SHORT COMMUNICATION

An *in vitro* study on the interaction between ethanol and imipramine at their high concentrations using human liver microsomes

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Abstract Imipramine is a tricyclic antidepressant widely used for the treatments of major depression, while ethanol is one of most joyful beverages for mankind. Sometimes, toxic interactions occur following combined administration of these two compounds. In this study, we have investigated the *in vitro* interaction between ethanol and imipramine at their high concentrations by observing a mixed-function oxidation reaction using human liver microsomes. Imipramine and its three main metabolites (desipramine, 2-hydroxyimipramine = 2-OHI and 2-hydroxydesipramine = 2-OHD) were measured by high-performance liquid chromatography with ultraviolet detection. As results, the production of 2-OHD, the main metabolite of imipramine, was significantly inhibited by 15-50% ($p<0.05$) by ethanol, but that of desipramine or 2-OHI was not. These results suggest that enhanced toxicity is attained by simultaneous administration of ethanol and high-dose imipramine in actual human body.

Keywords Drug interaction • Cytochrome P450 • Ethanol • Imipramine • *In vitro* experiments • Synergism

Introduction

The majority of serious cases of drug-drug interactions of toxicological and clinical interest appear attributable to pharmacokinetic phenomena. These are usually due to alterations in hepatic drug metabolic pathways catalyzed by the cytochrome P450 (CYP) system. The adverse effects can result from inhibition or induction of metabolic enzymes; the inhibition appearing is more important in many poisoning cases.

Imipramine is mainly metabolized by CYP2D6 [1] and it is the prototype of all tricyclic antidepressant drugs for the treatments of major depression. Adverse reactions to imipramine therapy include orthostatic hypotension, paresthesias, dry mouth, blurred vision, confusion, disorientation, insomnia, agranulocytosis and paralytic ileus. Its toxicity is characterized by hyperactivity, seizers, respiratory depression, hypertension, cardiac arrhythmias, hyperpyrexia, tachycardia, urinary retention, coma, circulatory collapse and death.

Ethanol can affect the pharmacokinetics of drugs by altering gastric emptying or liver metabolism (by inducing CYP2E1). Drugs may conversely affect the pharmacokinetics of ethanol by altering gastric emptying and inhibiting ethanol dehydrogenase (ADH). Long-term intake of large amounts of ethanol induces pathways of metabolism which are independent of ADH [2-4]. Other enzymes, especially the microsomal ethanol-oxidizing (MEOS) system, including CYP2E1, are also involved at higher doses of ethanol, and these metabolize up to 10% of the ingested ethanol [3,5,6]. After chronic ethanol consumption, there is 4- to 10-fold induction of CYP2E1 [6]. Ethanol metabolism by this enzyme results in the generation of acetaldehyde and oxygen radicals. The induction of CYP2E1 may cause increased metabolism of other xenobiotics to toxic metabolites by this enzyme [7].

We have already described the toxicological interactions between ethanol and three benzodiazepines (triazolam [8], flunitrazepam [9] and alprazolam [10]). Fatal poisoning

involving coadministration of alcohol and benzodiazepines, especially these three drugs continues to be a serious social problem.

In this study we have investigated the *in vitro* interaction between ethanol and imipramine by monitoring and its three main metabolites (desipramine, 2-hydroxyimipramine = 2-OHI, and 2-hydroxydesipramine = 2-OHD) at high-dose concentrations using human liver microsomes.

Materials and methods

Materials

NADPH was purchased from Oriental Yeast (Tokyo, Japan). Clomipramine (internal standard, IS) and ethanol were purchased from Sigma (St. Louis, MO, USA) and Wako (Osaka, Japan), respectively. Imipramine and its three metabolites (desipramine, 2-OHI and 2-OHD) were kindly provided by Mitsubishi Pharma (Osaka, Japan). All other chemicals and reagents used were of the highest quality commercially available.

Microsomes from three pooled human livers (catalog Nos : H003, H013 and H032) containing representative activities of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 were obtained from Daiichi Pure Chemical (Tokyo, Japan).

Enzyme assay

The incubation mixture contained enzyme protein (0.125 mg), 0.1 M potassium phosphate buffer (pH 7.4), 0.1 mM NADPH, imipramine (substrate concentration : 0-10 μM;

therapeutic level 0.155-0.465 μM, toxic level 3.27 μM and fatal level 4.74-6.33 μM) [5] and ethanol (0-80 mM; toxic level 20-40 mM and fatal level 70-80 mM) in a total volume of 0.5 ml. Incubations were initiated following a 3-min preincubation at 37℃ by the addition of NADPH and generally carried out for 20 min in a shaking water-bath at 37℃. The reaction was terminated by adding 100 μl acetonitrile and 3 ml *tert*-butyl methyl ether containing 9.5 μg/ml clomipramine (IS). After vortex mixing for 5 min, the tubes were centrifuged at 1,200 g for 3 min. The organic phase was transferred to a clean conical tube and evaporated in a water-bath at about 40℃ under a gentle stream of nitrogen. The residue was dissolved in 200 μl mobile phase and 50 μl injected into the HPLC system.

Determination of imipramine metabolites

The HPLC equipment consisted of a pump (Model CCPS, Tosho, Tokyo, Japan) and a variable-wavelength UV detector (Model UV-8020, Tosho, Tokyo, Japan). Separation was achieved using a C_{18} reversed-phase column (150 mm X 4.6 mm I.D., particle size 3 μm, Inertsil ODS-3, GL Sciences, Tokyo, Japan). The mobile phase was 50 mM K_2HPO_4/methanol/acetonitrile (50:10:40, v/v/v) and the flow rate was 0.7 ml/min. The absorbance of the eluent was monitored at 254 nm. All instruments were operated at ambient laboratory temperature (ca. 23℃). The retention times of 2-OHD, 2-OHI, desipramine and IS in a spiked sample of human liver microsomes was 3.2, 3.8, 6.3 and 16.2 min, respectively. The limits of detection (LOD) of desipramine, 2-OHI and 2-OHD were 20, 25 and 10 nM, respectively. The intra- and inter-assay coefficients of variation (C.V.) for the three metabolites were less than 5%.

Statistical analysis

To determine significant differences between group mean values, data were subjected to a one-way ANOVA test for repeated measures. Differences were considered significant at $p<0.05$. Results are expressed as means±SE.

Results and discussion

Table 1 shows *in vitro* production rates for imipramine metabolites desipramine, 2-OHI and 2-OHD according to various concentrations of ethanol and imipramine. Only the production of 2-OHD, the main metabolite of imipramine, was significantly inhibited by 15-50% by ethanol, but that of desipramine and 2-OHI was not. This inhibition was not dependent on ethanol concentrations tested.

Koyama et al. [1] reported that the metabolism of imipramine was most efficiently catalyzed by CYP2D6, followed by CYP1A2 and CYP2C19 in a recombinant human CYP isoform study. However, it is not known which isozyme(s) is responsible for the interaction between imipramine and ethanol at the present time.

Some reports for the *in vitro* experiments on the interaction between ethanol and drugs through CYP isozymes should be mentioned. Rubin et al. [11] reported that, ethanol (10, 50 and 100 mM) in vitro inhibited the activities of aniline (competitive) and pentobarbital (mixed type) hydroxylases, and the demethylation of aminopyrene (competitive) and ethylmorphine (mixed type). Schuppel and Kuthe [12] also studied the *in vitro* inhibition by ethanol of microsomal hydroxylation for a series of barbiturates (amobarbital, cyclobarbital and pentbarbital : mixed type), but hexobarbital hydroxylation remains

unaffected by ethanol (210 mM). In contrast, Cinti et al. [13] reported that, using liver slices, *N*-demethylation of aminopyrine was stimulated by 35-40% at a low ethanol concentration (2 mM), whereas no stimulation occurred at a high concentration (100 mM).

In conclusion, our results using a human liver microsomal preparation have showed that the formation of the 2-OHD metabolite of imipramine is inhibited by ethanol. To our knowledge, this kind of studies has not been reported. Therefore, enhanced toxicity may be attained by simultaneous administration of high-dose ethanol and imipramine in a human body.

In the near future, the specific CYP isozyme(s), which is responsible for interaction between ethanol and imipramine, should be identified; such a study will be useful for assessing the enhanced toxicity when both compounds are ingested simultaneously in forensic and clinical toxicology.

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Table 1 Effect of ethanol on *in vitro* production of imipramine metabolites by human liver microsomes

	Metabolite production rate (μmol/min/mg protein)[a]			
	Ethanol concntrations			
	0 mM(control) (%)	20 mM (%)	40 mM (%)	80 mM (%)
Imipramine (2 μM)				
Desipramine	0.08±0.036 (100)	0.05±0.010(63)	0.06±0.015(75)	0.08±0.025(100)
2-OHI	0.27±0.019 (100)	0.34±0.022 (125)	0.33±0.025(122)	0.31±0.028(114)
2-OHD	0.02±0.002 (100)	0.01±0.002(50)	0.04±0.018(200)	0.02±0.003(100)
Imipramine (4 μM)				
Desipramine	0.18±0.047(100)	0.12±0.014(66)	0.11±0.012(61)	0.15±0.037(83)
2-OHI	0.54±0.029 (100)	0.44±0.036 (81)	0.44±0.015(81)	0.44±0.052 (81)
2-OHD	0.03±0.001 (100)	0.04±0.005(133)	0.03±0.004(100)	0.04±0.016(133)
Imipramine (6 μM)				
Desipramine	0.24±0.038(100)	0.19±0.016(79)	0.18±0.017(75)	0.22±0.032(91)
2-OHI	0.65±0.102 (100)	0.56±0.019(86)	0.52±0.044 (80)	0.61±0.052(93)
2-OHD	0.07±0.009 (100)	0.05±0.005(71) *	0.06±0.008(85) *	0.05±0.032(71) *
Imipramine (8 μM)				
Desipramine	0.29±0.038 (100)	0.26±0.013(89)	0.26±0.056(89)	0.25±0.054(86)
2-OHI	0.69±0.151(100)	0.61±0.021(88)	0.61±0.034(88)	0.63±0.079(91)
2-OHD	0.10±0.003 (100)	0.05±0.002(50) *	0.06±0.002(60) *	0.07±0.002(70) *
Imipramine (10 μM)				
Desipramine	0.31±0.021 (100)	0.30±0.13(96)	0.31±0.028(100)	0.31±0.023(100)
2-OHI	0.78±0.108 (100)	0.66±0.022(84)	0.70±0.067 (89)	0.64±0.080(82)
2-OHD	0.13±0.018 (100)	0.07±0.006(53) *	0.08±0.008(61) *	0.07±0.003(53) *

[a] Each values are the mean±SE of triplicate determinations. 2-OHI = 2-hydroxyimipramine, 2-OHD = 2-hydroxydesipramine, * = $p<0.05$ (vs control)