Effect of ethanol on levels of isoniazid, sulfanilamide and sulfapyridin in mouse blood

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Summary. The effect of ethanol on blood levels of free and conjugated sulfonamides (sulfanilamide and sulfapyridin) and isoniazid was investigated in mice. Ethanol (1.5 and 4 mg/g i.v.) enhanced the amount of conjugated isoniazid without affecting the total amount of isoniazid in blood, and tended to raise the total amount of the sulfonamides.

During the course of the metabolic degradation of ethanol, a considerable amount of acetate is formed, which may enter the acetate pool of the liver[2,3]. On the other hand, studies by Ammon et al.[4-6] have shown that in mice ethanol decreases the coenzyme A activity in vivo and reduces the acetylating properties of liver preparations in vitro. The authors attributed this effect to the formation of a hemiacetal between the SH-group of coenzyme A and the acetaldehyde derived from ethanol degradation, thus converting coenzyme A into a metabolically inactive compound[6].

Since acetate and coenzyme A play an important role in the biotransformation of xenobiotics, it was interesting to know if ethanol might interfere with the conjugation of drugs. For this reason the effect of ethanol on the blood levels of isoniazid and 2 sulfonamides (free and conjugated drugs) was determined in mice. These drugs are known to be transformed almost exclusively into acetate conjugates in most species[7,8].

Methods. All experiments were performed on female NMRI mice of 25-30 g b.wt housed in plastic cages on soft-wood bedding and fed ad libitum with standard diet (Herilan®) and tap water. The animals were treated either with isoniazid (16 µg/g i.v.) or with sulfanilamide or sulfapyridin (30 µg/g i.p.). Part of the animals was injected i.v. with 1.5 or 4 mg/g ethanol simultaneously with the drugs. Other drug-treated animals received saline instead of ethanol and served as controls. The volume of the drug solutions or saline injected was 0.01 ml/g b.wt. At different times after the drug injections (for detail see table), animals were killed by decapitation and blood was collected for the analysis of isoniazid or the sulfonamides.

The sulfonamides, free and conjugated fractions, were determined according to the colorimetric micromethod by Gladtke[9]. The conjugated fraction was calculated as the difference between the total and the free fractions, and considered to represent the acetylated drug, since both sulfonamides are known to be conjugated almost exclusively by acetylation[8]. Isoniazid, free and conjugated fraction, was determined according to Maher et al.[10]. According to the authors, the difference between both fractions represents acetylisoniazid.

Mean values and their SE were calculated for each treatment group. Comparisons of 2 mean values were made by means of Student's t-test. Differences were regarded to be significant if $p \leq 0.05$.

Results and discussion. All results are presented in the table. Isoniazid: Both doses of ethanol do not affect the total amount of isoniazid (free + conjugated) in the blood at any time investigated. This is in agreement with results of Lester[11], who found no changes in the plasma half-life of isoniazid in alcoholics. The fact that the fraction of conjugated isoniazid is increased by ethanol suggests that ethanol stimulates the acetylation of this drug due to its ability to enhance the acetate pool[2,3]. It provides a reasonable explanation for the observation that isoniazid toxicity is reduced in ethanol-treated mice[12], because acetylated isoniazid is supposed to be less toxic than the free drug.

Sulfonamides. In the mouse, both sulfonamides tested are conjugated to a much smaller extent than isoniazid. This may be the reason why the effect of ethanol on the conjugation of these drugs is only at the margin of significance: Ethanol enhances the amount of conjugated sulfonamides - if at all - only at the lower dose. Thus the relevance of the results is somewhat dubious, even if they may be explained rationally by a dual action of ethanol: a) promotion of acetylation by increased formation of acetate taking effect at both doses, and b) inhibition of acetylation, which should neutralize the first effect, becoming effective only at the higher dose. Inhibition of acetylation of sulfanilamide has been shown to occur in vitro[5,6]. In addition, both doses of ethanol tend to increase the total blood levels (free + conjugated drug) of both sulfonamides.

Effect of ethanol on blood levels of free and conjugated isoniazid and sulfonamides

Treatment	15 min after drug				60 min after drug				120 min after drug			
	N	Total (µg/ml)	Conjugated (µg/ml)	(%)	N	Total (µg/ml)	Conjugated (µg/ml)	(%)	N	Total (µg/ml)	Conjugated (µg/ml)	(%)
Isoniazid												
Saline	16	14.7±0.9	7.2±0.9	49	15	6.4±0.8	3.4±0.7	53	16	2.4±0.8	0.7±0.2	28
Ethanol (1.5 mg/g)	13	11.4±1.2	4.9±1.0	43	15	5.7±0.6	3.4±0.8	60	16	3.4±1.1	2.0±0.5*	60
Ethanol (4 mg/g)	23	13.4±0.8	9.4±0.8	70	24	5.5±0.6	4.2±0.4	76	24	2.4±0.6	2.0±0.5*	86
Sulfapyridin												
Saline	16	79.2±1.6	14.4±0.6	18	16	65.5±1.8	12.3±1.1	19	11	45.5±1.6	6.9±1.3	15
Ethanol (1.5 mg/g)	16	80.0±4.5	12.6±1.3	16	16	71.2±2.0*	13.8±0.8	19	13	50.7±1.7*	10.2±0.9*	20
Ethanol (4 mg/g)	14	79.2±3.4	12.2±1.0	15	15	72.9±2.6*	14.6±0.9	20	14	56.3±1.8*	9.3±0.9	16
Sulfanilamide												
Saline	19	20.5±0.7	1.7±0.3	8	14	14.0±0.7	1.8±0.4	13	14	8.6±0.3	2.0±0.2	22
Ethanol (1.5 mg/g)	19	22.9±1.2	3.2±0.6*	14	14	16.5±0.8*	1.7±0.3	10	14	11.3±0.7*	2.7±0.3*	24
Ethanol (4 mg/g)	19	22.2±1.4	1.8±0.4	8	14	17.3±0.5*	2.3±0.4	13	14	10.2±1.0	2.3±0.5	23

* p vs saline ≤ 0.05.

The reason for the retarded elimination is not apparent from our data.

Conclusion. Our results show that in fact ethanol can affect the conjugation of isoniazid in vivo. As mice oxidize ethanol much faster than men[13], our data may be valid only for this species and the high dose of ethanol that had to be applied, but considering the different toxicity and therapeutic efficacy of free and conjugated isoniazid, and the fact that pharmacodynamic interactions have been reported[12], it appears to be worthwhile to search for pharmacokinetic interactions of ethanol and isoniazid also in humans. The results obtained with the sulfonamides, being less clear-cut, are not indicative of a significant interaction of these drugs and ethanol.

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Pharmacological studies of a new analgesic, dl-erythro-1-phenyl-2-(o-chlorophenyl)-2-[4-(p-methoxybenzyl)-1-piperazinyl] ethanol dihydrochloride, in experimental animals

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Summary. dl-Erythro-1-phenyl-2-(o-chlorophenyl)-2-[4-(p-methoxybenzyl)-1-piperazinyl] ethanol dihydrochloride showed orally a definite analgesic activity, without producing the significant morphine-like physical dependence liability, and its analgesic potency was about a half that of codeine and far superior to aminopyrine in experimental animals.

Analgesic activity of derivatives of 1,2-diphenyl-2-piperazinyl-ethanol has been tested in experimental animals. Subsequently, it was found that dl-erythro-1-phenyl-2-(o-chlorophenyl)-2-[4-(p-methoxybenzyl)-1-piperazinyl] ethanol dihydrochloride (DU-608, figure 1) possessed a comparatively potent analgesic activity by oral application without producing the significant morphine-like physical dependence liability.

Methods and results. I.v. analgesic activity of DU-608, codeine phosphate(codeine) and d-propoxyphene hydrochloride (d-propoxyphene), by the method of mechanical pressure stimuli[1,2], is shown in figure 2. Analgesic ED50-values (mg/kg) and 95% confidence limits of DU-608 in rats and mice were 2.94 (2.00–4.40, n = 40) and 4.04 (2.99–5.46, n = 51), respectively, and its activity was approximately comparable to that of d-propoxyphene and codeine, and far superior to that (ED50 = 73.0, n = 30 in mice) of aminopyrine. As shown in figure 3, DU-608, administered orally, produced a dose-dependent inhibition of the pain-like response induced by stimulating electrically a tooth pulp through the electrodes implanted chronically in conscious dogs[3,4]. The potency of DU-608 (ED50 = 50.1 mg/kg) was about a half that of codeine (28.1 mg/kg) and superior to that of aminopyrine (almost inactive). The oral activity of DU-608 to abolish the pain-like responses induced by various nociceptive stimuli (chemical[5], mechanical pressure[1,2], radiant heat[1,6]) in mice and rats was superior to that of pentazocine and aminopyrine, and a half or one-third that of codeine and d-propoxyphene. Its therapeutic index (LD50/ED50) was higher than that of pentazocine and aminopyrine, and higher than or at least equal to that of d-propoxyphene.

Anti-writhing (phenylquinone induction) activity of DU-608 was antagonized by naloxone hydrochloride similar to that of pentazocine, codeine and d-propoxyphene in mice. On the other hand, analgesic activity (by the radiant heat method) of 5 mg/kg, s.c., of morphine hydrochloride(morphine) was only slightly enhanced by the simultaneous administration of 160 mg/kg, s.c., of DU-608 (ED50 = 80 mg/kg, s.c.), but not affected by the administration of 20 to 80 mg/kg in mice. Furthermore, Straub tail elevating activity of morphine (10 mg/kg, i.v.) was neither additively enhanced nor antagonized by a s.c. administration of up to 160 mg/kg of DU-608. The activities of morphine were enhanced or antagonized by the simultaneous administration of codeine or pentazocine, respectively.

No significant withdrawal syndrome was observed by an abrupt withdrawal of DU-608 or by an administration of levallorphan tartrate (10 mg/kg, s.c.) instead of DU-608 in the rats, receiving the chronic administration of DU-608 (the maximal daily tolerant dose; 48 mg/kg, s.c., or 400 mg/kg, p.o.) twice daily for 7–8 weeks. The rats, receiving chronically codeine (60 mg/kg/day, s.c., or 80 mg/kg day,

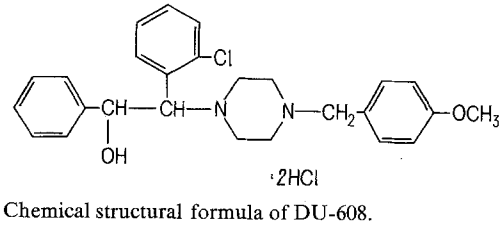

Fig. 1. Chemical structural formula of DU-608.