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Studies on ethanol-inducible cytochrome P-450 in rabbit liver, lungs and kidneys

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In humans, chronic ethanol consumption may alter rates of drug metabolism and increase the incidence of liver diseases [1]. Studies in rats have shown that ethanol-induced cytochrome P-450, termed P-450j, is associated with increased metabolism of aniline [2, 3], the carcinogen *N*-nitrosodimethylamine [4], and hepatotoxicity of CCl_4 [5]. In rabbits, a new form of cytochrome P-450, termed 3a, has been purified from the livers of rabbits chronically treated with ethanol [6]. Form 3a is also inducible by a diversity of other agents including acetone and isoniazid [7] and, in a reconstituted system, 3a is highly active in the hydroxylation of aniline and the oxidation of alcohols [8].

In contrast to the extensive studies of ethanol-induced liver cytochromes P-450 in various species, there is a paucity of data on the ability of ethanol to induce cytochrome P-450 in extrahepatic tissues. The present study compares the inductive effects of ethanol in rabbit liver with those in the lung and kidney, these being important entry and excretion sites, respectively, of many foreign chemicals. The results of enzymatic and immunochemical studies demonstrate that ethanol treatment of rabbits induces specific cytochrome P-450 isozymes in the liver and kidney, but not in the lung.

Materials and methods

Adult male New Zealand White rabbits, weighting 2.6 to 3.2 kg, were treated with 10% ethanol (v/v) in their drinking water for 15 days. At the end of 15 days, the rabbits were killed, tissues were removed, and washed microsomes were prepared. Microsomal cytochrome P-450 content was determined by the method of Omura and Sato [9]. Aniline hydroxylase [10], 7-ethoxycoumarin *O*-deethylase [11], 7-ethoxyresorufin *O*-deethylase [12], and NADPH-cytochrome *c* reductase [13] activities were determined as previously described.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis [14] and transfer of the microsomal proteins from slab gels to nitrocellulose membrane [15] were carried out as described previously. Immunochemical detection of P-450 was performed using a 1 : 1000 dilution of mouse antiserum ethanol-inducible rat liver P-450et, homologous to P-450j [16].

Results and discussion

The effects of ethanol ingestion on monooxygenase activities in rabbit liver, lung and kidney are shown in Table 1. In the liver, pretreatment with ethanol caused a 2.6-fold increase in aniline hydroxylase activity, in confirmation of previous data [8]. In contrast, ethanol caused statistically significant ($P < 0.05$) decreases of 21 and 43% in total cytochrome P-450 content and 7-ethoxyresorufin *O*-deethylase activity respectively. Koop *et al.* [7] also observed a small, but statistically insignificant decrease in total hepatic cytochrome P-450 in livers of ethanol-treated rabbits. The chronic ethanol treatment had no significant effect on hepatic microsomal 7-ethoxycoumarin *O*-deethylase activity and NADPH–cytochrome *c* reductase activity.

In the kidney, ethanol caused a 50% increase in total cytochrome P-450 content (Table 1). The increase was associated with 4- and 2-fold increases in aniline hydroxylase and 7-ethoxycoumarin *O*-deethylase activities respectively. Ethanol treatment caused an 18% increase in kidney NADPH–cytochrome *c* reductase activity. Renal 7-ethoxyresorufin *O*-deethylase activity was not detected in ethanol-treated rabbits. In contrast to liver and kidney, ethanol caused no significant alterations in pulmonary cytochrome P-450 content, NADPH–cytochrome *c* reductase activity, or metabolism of aniline, 7-ethoxycoumarin and 7-ethoxyresorufin (Table 1).

In data not shown, cytochrome b_5 content, benzo[a]pyrene hydroxylase and benzphetamine *N*-demethylase activities were similar in tissues of untreated and ethanol-treated rabbits. The results of the above monooxygenase studies demonstrate that chronic ethanol pretreatment results in the induction of aniline hydroxylase activity and the depression of 7-ethoxyresorufin *O*-deethylase activity in the

Table 1. Effect of ethanol on microsomal monooxygenases of rabbit tissues

	Cytochrome P450 (nmol/mg protein)	Aniline hydroxylase (nmol *p*-aminophenol/ min/mg protein)	7-Ethoxycoumarin *O*-deethylase (nmol hydroxycoumarin/ min/mg protein)	7-Ethoxyresorufin *O*-deethylase (pmol resorufin/ min/mg protein)	NADPH–cytochrome *c* reductase (nmol cyt. *c* reduced/min/ mg protein)
Liver					
Control	1.39 ± 0.05	0.53 ± 0.03	3.06 ± 0.28	124 ±13	172 ± 3
EtOH	1.10 ± 0.05*	1.39 ± 0.25*	3.63 ± 0.32	70 ± 9*	177 ± 11
Kidney					
Control	0.06 ± 0.01	0.02 ± 0.01	0.08 ± 0.02	0.2 ± 0.1	14.3 ± 0.5
EtOH	0.09 ± 0.01*	0.08 ± 0.01*	0.17 ± 0.02	ND†	16.8 ± 1.0*
Lung					
Control	0.32 ± 0.03	0.16 ± 0.02	2.35 ± 0.27	1.6 ± 0.3	93 ± 6
EtOH	0.36 ± 0.02	0.19 ± 0.02	2.65 ± 0.12	1.2 ± 0.5	103 ± 6

Male New Zealand White rabbits weighing 2.6 to 3.2 kg were given 10% ethanol (v/v) in their drinking water for 15 days. At the end of 15 days, the animals were killed, tissues were removed, and microsomes were prepared. Each value represents the mean ± SE for six rabbits.

* Significantly different from the respective control value, $P < 0.05$.

† Not detectable.

liver and kidneys, possibly owing to selective induction of certain hepatic and renal cytochrome P-450 isozymes along with repression of other isozymes.

Liver, kidney and lung microsomes of control and ethanol-treated rabbits were solubilized with SDS and subjected to electrophoresis followed by "Western blots" and immunochemical detection procedures. Since the ethanol-inducible rabbit liver P-450 3a and rat liver P-450j appear to be identical by several criteria, including immunochemical reactivities [17], in the present studies mouse antiserum against P-450et was used to probe for immunorelated P-450s. Figure 1, lane 1, shows that a single band was detected with liver microsomes from rats induced by acetone. Acetone has been shown to be a potent inducer of the ethanol-inducible form of P-450 in both rabbits [7] and rats*. In rabbit liver, two bands were observed with the control microsomes (lane 2). The intensities of both these bands were increased markedly in the liver microsomal preparation from ethanol-treated rabbits (lane 3). In the kidney, a single immunoprecipitin band was observed with microsomes from untreated rabbits, as shown in Fig. 1, lane 4. The migration of this renal protein was identical to the lower protein band observed with liver microsomes. Based on the relative staining intensities, chronic ethanol treatment of rabbits caused a marked increase in the renal protein (lane 5). In contrast to liver and kidney, lanes 6 and 7 of Fig. 1, show that, in the lung, no immunoreactive protein band was detected with microsomes prepared from control or ethanol-treated rabbits, even though the microsomal protein load was 150-fold greater than that of liver microsomes.

The results of immunochemical studies are in agreement with the catalytic studies which showed that aniline hydroxylase which is associated with ethanol-induced P-450s is inducible in the liver and kidneys of ethanol-treated rabbits. Although the immunoblot of rabbit lung showed an absence of immunoreactive protein band, rabbit lung microsomes had significantly greater aniline hydroxylase activity than rabbit kidney microsomes. Unlike kidney, however, the pulmonary activity was not ethanol-inducible. These results indicate that the pulmonary P-450s responsible for the metabolism of aniline are immunologically different from the hepatic and kidney P-450s. The multiplicity of protein bands observed in the immunoblots of rabbit liver is of interest. Ryan *et al.* [17] recently used sheep antiserum against rabbit P-450 isozyme 3a to probe immunoblots of rat liver microsomes and purified P-450 isozymes. Two species of immunoreactive proteins differing in apparent molecular weights were observed using microsomes from untreated rats but not from ethanol-induced rats. One species of the rat proteins corresponds to the apparent molecular weight of rabbit isozyme 3a; however, the other species of protein is inducible by ethanol.

In summary, the present report demonstrates tissue specificity in the induction of rabbit cytochrome P-450 isozyme 3a by ethanol. The microsomal enzymes of rabbit liver and kidney are responsive to the inductive effect of ethanol. That chronic ethanol treatment lacks an effect on the pulmonary enzymes differs markedly from the effects of other prototypic inducing agents such as phenobarbital and 3-methylcholanthrene [18]. It will be of interest to extend the present studies to determine the responses of rabbit extrahepatic tissues to acetone, a more potent inducer of isozymes 3a [7]. Following the completion of the present studies, Ding *et al.* [19] have shown that cytochrome P-450 form 3a is present in microsomes prepared from kidney and nasal mucosa, but not from lung and other extrahepatic tissues from both untreated and ethanol-treated rabbits.

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*S. S. Park and H. V. Gelboin, manuscript in preparation.

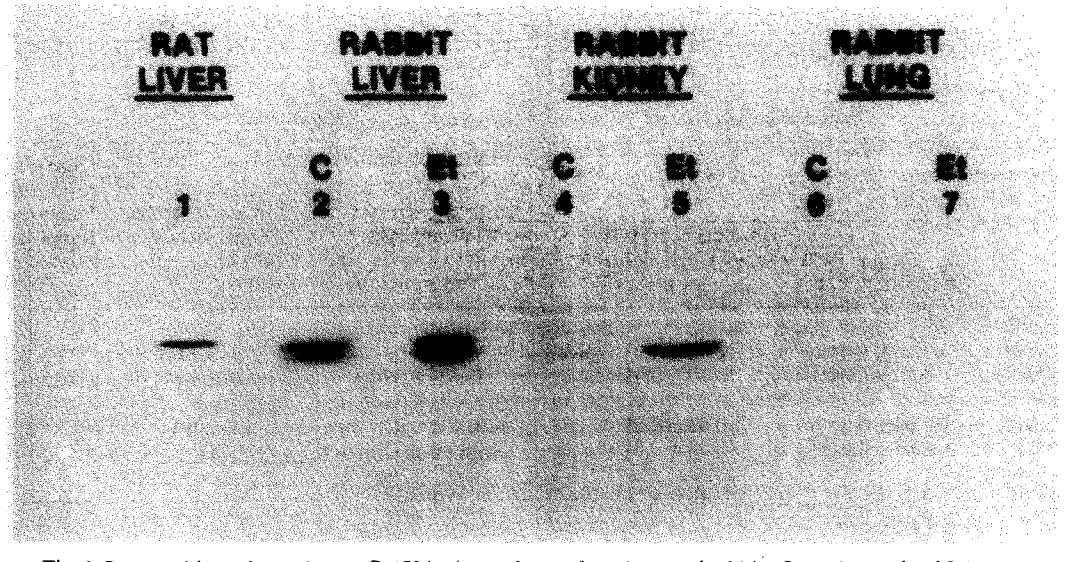

Fig. 1. Immunoblots of cytochrome P-450 in tissues from ethanol-treated rabbits. Lane 1 contained 0.1 μg of liver microsomal protein from acetone-treated rats. The amounts of rabbit microsomal protein loads were: 1 μg in lanes 2 and 3; 15 μg in lanes 4 and 5; and 150 μg in lanes 6 and 7. Electrophoresis was performed, and the proteins were electrophoretically transferred to nitrocellulose membrane and immunochemically stained with antiserum to P-450et.

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Josamycin and troleandomycin increase hepatic glutathione turnover in rats

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The concomitant administration of troleandomycin with a number of other drugs is associated with a high incidence of adverse side-effects [1, 4] that result from the inhibition of the hepatic monoxygenase system by the antibiotic [3, 5, 6]. A metabolite of troleandomycin forms a complex with the iron of cytochrome P-450 thereby inactivating the monooxygenase [7]. This metabolic activation is associated with a decrease in hepatic glutathione (GSH) suggesting that GSH might play a role in the detoxification of the reactive metabolite [8]. However, inhibition of the synthesis of GSH, as well as loss of GSH by formation of a troleandomycin–glutathione adduct, or by some other mechanism, could be responsible for the observed depletion of hepatic GSH.

In order to better understand the mechanism underlying the depletion of GSH we studied the effect of troleandomycin and josamycin on hepatic GSH turnover in rats. Josamycin does not form cytochrome P-450 complexes [9] and does not inhibit drugs metabolism [10] but its effect on hepatic GSH is not known. If this compound affected GSH homeostasis without forming a metabolite reacting with cytochrome P-450 it might provide new insights into the relationship between depletion of GSH and inactivation of cytochrome P-450 following the administration of macrolide antibiotics.

Material and methods

Male Sprague-Dawley rats (Charles River, Wiga GmbH, F.R.G.) weighing 130–250 g had free access to food and water and were kept in an air-conditioned room with a 12 hr light–dark cycle. Josamycin and troleandomycin (Pfizer Laboratories) were dissolved in 1 ml of arachis oil, and 400 μmol/100 g b.w. were administered by gavage. Control animals received the vehicle only. At 24, 48, and 120 hr, portions of the liver were obtained for the measurement of hepatic GSH [11].

To determine GSH turnover [12, 13] identical doses of troleandomycin, josamycin, and vehicle were administered to three other groups of rats. The rats were fasted after administration of the drugs and were studied 24 hr later under pentobarbital anaesthesia (50 mg/kg i.p.). The common bile duct was cannulated with a PE-10 catheter. Throughout the experiment the temperature of the animals measured rectally was maintained between 37 and 38° with a heating lamp. At time zero 30 μCi of ^{35}S-L-cysteine (300 Ci/mmol, NEN, U.S.A.) were injected i.v. Bile samples were then collected into preweighed tubes containing 0.1 ml of 4% sulphosalicylic acid at timed intervals for the determination of the biliary excretion of GSH. For the subsequent determination of the specific activity of GSH, aliquots of bile were collected into 5 mM monobromobimane in acetonitrile which derivatizes GSH for HPLC analysis [14]. At the end of the experiment a portion of the liver was removed for the determination of hepatic GSH.

Analytical methods. For the determination of the specific activity of GSH, the bile samples collected into monobromobimane were analyzed by HPLC using a 7 μm R.P-18 column (E. Merck, Darmstadt, F.R.G.) and water/methanol/acetic acid (89.75 : 10 : 0.25, v : v, pH 3.9) at a flow rate of 1.5 ml/min as the mobile phase [14]. The column effluent was monitored at 254 nm and the GSH peaks were collected and assayed for radioactivity by liquid scintillation spectrometry. The specific activity of GSH was calculated from the radioactivity and the mass of GSH, which was obtained by comparing the area of the GSH peak with a standard curve. Hepatic glutathione (GSH plus GSSG) was assayed by the enzymatic recycling method of Tietze [15].

Calculations. The slope of the declining phase of the specific activity–time curve of GSH in bile was calculated by least-square regression analysis following logarithmic transformation of the measured values. The calculated slope of the monoexponential phase of each curve corresponds to the fractional rate of turnover of hepatic GSH.

Statistics. All values are expressed as mean ± SD. Group means were compared using Student's *t*-test.