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Ethanol Oxidation and Toxicity: Role of Alcohol P-450 Oxygenase

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The isolation and characterization of ethanol-inducible rabbit liver microsomal cytochrome P-450, termed P-450 3a or P-450ALC, has provided definitive evidence for the role of this enzyme in alcohol oxidation. From findings on the distribution, substrate specificity, and mechanism of action of P-450ALC we have suggested "alcohol P-450 oxygenase" as a more biochemically accurate name than "microsomal ethanol-oxidizing system." The present review is concerned with studies in this and other laboratories on activities and inducers associated with this versatile enzyme. Numerous xenobiotics, including alcohols and ketones, nitrosamines, aromatic compounds, and halogenated alkanes, alkenes, and ethers, are known to undergo increased microsomal metabolism after chronic exposure of various species to ethanol. Diverse compounds and treatments may induce P-450ALC, including the administration of ten or more chemically different compounds, fasting, or the diabetic state. Whether a common mechanism of induction is involved is unknown at this time. As direct evidence that P-450ALC catalyzes numerous metabolic reactions, the purified rabbit enzyme has been used in a reconstituted system to demonstrate various metabolic transformations, including the oxidation of various alcohols, acetone, acetol, *p*-nitrophenol, and aniline, the dealkylation of substituted nitrosamines, the reductive dechlorination of carbon tetrachloride, carbon tetrachloride-induced lipid peroxidation, and acetaminophen activation to form the glutathione conjugate.

ETHANOL consumption is known to have profound biochemical and physiological effects in animals and man.[1] The mechanisms by which ethanol perturbs cellular homeostasis are not yet well understood but appear to involve interactions with cellular and subcellular membranes[2] as well as secondary changes resulting from ethanol metabolism.[3,4] Considerable evidence exists that alcoholics have enhanced susceptibility to potential toxins, such as commonly used drugs and industrial solvents, which may be hepatotoxic, mutagenic, or carcinogenic.[5-8] Most such compounds are not toxic or carcinogenic per se, but elicit their effects after metabolic activation by the cytochrome P-450-dependent mixed function oxidase system.[9] Ethanol can both stimulate and inhibit P-450-dependent metabolism. The inhibitory effects of an acute dose are due, in part, to the ability of ethanol to bind to P-450 isozymes and thus compete with the metabolism of other substrates.[6,10] In addition, ethanol decreases the availability of NADPH.[11,12] The stimulation of P-450-dependent metabolism by the chronic administration of ethanol, which can result from the induction of specific isozymes of cytochrome P-450, is the subject of this review.

The enzyme system primarily responsible for both the bioactivation and the inactivation of xenobiotics is the microsomal P-450-dependent mixed function oxidase system.[13] The terminal oxidases of the system are collectively referred to as P-450, although it has been clearly established that distinct isozymes of P-450 are present in hepatic as well as extrahepatic tissues.[9,14-16] Each isozyme has a characteristic substrate preference although, in most cases, considerable overlap in metabolic activity exists. Until recently, it was difficult if not impossible to attribute an observed metabolic activity to a specific isozyme. However, the purification of individual P-450 isozymes has provided the necessary antigens for production of specific inhibitory monoclonal and polyclonal antibodies to be used in identifying the activities of each such catalyst. Each species has its own complement of P-450's, but recent comparisons of the primary structures by direct sequence analysis or by prediction from the cDNA structures indicate that homologous isozymes are present in different species.[16,17] Structural homology is also evident from the cross-reactivity of monoclonal and polyclonal antibodies[18,19] with the cytochromes from different organisms. Furthermore, the homologous isozymes in different species have very similar metabolic activities. For example, rat P-450c, rabbit P-450 6, and mouse P_1-450 are structurally and immunochemically similar and are the most active isozymes in the metabolism of benzo(*a*)pyrene.[18,20] In addition, all three isozymes are induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.[21]

The exposure of experimental animals and man to drugs and xenobiotics results in the induction of one or more isozymes of cytochrome P-450.[17,22,23] In the late 1960s and early 1970s, Lieber and coworkers reported that ethanol treatment of humans and rats resulted in proliferation of the hepatic smooth endoplasmic reticulum and an increase in the drug-metabolizing activity of microsomal preparations.[24-26] These effects were accompanied by an increase in the microsomal P-450 concentration and

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an increase in the ability of hepatic microsomes to catalyze the oxidation of ethanol.[27-30] The microsomal ethanol-oxidizing activity was shown to be present in a P-450 fraction by solubilization and reconstitution of components free of catalase and alcohol dehydrogenase.[29, 31-33] An observed increase in microsomal ethanol oxidation activity and a change in the banding pattern of hepatic microsomes on sodium dodecyl sulfate-polyacrylamide gels distinct from that produced by phenobarbital or 3-methylcholanthrene led to the hypothesis that ethanol induced a unique isozyme of cytochrome P-450.[29, 34] Definitive proof for the existence of a unique ethanol-inducible isozyme of cytochrome P-450 was provided by the purification and characterization of an isozyme from ethanol-treated rabbits,[35, 36] about 12 years after the effects of ethanol on P-450 were first documented.

Rubin et al.[26] reported that, in addition to an increase in cytochrome P-450, the microsomal *p*-hydroxylation of aniline was increased about 7-fold by ethanol administration. The increase in aniline hydroxylation is an excellent marker for ethanol-dependent increases in P-450-dependent metabolism and is more reliable than measurement of ethanol oxidation due to lower blank values. Aniline hydroxylation is elevated 16–24 hr after a single dose of ethanol[37-39] or after vapor exposure,[40] and is induced by ethanol even though no significant increase is seen in total P-450 in liver from rabbits[36, 41] and chick embryos.[42] The metabolism of a large number of structurally diverse compounds, in addition to aniline and ethanol, is increased by ethanol consumption. Table 1 provides a partial list of compounds where in vitro metabolism and/or in vivo toxicity is enhanced by ethanol treatment. The list is not meant to be comprehensive but to provide representative examples. As can be seen, the reactions attributable to an ethanol-inducible isozyme include aromatic hydroxylation (aniline), aliphatic hydroxylation (acetone), dehalogenation (enflurane), *N*-demethylation (*N*-nitrosodimethylamine), and alcohol oxidation (ethanol), all characterized as monooxygenase reactions of P-450.[9, 72]

While all of the activities indicated above are induced by ethanol treatment, thus implicating an ethanol-inducible isozyme of P-450, many are also induced by other agents or treatments as summarized in Table 2. Such results bring to mind many possible interpretations, the simplest being either that each compound induces a unique isozyme that exhibits an overlapping substrate preference to that of other isozymes, or that all compounds induce the same isozyme, in this case the alcohol-inducible form. The availability of the purified ethanol-inducible isozyme 3a from rabbits, along with specific antibodies to this enzyme, has enabled us to test these two contrasting hypotheses. The direct structural comparison of the enzyme purified from ethanol-, imidazole-, and acetone-treated rabbits indicated that the same isozyme is induced by all three compounds.[48, 79, 93] Furthermore, an immunochemically identical isozyme is induced separately by

Table 1. Substrates That Undergo Increased Metabolism after Ethanol Treatment of Animals

Substrate tested	Species examined	References
Alcohols and ketones		
Ethanol	Rat	27
	Rabbit	36
	Hamster	43
	Deermouse	44, 45
Acetol	Rat	46, 47
	Rabbit	48
n-Propyl alcohol	Rat	49
	Deermouse	44
n-Butyl alcohol	Rat	49
	Rabbit	50
2-Butanol	Rat	51
n-Pentanol	Rabbit	50
Acetone	Rat	46, 47
	Rabbit	48
Nitrosamines		
N-Nitrosodimethylamine	Rat	52–55
	Rabbit	56
N-Nitrosopyrrolidine	Rat	57
	Hamster	58
N-Nitroso-2,6-dimethylmorpholine	Rabbit	59
N-Nitrosonornicotine	Rat	60
Halogenated alkanes, alkenes, and ethers		
Enflurane	Rat	61
Carbon tetrachloride	Rat	54, 62–64
Chloroform	Rat	63
Trichloroethylene	Rat	63
1,1-Dichloroethylene	Rat	63
1,2-Dichloroethane	Rat	63
Aromatic compounds		
Aniline	Rat	26, 31, 40
	Rabbit	36
	Hamster	43, 65
	Mouse	66
Acetaminophen	Rat	67
	Mouse	68, 69
	Hamster	65
Benzene	Rat	63
Toluene	Rat	63
p-Nitrophenol	Rat	70, 71
	Rabbit	

trichloroethylene, isoniazid, benzene, pyrazole, acetone, and imidazole in this species.[48, 79] Thus, the results strongly suggest that these structurally diverse compounds induce the same isozyme.

Antibody inhibition in conjunction with reconstitution experiments demonstrated the role of P-450 isozyme 3a in microsomal preparations. The reactions catalyzed by this cytochrome in rabbit microsomes are shown in Table 3. It is important to emphasize that for most of the reactions isozyme 3a is not the sole catalyst, since other isozymes are known to exhibit significant activity. However, the results of antibody inhibition experiments indicate that, after ethanol treatment, isozyme 3a is the principal catalyst. For example, anti-3a IgG inhibits 90% of the *N*-nitrosodimethylamine demethylase activity, 75% of the ethanol oxidation activity, and 60% of the aniline hydroxylase activity of microsomes from ethanol-treated rabbits, and this antibody inhibits 70%, 35%, and 30% of the respective activities in microsomes from untreated rabbits.[50, 75] Since other isozymes exhibit significant activ-

Table 2. Agents or Treatments Having Inducing Effects Similar to That of Ethanol

Treatment or inducer administered to animal	Substrate examined for activity of microsomes	Species examined	Reference
Acetone	*N*-Nitrosodimethylamine	Rat	73, 74
		Guinea pig	75
	n-Butyl alcohol	Rabbit	41
	Acetone	Rat	46
	Acetone	Rabbit	48
	Carbon tetrachloride	Rat	76
Pyrazole	*N*-Nitrosodimethylamine	Rat	77, 78
	n-Butyl alcohol, aniline	Rabbit	41, 79
	2-Butanol	Rat	80, 81
	Carbon tetrachloride	Rabbit	82
Isopropyl alcohol	*N*-Nitrosodimethylamine	Rat	73, 74
	Carbon tetrachloride	Rat	83, 84
	Aniline	Rat	84
4-Methylpyrazole	2-Butanol	Rat	80
Imidazole	Aniline	Rabbit	79
	n-Butyl alcohol	Rabbit	41
	N-Nitrosodimethylamine	Rabbit	85
Fasting	*N*-Nitrosodimethylamine	Rat	86
Trichloroethylene	*n*-Butyl alcohol	Rabbit	41
Benzene	Aniline	Rabbit	87
Diabetic state	Aniline	Rat	88
	N-Nitrosodimethylamine	Rat	89
Isoniazid	Aniline	Rat	90, 91
	Enflurane	Rat	90
	n-Butyl alcohol	Rabbit	41
Ethylene thiourea	Aniline	Mouse	92

Table 3. Reactions Catalyzed by Rabbit Liver Microsomal P-450 Isozyme 3a

Reaction catalyzed	References
N-Nitrosodimethylamine demethylation (low K_m)	56, 75
Alcohol oxidation (ethanol, butanol, and pentanol)	50
Acetone hydroxylation	48
Acetol oxidation	48
p-Nitrophenol hydroxylation	94
Aniline hydroxylation	50
Carbon tetrachloride reductive dechlorination	82
N-Nitroso-2,6-dimethylmorpholine hydroxylation	59
Carbon tetrachloride-induced lipid peroxidation	82
Acetaminophen activation (to form glutathione conjugate)	95

ity toward some substrates, the induction of a particular microsomal activity does not necessarily mean that the level of isozyme 3a has been increased; such a conclusion requires confirmation by antibody inhibition. The effect of substrate concentration is also a highly important consideration in an evaluation of the relative roles of different isozymes, as strikingly demonstrated with *N*-nitrosodimethylamine. Ethanol and certain other compounds induce a demethylase with a low K_m for this substrate in rats[52, 53] and rabbits.[56] Of six rabbit isozymes tested, only 3a exhibited activity with 0.1 mM *N*-nitrosodimethylamine, whereas isozymes 2, 3a, 3b, 3c, and 6 displayed activity with 4 mM, and all six isozymes were active, with isozyme 3a being only slightly more effective than isozyme 6, at the highest substrate concentration, 100 mM. Thus, depending upon the substrate concentration, isozyme 3a could be the sole catalyst or one of numerous isozymes effecting the demethylation reaction. This cytochrome appears to be the sole catalyst for acetone hydroxylation since the antibody inhibited 90% or more of the activity in microsomes from both untreated and ethanol-treated rabbits.[48] Similar results have recently been obtained for the hydroxylation of *p*-nitrophenol to give 4-nitrocatechol.[94]

The antibody to rabbit isozyme 3a was also shown to be inhibitory in microsomes from other species, suggesting the presence of a homologous isozyme. The antibody inhibited 71% and 92% of the *N*-nitrosodimethylamine demethylase activity of microsomes from untreated and ethanol-treated rats, respectively, at 5 mM substrate.[75] Tu and Yang[96] have recently reported that an isozyme termed P-450et from ethanol-treated rats has significant activity toward *N*-nitrosodimethylamine. Anti-3a IgG also was an effective inhibitor of acetone hydroxylation by microsomes from acetone-treated rats[48] and of *N*-nitrosodimethylamine demethylation by microsomes from untreated mice and guinea pigs and acetone-treated guinea pigs,[75] thus indicating the likely presence of a homologous isozyme in these species.

Ryan et al.[91] reported the isolation from isoniazid-treated rats of P-450j, a cytochrome with activity in aniline hydroxylation and spectral properties similar to those of rabbit isozyme 3a. As a further similarity, the first 19 residues of the amino terminus of P-450j exhibit 68% sequence homology with isozyme 3a, which is also known to be induced by isoniazid.[41] We have exchanged purified proteins and antibody with Ryan and co-workers and found that, in all respects, P-450j and isozyme 3a are highly similar enzymes.[97] The degree of structural homology can be determined only when the complete amino acid sequences are known.

The immunochemical and structural comparison of P-450j and isozyme 3a and the results of antibody inhibition experiments suggest that ethanol, isoniazid, and acetone induce P-450j and isozyme 3a, as indicated above, and that a homologous isozyme is induced by acetone in guinea pigs.[75, 97] However, compounds that induce isozyme 3a in rabbits need not necessarily have comparable effects in other species, as shown by the following examples. Reinke et al.[71] reported that, in the rat, imidazole treatment resulted in an increase in 7-ethoxycoumarin deethylation and aminopyrine demethylation with no ac-

companying increase in aniline hydroxylation or *p*-nitrophenol hydroxylation. The protein profiles on sodium dodecyl sulfate-polyacrylamide gels also suggested marked differences in induction by imidazole or ethanol. Pyrazole induces isozyme 3a in rabbits[41] and *N*-nitrosodimethylamine demethylation[77, 78] and alcohol oxidation activities in rats.[80] In contrast, in three strains of mice, DBA/2N, C57BL/6N, and AKR/N, pyrazole had no effect on *N*-nitrosodimethylamine demethylation, while coumarin 7-hydroxylase activity was induced in the DBA/2 strain only.[98] Furthermore, Elves et al.[66] reported that ethanol treatment induces aniline hydroxylation in C57BL/6J and A/J mice. The results of such experiments indicate that the effect of an administered agent is both species- and strain-dependent, and that more than one isozyme may be induced. Feierman and Cederbaum[80] have recently compared the inductive effects of pyrazole and 4-methylpyrazole in rats. Pyrazole treatment had no effect on total microsomal P-450 or aminopyrine demethylation, while 4-methylpyrazole treatment resulted in about a 2-fold increase in both as well as an increase in the microsomal rates of ethanol and 2-butanol oxidation. These results are suggestive that, in the rat, 4-methylpyrazole treatment results in the induction of more than one P-450 isozyme.

At the present time the available evidence indicates that ethanol induces a single isozyme in hepatic microsomes from rabbits. However, until all of the isozymes present in the microsomal fraction have been purified and characterized, the coinduction of other cytochromes cannot be excluded. There is suggestive evidence that ethanol-treatment may induce other isozymes in extrahepatic tissues. Rat P-450j has no significant activity toward benzo(*a*)pyrene,[91] yet Lieber and co-workers reported that chronic ethanol treatment significantly enhanced 3-hydroxybenzo(*a*)pyrene formation in microsomes from rat intestinal mucosa.[99] These results may be taken to mean that either a different isozyme is induced by ethanol in the intestinal mucosa or that the effect of ethanol is to stimulate in some manner constitutive isozymes that hydroxylate benzo(*a*)pyrene. Ethanol treatment was reported to have no effect on lung microsomal benzo(*a*)pyrene metabolism, although the activation of tobacco smoke condensate was enhanced.[100] Ding et al. (X. Ding, D. R. Koop, B. L. Crump, and M. J. Coon, unpublished results) have examined a variety of extrahepatic rabbit tissues for the presence of isozyme 3a, which was identified by immunoblots in microsomes from the kidney and nasal membranes; the enzyme was induced by ethanol in the former but not the latter tissue. Due to species differences in the inducibility of this isozyme, it will be necessary to determine whether the enzyme is induced extrahepatically in the rat, as possibly indicated from mutagenesis studies.[99, 100]

Much progress has been made toward an understanding of the effects of ethanol on P-450 since the initial observations of Lieber and coworkers in 1968. In addition to ethanol, a large number of other chemical agents have a similar effect on the P-450 system. Although the mechanisms by which such structurally diverse compounds induce one or more P-450 isozymes in a species- and tissue-specific manner remain to be elucidated, it has become clear that a particular ethanol-inducible isozyme is responsible for the metabolism and activation of many foreign compounds.

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