.3 (± 0.7) [1.7, 2.9]	NS	1.2 [0.2, 2.1]	−0.2 [−0.9, 0.6]

95% confidence intervals are shown in square brackets.

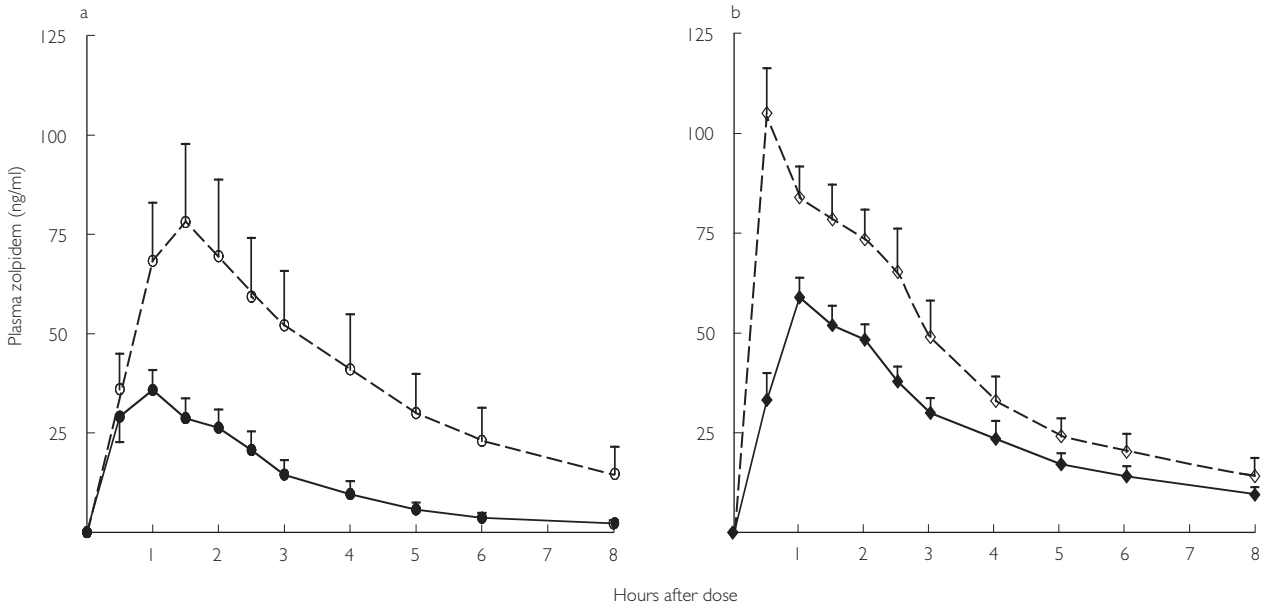

Figure 1 Mean (± SEM) serum zolpidem concentrations–time plots for young (●, ◆) and elderly (○, ◇) male (a) and female (b) volunteers.

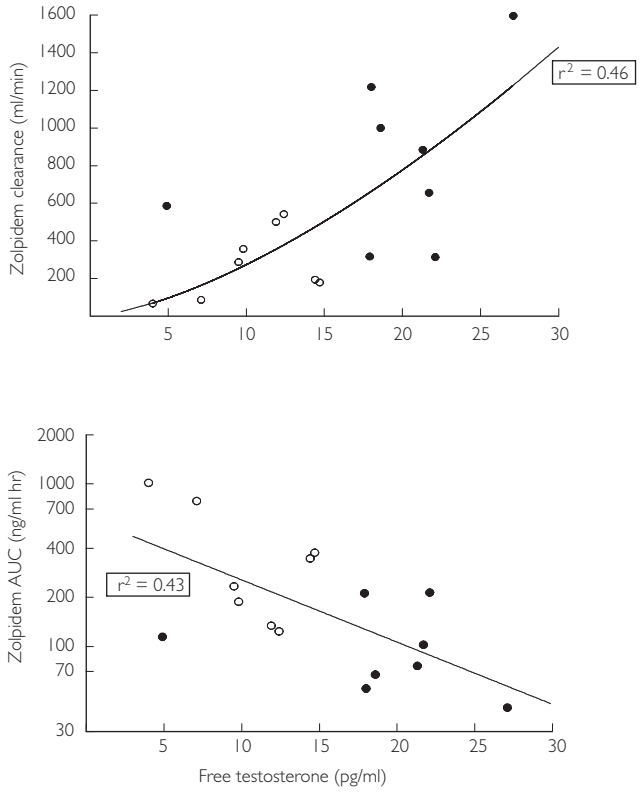

Figure 2 Relationship between free serum testosterone concentration and zolpidem oral clearance (top) and zolpidem AUC (bottom) in male subjects. For zolpidem AUC, linear regression was used after logarithmic transformation of AUC values ($r^2 = 0.43$). For zolpidem clearance, the line represents a function of the form: $y = Bx^A$ ($r^2 = 0.46$). Elderly male (○) and young male (●).

dem AUC ($r^2 = 0.43$), and with oral clearance of zolpidem, based on a nonlinear function of the form: $y = bx^A$ ($r^2 = 0.46$, $P < 0.001$) (Figure 2). Multiple regression analysis indicated that age and free testosterone collectively accounted for a significant proportion of the variance in zolpidem oral clearance (multiple $r^2 = 0.48$, $P < 0.02$). However, standardized regression coefficients suggested that the contribution of free testosterone was greater than that of age, though the difference was not statistically significant. Free testosterone concentration was low in all females and was unrelated to age or zolpidem clearance. Human growth hormone concentrations, though higher in young women, were not significantly related to age and gender.

Human liver microsomal study

Biotransformation of zolpidem to its principal hydroxylated metabolite by human liver microsomes *in vitro* was modulated by testosterone. Testosterone (0-10 μM) caused a concentration-dependent increase in the rate of zolpidem biotransformation (Figure 3). At 10 μM testosterone (the same concentration as that of the substrate, zolpidem), zolpidem metabolite formation was increased by a mean factor of 1.7 compared with the control reaction velocity. At higher concentrations of testosterone, no additional increase was seen.

Cultured human hepatocyte study

Exposure to rifampicin 20 μM increased the rate of triazolam α-hydroxylation by a factor of 3.7 compared with

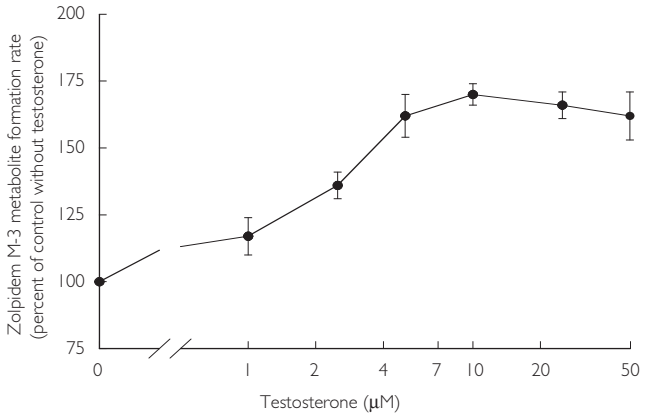

Figure 3 Effect of testosterone on the rate of formation of the zolpidem M-3 metabolite from zolpidem (10 μM, equivalent to 2.88 μg ml^{-1}) by human liver microsomes. Rates of formation are expressed as a percent of the control velocity with no testosterone present. Each point is the mean (± SEM) of four separate human liver microsomal preparations.

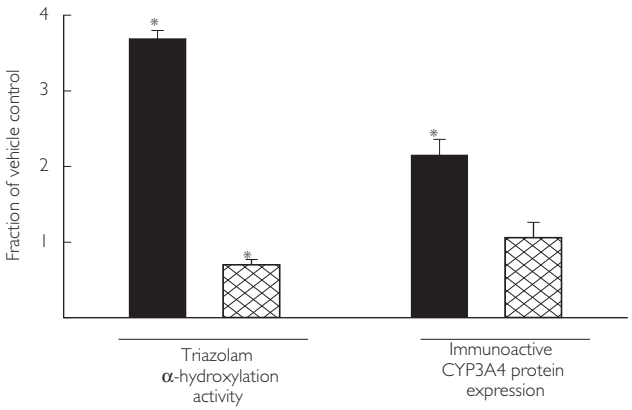

Figure 4 Effect of 48 h of exposure to rifampin (20 μM) (▬) or testosterone (10 μM) (▨) on triazolam α-hydroxylation activity and on the expression of immunoactive CYP3A4 protein in human hepatocytes in cell culture. Bars indicate the mean (± SEM) values relative to cells cultured with vehicle control (0.5% DMSO). Asterisk (*) indicates a significant difference from 1.0 based on Student's *t*-test ($n = 4$ separate experiments for triazolam α-hydroxylation, $n = 2$ for immunoactive protein).

vehicle control. In contrast, exposure to testosterone 10 μM did not increase activity compared with control (Figure 4). Rifampicin also increased immunoactive CYP3A protein expression by 2.1-fold compared with control, whereas the change attributable to testosterone was negligible (1.1-fold).

Discussion

During the last decade there has been a trend towards the use of short half-life hypnotics to minimize daytime residual effects and drug accumulation after multiple dosage, both of which are of particular concern for the elderly. Older benzodiazepine hypnotics, such as flurazepam, are eliminated slowly [46], cause residual daytime sedation, and accumulate during multiple dosage, and have been associated with sedation, cognitive impairment and injuries in the elderly [47]. Zolpidem is now prescribed extensively as a hypnotic, particularly because of its shorter half-life. Although reports indicate that the clearance of zolpidem may diminish in the elderly [16, 26], the effect of age on its pharmacokinetics is not fully understood.

Our study shows that the elderly clear zolpidem much more slowly than younger subjects. Oral clearance in elderly men was about one third of that in younger men, and AUC was about four times higher than in younger men. C_{max} was increased more than two fold in the elderly and half-life was approximately doubled. A similar difference, though less pronounced, was evident in elderly women compared with the younger group with regard to oral clearance and, to a lesser extent, C_{max}. We also observed higher values for oral clearance and lower C_{max} concentrations in men compared with women within both age groups. These latter differences were not subjected to separate statistical comparison, but are consistent with previous reports [48].

It is known that elderly men have lower testosterone concentrations than younger men [49–52], as verified in this study. Regression analysis suggested that lower concentrations of testosterone were associated with the reduced clearance of zolpidem in ageing men. Therefore it is possible that the lower plasma concentrations of free testosterone present in the elderly may contribute to lower CYP3A activity. Testosterone, itself a CYP3A4 substrate, has complex effects on hepatic microsomal activity. It does not induce expression of CYP3A protein in humans, which was confirmed in our *in vitro* study using cultured human hepatocytes. However, exposure to testosterone is known to activate the biotransformation of a number of CYP3A substrates *in vitro* [27–35], such as the CYP3A-mediated production of both 3-OH diazepam and N-desmethyldiazepam from diazepam [27]. Testosterone activates 4-OH triazolam formation and at the same time inhibits α-OH triazolam production from triazolam [28, 31]. A similar pattern is observed with midazolam [27, 35]. We found that the rate of transformation of zolpidem to its major hydroxylated metabolite in human liver microsomes was increased by an average of 70% by co-incubation with equimolar amounts of testosterone. This study has important limitations, in that the ratio of testosterone relative to zolpidem concentrations is much higher than would be encountered *in vivo*. Nonetheless, these *in vitro* data, together with the clinical

results, raise the possibility that testosterone can modulate age-related changes in the clearance of zolpidem, and possibly other CYP3A substrates, in men. This hypothesis will require further clinical investigation. It remains unclear why age-dependent differences in zolpidem clearance among women were unrelated to testosterone concentration.

Our study also suggests that human growth hormone (HGH) does not appear to exert any modulatory effect on the metabolism of zolpidem across age groups and gender. This is consistent with a recent study that showed that HGH has no effect on CYP3A4 [53].

In conclusion, we have demonstrated substantially diminished clearance of zolpidem in the elderly, particularly in elderly men. This finding supports the recommendation that zolpidem dosage should be decreased in the elderly, to minimize the likelihood of elevated serum concentrations [54].

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