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Ethanol inhibits in-vitro metabolism of nifedipine, triazolam and testosterone in human liver microsomes

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Abstract

Although extended exposure to ethanol induces CYP3A metabolism in-vivo, the acute effects of ethanol on CYP3A metabolism have not been fully evaluated in-vitro. We assessed the effect of ethanol on CYP3A-mediated biotransformation using human liver microsomes in-vitro with three prototypic CYP3A-mediated reactions: nifedipine to oxidized nifedipine, triazolam to its 1-hydroxy (1-OH TRZ) and 4-hydroxy (4-OH TRZ) metabolites, and testosterone to 6β-hydroxytestosterone (6β-OH TST). Ethanol inhibited metabolism of nifedipine (oxidized nifedipine IC50 3 mg dL^{-1}, where the IC50 value is the inhibitor concentration corresponding to a 50% reduction in metabolite formation velocity), triazolam (1-OH TRZ IC50 1.1 mg dL^{-1}, 4-OH TRZ IC50 2.7 mg dL^{-1}) and testosterone (6β-OH TST IC50 2.4 mg dL^{-1}). The inhibitory potency of ethanol was similar for the three substrates representing the three hypothetical CYP3A substrate categories. The IC50 values obtained were lower than clinically relevant blood alcohol concentrations. In conclusion, ethanol is an inhibitor of human CYP3A metabolism and may contribute to clinically important interactions.

Introduction

Approximately 10% of adults drink alcohol daily and about 70% of the adult population consumes alcohol at least occasionally (Midanik & Room 1992). These data suggest that concurrent use of alcohol and medications is inevitable. The CYP3A subfamily is the most abundantly expressed P450 in human liver, and CYP3A is involved in the biotransformation of approximately 50% of drugs that are metabolized (Komori et al 1990). As a result, drug interactions associated with modulation of CYP3A-mediated metabolism can be of substantial clinical importance.

Previous studies have shown that ethanol inhibits metabolism of substrates not exclusively metabolized by CYP3A in-vitro, and the inhibitory potency can be substrate specific (Rubin et al 1970; Thummel et al 1989). Extended exposure to ethanol induces CYP3A metabolism in-vivo (Hoshino & Kawazaki 1995; Roberts et al 1995; Feierman et al 2003). However, acute effects of ethanol on CYP3A metabolism in-vitro have not been completely evaluated. Kenworthy et al (1999), using coefficients of determination and cluster analysis, classified CYP3A substrates into three hypothetical groups. It has been suggested that one or more CYP3A substrates from each category should be used to fully evaluate the inhibitory effect of a specific inhibitor on CYP3A metabolism. Hence, in our study, we used one substrate from each group (nifedipine, triazolam and testosterone) to evaluate the effect of ethanol on CYP3A-mediated biotransformation using human liver microsomes (HLMs) in-vitro.

Materials and Methods

All chemicals were were kindly provided by their pharmaceutical manufacturers or purchased from Ultrafine Chemicals (Oxford, UK) or Sigma-Aldrich (St Louis, MO). Acquisition of de-identified discarded human liver tissue for purposes of in-vitro studies was determined to be exempted from review by the local institutional review board. Preparations of HLMs, incubation techniques and HPLC details have been

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described previously (von Moltke et al 1993). Briefly, incubations were performed at fixed substrate concentrations of the three substrates (10 μM triazolam, 50 μM testosterone and 20 μM nifedipine) around or below the corresponding K_m values. Varying concentrations (0–11 mg dL^{-1}) of ethanol were added for metabolite formation studies in human liver microsomes (von Moltke et al 1996; Patki et al 2003). Phenacetin served as the internal standard for triazolam and testosterone, and diazepam for nifedipine. Controls with no inhibitor were performed. Since blood alcohol concentrations are commonly expressed as mg dL^{-1} in-vivo, we used these units for ethanol in-vitro, for better appreciation of the clinical relevance of these values.

Three human livers (L1–L3), characterized as relatively high CYP3A metabolizers from a library of livers, were used for all inhibition studies. Metabolite formation was expressed as a percentage of control without inhibitor. IC50 (the inhibitor concentration corresponding to a 50% reduction in metabolite formation velocity) values were determined through non-linear regression of relative reaction velocities at a single substrate concentration in the presence of varying inhibitor concentrations (von Moltke et al 1998).

Results and Discussion

Ethanol was an inhibitor of both nifedipine oxidation and testosterone 6β-hydroxylation in HLMs, with mean IC50 value of 3 mg dL^{-1} and 2.4 mg dL^{-1}, respectively (Figure 1A, 1C, Table 1). Similarly, it inhibited formation of both 1-OH and 4-OH metabolites of triazolam, with mean IC50 values of 1.1 mg dL^{-1} and 2.7 mg dL^{-1}, respectively (Figure 1B, Table 1) (2 mg dL^{-1} equals approximately 0.43 mmol L^{-1}). Our results differ from those of two previous studies (Feierman 1996; Feierman & Lasker 1996), which showed that ethanol does not inhibit CYP3A in-vitro. However, in those studies fentanyl was used as a CYP3A substrate. Laws making a blood alcohol content of 100 mg dL^{-1} illegal for motor vehicle operation have been enacted in most states in the USA (Voas et al 2003). When we compare the IC50 values obtained for all three CYP3A substrates in our study and the illegal blood alcohol content limit, it appears that clinically relevant alcohol-mediated interactions, secondary to inhibition of CYP3A, are possible (Voas et al 2003; Table 1). In previous human studies, the blood alcohol levels achieved were dependent upon the dose as well as the timing of ethanol consumption; peak blood alcohol levels generally ranged from 50 to 70 mg dL^{-1} (Rubin et al 1970; Auty & Branch 1977). These levels, compared with the IC50 values obtained from our study, further indicate that clinically significant interactions secondary to inhibition of CYP3A metabolism by ethanol are possible.

Previous studies have shown that extended exposure to ethanol induces CYP3A in a dose-dependent manner (Hoshino & Kawasaki 1995; Kostrubsky et al 1995; Roberts et al 1995; Feierman et al 2003). It has been suggested that when acute doses of alcohol are taken with benzodiazepines, Phase I metabolism is usually inhibited

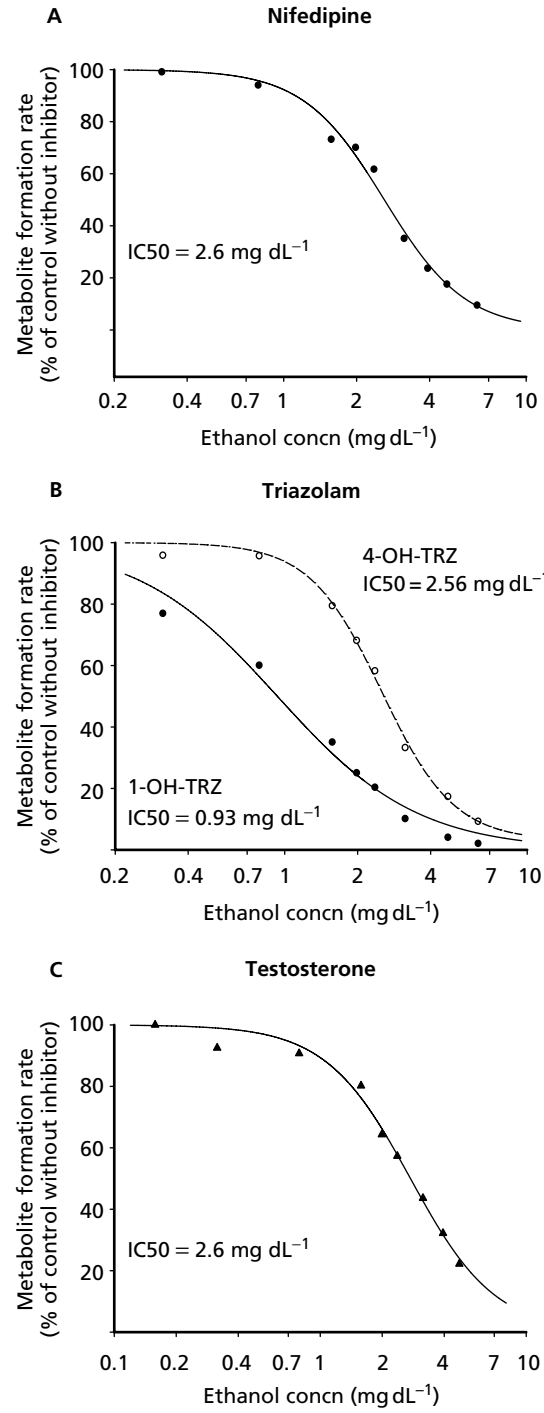

Figure 1 Representative graph showing effect of ethanol on oxidized nifedipine formation from nifedipine (A), 1-OH TRZ and 4-OH TRZ formation from triazolam (B) and 6β-OH TST metabolite formation from testosterone (C) in HLMs. A fixed concentration of nifedipine (20 μM), testosterone (50 μM) and triazolam (10 μM) was incubated with ethanol in concentrations ranging from 0 to 11 mg dL^{-1}. Reaction velocities were expressed as a ratio (%) relative to the velocity with no ethanol present. The reaction velocities for 1-OH TRZ, 4-OH TRZ, 6β-OH TST and oxidized nifedipine formation in the control condition without co-addition of ethanol were 300, 100, 4030 and 2800 pmol min^{-1} (mg protein)$^{-1}$, respectively.

Table 1 Effect of ethanol on nifedipine, triazolam and testosterone metabolism

Substrate Product(s)	IC50 (mg dL^{-1} of ethanol)			
	Nifedipine	Triazolam		Testosterone
	Oxidized nifedipine	1-OH TRZ	4-OH TRZ	6β-OH TST
L1	2.6	1.3	3.3	2.7
L2	3.1	1	2.2	2.1
L3	3.1	0.9	2.6	2.4
Mean	3	1.1	2.7	2.4
s.d.	0.35	0.17	0.52	0.35

Results are expressed as mean ± s.d. of three human liver microsomal preparations.

while Phase II metabolism is unaffected (Abernethy et al 1984; Hollister 1990). Given the results of our study and the dose-dependent CYP3A induction by ethanol (Feierman et al 2003), the net effects of ethanol on CYP3A are likely to be complicated and may depend on the extent, as well as the duration, of ethanol consumption (Sellers et al 1978). However, in acute alcohol consumption/intoxication, inhibitory effects may contribute significantly to clinically important interactions (Abernethy et al 1984; Hollister 1990).

The exact mechanism by which ethanol inhibits CYP3A is not fully understood. However, possible mechanisms could include an effect on hepatic microsomal oxygen consumption, CYP reductase levels (Ross et al 1995), interference with the microsomal site of drugs (Rubin et al 1970), inhibition of cytochrome P450 reduction (Gigon et al 1969), depletion of free NADPH in cytosol (Thummel et al 1989) or modification of the lipophilic milieu that surrounds cytochrome P450 in the microsomal membrane (Khanna et al 1979).

We reviewed the literature for reports and studies of interactions of ethanol with CYP3A, more specifically with the substrates we used. With concurrent alcohol ingestion in rats, nifedipine clearance was decreased by 49%, the area under the nifedipine plasma concentration curve (AUC) was increased and the elimination rate constant was significantly decreased ($P < 0.05$) (Boje et al 1984; Boje & Fung 1989). In a clinical study (Qureshi et al 1992), an acute dose of alcohol increased the AUC of nifedipine by 54%, and the time to reach peak heart rate was faster in the group treated with alcohol. In a study by Krecic-Shepard et al (2000), alcoholics had significantly reduced clearance of nifedipine compared with non-alcoholics. Dorian et al (1985) showed that triazolam total AUC was increased by 21% in six subjects after ethanol consumption. However, Ochs et al (1984) observed no pharmacokinetic interaction between ethanol and triazolam. Tanaka (2002) reviewed pharmacokinetic interactions between ethanol and benzodiazepines. In many fatal poisonings, triazolam levels were much higher than the therapeutic levels in the presence of alcohol (Steentoft & Worm 1993; Tanaka et al 2002). A study by Barbone et al (1998) reviewed the association of road-traffic accidents with benzodiazepine use. The odds ratio for road-traffic accidents in benzodiazepine users with a positive blood alcohol test was significantly higher than those on benzodiazepines with negative blood alcohol. Although the additive sedative effects of alcohol and benzodiazepines may be responsible for the significantly higher odds ratio of accidents in subjects on benzodiazepines and alcohol, our results, as well as the studies reviewed, suggest a possible contribution of inhibited CYP3A metabolism to the increased odds ratio of road-traffic accidents. Sarkola et al (2001a, b) reported an acute elevation of plasma testosterone levels (~400%) in premenopausal women using oral contraceptives; this elevation closely followed the kinetics of plasma ethanol. In premenopausal women, including those taking and not taking oral contraceptives, total testosterone levels were significantly elevated during alcohol exposure sessions compared with placebo (Sarkola et al 2000). Frias et al (2002) found that testosterone levels were significantly higher in women after acute alcohol intoxication compared with controls; this was associated with essentially unchanged progesterone levels. In contrast, testosterone levels in men were significantly lower after acute alcohol intoxication compared with controls, and this was associated with significantly higher progesterone levels compared with controls (Frias et al 2002). However, a recent study by Sarkola & Eriksson (2003) reported a significant acute increase in plasma testosterone in men after a low dose of alcohol. The gender differences in the effect of ethanol on testosterone levels may indicate that these effects are mediated by the pituitary–adrenal axis, due to inhibited testosterone synthesis in the testis, or a change in androgen balance in men secondary to a change in the redox state in the liver (Sarkola & Eriksson 2003).

Conclusion

In conclusion, ethanol inhibits CYP3A metabolism in-vitro. The inhibitory effect appears to be similar regardless of the specific CYP3A substrate category. The acute effects of ethanol secondary to inhibition of CYP3A metabolism may be clinically relevant. However, the net effects of ethanol on CYP3A are likely to be complicated and may depend on the extent, as well as the duration, of ethanol consumption.

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