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Influence of Aliphatic Alcohols on the Hepatic Response to Halogenated Olefins

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The role of alcohols in potentiating the toxicity of halogenated hydrocarbon solvents has been reviewed. The toxicity of carbon tetrachloride and chloroform can be markedly potentiated by prior treatment with ethanol or phenobarbital. Trichloroethylene toxicity may also be potentiated by ethanol ingestion. Prior ethanol ingestion acts by altering biochemical parameters that result in an increased response to subsequent solvent exposure. Simultaneous exposure to both ethanol and trichloroethylene allows for competitive substrate inhibition of metabolism since these compounds share several common enzymatic pathways. Thus the toxic response to multiple exposures varies depending upon the time sequence and the comparative levels of the individual components. Phenobarbital apparently potentiates solvent toxicity by induction of the microsomal mixed function oxidase system. Ethanol, either on a chronic or single dose basis, also has the ability to stimulate this enzyme system. Although alteration of the microsomal mixed function oxidase system by chronic ethanol ingestion may play an important role in potentiation of solvent toxicity, the potentiation seen following a single dose of ethanol cannot be fully accounted for by the known effects of ethanol on the mixed function oxidase system. In addition to ethanol a large number of other alcohols will markedly potentiate the hepatotoxic response to solvents such as carbon tetrachloride and chloroform. The mechanisms involved in such potentiation are not known at the present time.

Introduction

Pretreatment of experimental animals with either ethanol or phenobarbital prior to carbon tetrachloride or chloroform exposure markedly increases the hepatotoxicity of these halogenated hydrocarbons (*1–6*). Phenobarbital potentiation of toxicity did not occur, however, in rats injected with methylene chloride, methyl chloroform, trichloroethylene or perchloroethylene at IP dose levels up to 2.0 ml/kg (*1*). Conversely, phenobarbital has been reported to protect against the hepatotoxic response to PO doses of vinylidene chloride (1,1-dichloroethylene) (*7*). That phenobarbital alters the toxic response to certain halogenated hydrocarbon solvents by induction of the liver microsomal mixed function oxidase system (MFOS) appears well documented, since a number of studies with microsomal enzyme inducers and inhibitors generally support such a mechanism (*5, 8, 9*). Apparently, the increased rate of metabolism of hepatotoxic solvents such as carbon tetrachloride and chloroform increases the rate of formation of more oxic metabolites, possibly free radicals or epoxides, resulting in a greater hepatotoxic response.

Ethanol Potentiation

Ethanol potentiation of carbon tetrachloride and chloroform toxicity in experimental animals may elevate serum glutamic oxaloacetic transaminase activity (SGOT) as much as 10-fold when ethanol is ingested prior to solvent exposure (*2, 10*). Trichloroethylene hepatotoxicity is also increased by ethanol, but rather high levels of solvent exposure are required to produce a response, even with prior ethanol treatment (*2*). The simultaneous ingestion of ethanol and trichloroethylene in man has been shown to alter the excretory pattern of metabolites of both chemicals depending upon the relative concentrations of the two solvents. Recent information

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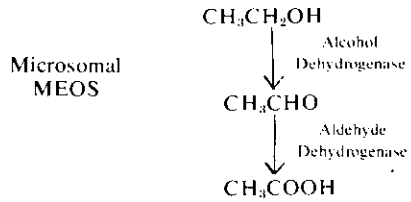
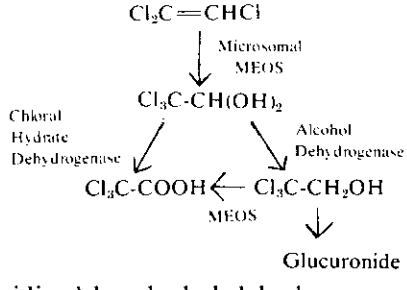
on these interactions *in vivo* have been provided by Müller et al. (*11*). Ethanol is metabolized [Eq. (1)] to acetaldehyde primarily by the action of alcohol dehydrogenase, and the acetaldehyde is rapidly converted to acetate. In addition, a microsomal ethanol oxidizing system (MEOS) has also been shown to function, particularly at levels of ethanol which may saturate the alcohol dehydrogenase system (*12, 13*). Whether or not this system is identical to the microsomal mixed function oxidase system is still the subject of considerable research, as in the *in vivo* significance of the system.



(1)

Trichloroethylene Metabolism

The metabolism of trichloroethylene [Eq. (2)] may involve an epoxide yielding chloral hydrate by way of a microsomal oxidizing system requiring DPNH and O_2 (*14, 15*). Chloral hydrate is subsequently oxidized by alcohol dehydrogenase to trichloroethanol or by chloral hydrate dehydrogenase to trichloroacetic acid. These enzymes are apparently not identical (*11*). The conversion of trichloroethylene to trichloroacetic acid by a microsomal system apparently also occurs. Since common metabolic pathways exist for trichloroethylene and ethanol it is apparent that several interactions may occur, with various effects, depending upon dose levels and the sequence of exposures. When simultaneous exposure occurs, substrate competition for both alcohol dehydrogenase and the microsomal enzyme system is possible. That such competition does, in fact, occur is consistent with the altered excretory pattern of trichloroethylene metabolites reported by Müller et al. (*11*) in individuals exposed to both trichloroethylene and ethanol. That trichloroethanol can inhibit the metabolism of ethanol is also suggested in man as reported by Stewart et al. (*16*). In individuals exposed to trichloroethylene vapor as little as 0.5 ml/kg of ethanol elicited "degreaser's flush," reaching in 30 min, a maximum vasodilation of superficial skin vessels primarily on the face, neck, shoulders, and back.



(2)

Alcohols as Potentiators of Toxicity

Cornish and Adefuin (*17*) have also reported that a wide variety of alcohols potentiate the hepatotoxicity of carbon tetrachloride when ingested 16 to 18 hr prior to the halogenated hydrocarbon exposure. Methanol, isopropanol, and secondary and tertiary butanols were better potentiators of carbon tetrachloride toxicity than ethanol, as measured by the elevation of serum glutamic-oxaloacetic transaminase (SGOT) levels. A comparison of *n*-butyl, isobutyl, *sec*-butyl, and *tert*-butyl alcohols given on an equimolar basis 16–18 hr prior to a 2.5 hr exposure to 1000 ppm of carbon tetrachloride resulted in SGOT levels of 707, 1380, 37100, and 48900 units/ml, respectively (Table 1). This exposure to CCl_4 alone did not alter SGOT levels (240 units). Normal and isobutyl alcohol are metabolized extensively while *sec*- and *tert*-butanol are more slowly metabolized and excreted. Traiger and Plaa have further investigated carbon tetrachloride potentiation by isopropyl alcohol (*18*). These data suggest that in addition to ethanol ingestion, mixed solvent exposures involving other alcohols and halogenated hydrocarbon solvents may be of industrial concern. Since in these studies ethanol was given 16–24 hr prior to solvent exposure, residual alcohol should be insignificant at the time of solvent exposure. Thus, under such conditions, with ethanol metabolism essentially complete, direct substrate competition is unlikely and other mechanisms must be considered. Evidence has now been published which indicates that both chronic and single exposures to ethanol can increase the activity of the liver microsomal mixed function oxidase system (*19, 20*).

Table 1. Effect of butyl alcohols on carbon tetrachloride hepatotoxicity.[a]

Alcohol	Dose, nmole/kg	CCl_4 (1000 ppm)[b]	SGOT, units/ml[c]
None	0	0	246 ± 20
None	0	+	241 ± 25
n-Butyl	20	+	707 ± 42
Isobutyl	20	+	1,380 ± 390
sec-Butyl	20	+	37,100 + 430
tert-Butyl	20	+	48,900 ± 470

[a]Data from Cornish and Adefuin (*17*).
[b]Vapor exposure 2.5 hr, at 16–18 hr after alcohol ingestion.
[c]Six animals per group.

Combined Effects of Phenobarbital and Ethanol on Several Halogenated Solvents

The effect of pretreatment with phenobarbital and/or ethanol on the toxicity of 0.2 ml/kg carbon tetrachloride is shown in Table 2. Phenobarbital and ethanol, given separately in the doses specified, potentiated the carbon tetrachloride-induced elevation in SGOT activity to approximately 1000 units/ml. SGOT activity was approximately 3500 units in the group given both inducers prior to CCl_4. Phenobarbital or ethanol alone produced no elevations of SGOT activity.

Table 2. Effect of phenobarbital and/or ethanol pretreatment on carbon tetrachloride-induced hepatotoxicity.

Phenobarbital (2 doses) 15 mg/kg[a]	EtOH, 5 ml/kg[b]	CCl_4, 0.2 ml/kg[c]	SGOT, units/ml
+	−	−	70 ± 4
+	+	−	63 ± 2
+	+	+	3,498 ± 690
+	−	+	1,148 ± 259
−	+	−	73 ± 7
−	+	+	1,050 ± 202
−	−	+	193 ± 60
−	−	−	74 ± 2

[a]As 15 mg/kg IP daily for 2 days prior to CCl_4, 4 to 6 rats per group.
[b]As 5 ml/kg PO, 18 hr prior to CCl_4.
[c]As 0.2 ml/kg in corn oil, IP, animals sacrificed 24 hr later.

Histological examination of livers from these animals showed that 0.2 ml/kg CCl_4 produced a mild centrolobular fatty infiltration with the formation of "balloon" cells, and a mild inflammatory response immediately surrounding the central veins. Pretreatment with phenobarbital and ethanol, individually or simultaneously, resulted in centrolobular necrosis usually involving one third to one half the lobule. Scattered balloon cells were seen at the margin of the necrotic area. Moderate fatty infiltration occurred throughout the necrotic area, and was sometimes intensified at the junction of necrotic and viable cells. While the difference in histologic appearance of the liver between groups receiving CCl_4 only and groups also given a potentiator was pronounced, it was not possible to distinguish the livers showing potentiation of hepatotoxicity according to the pretreatment given.

Data from a similar study with chloroform is given in Table 3. Chloroform (0.3 ml/kg) increased SGOT activity threefold. Ethanol pretreatment had no additional effect on SGOT activity. The lack of potentiation of ethanol pretreatment may be due to the relatively low dose of ethanol utilized in this study. Phenobarbital pretreatment caused a marked potentiation of chloroform toxicity. Pretreatment with ethanol did not alter the potentiating effect of phenobarbital pretreatment.

Table 3. Effect of phenobarbital and/or ethanol pretreatment on chloroform-induced hepatotoxicity.

Phenobarbital (2 doses) 15 mg/kg[a]	EtOH, 5 ml/kg[b]	$CHCl_3$, 0.3 ml/kg[c]	SGOT, units/ml
+	−	−	106 ± 2
+	+	−	102 ± 6
+	+	+	2172 ± 1645
+	−	+	2285 ± 1980
−	+	−	89 ± 6
−	+	+	210 ± 21
−	−	+	219 ± 49
−	−	−	78 ± 3

[a]As 15 mg/kg IP daily for 2 days prior to $CHCl_3$, 4 to 6 rats per group.
[b]As 5 ml/kg PO, 18 hr prior to $CHCl_3$.
[c]As 0.3 ml/kg in corn oil, IP, animals sacrificed 24 hr later.

A large IP dose of trichloroethylene (2.0 ml/kg) markedly increased SGOT activity (Table 4). Pretreatment with ethanol did not alter this response. Rats pretreated with either phenobarbital or phenobarbital and ethanol had a somewhat lower average SGOT activity than rats given only trichloroethylene, but the differences were not significant when the range of the individual values is considered. In similar studies, lower doses of trichloroethylene (1.0 and 0.5 ml/kg) produced smaller elevations in SGOT activity. Again, pretreatment with either phenobarbital or ethanol or both had no effect on this response.

Table 4. Effect of phenobarbital and/or ethanol pretreatment on trichloroethylene-induced hepatotoxicity.

Phenobarbital (2 doses) 15 ml/kg[a]	EtOH, 5 ml/kg[b]	Trichloroethylene, 2.0 ml/kg[c]	SGOT, units/ml
+	−	−	68 ± 1
+	+	−	67 ± 4
+	+	−	983 ± 126
+	−	+	797 ± 376
−	+	−	69 ± 2
−	+	+	1,160 ± 62
−	−	+	1,277 ± 339
−	−	−	79 ± 4

[a]As 15 mg/kg IP daily for 2 days prior to trichloroethylene, 4 to 6 rats per group.
[b]As 5 ml/kg PO, 18 hr prior to trichloroethylene.
[c]As 2.0 ml/kg in corn oil, IP, animals sacrificed 24 hr later.

The lack of potentiation of trichloroethylene toxicity in these studies may relate to the route of administration which was by intraperitoneal injection. A previous study (*2*) demonstrated a marked potentiation of trichloroethylene hepatotoxicity by prior ethanol treatment (Table 5). SGOT levels were increased from 232 units/ml to 4408 unit/ml in

Table 5. Effect of ethanol pretreatment on trichloroethylene-induced hepatotoxicity.[a]

Exposure			
Concn, ppm	Time, hr	Ethanol[b]	SGOT, units/ml
0	0	−	246 ± 20
		+	266 ± 32
2,000	4	−	210 ± 18
		+	283 ± 25
5,000	4	−	232 ± 16
		+	4408 ± 1810[c]
10,000[d]	1.5	−	4490 ± 975
		+	7953 ± 814

[a]Data from Cornish and Adefuin (*2*).
[b]Ethanol 5 g/kg oral dose.
[c]$p < 0.05$.
[d]Three of six rats in each group died.

ethanol-pretreated animals exposed for 4 hr to 5000 ppm trichloroethylene. At lower or higher exposure levels of trichloroethylene, this potentiating effect may not have been detected. Powis (*20*) has recently shown that, using a larger single dose of ethanol, aniline hydroxylation was significantly increased but aminopyrine demethylation was depressed in the hepatic microsomal fraction prepared 24 hr after ethanol ingestion. In addition, microsomal P-450, and NADPH-cytochrome c reductase and NADPH-cytochrome P-450 activities were unchanged by ethanol pretreatment. Powis also indicates that an attempt to increase the phenobarbital induction of aniline hydroxylation by simultaneous treatment with ethanol was unsuccessful. This is consistent with the SGOT data reported here for chloroform and trichloroethylene. Carbon tetrachloride, however, did produce an elevation of SGOT when animals were pretreated with both ethanol and phenobarbital. Whether or not this additional response may result from an effect of ethanol unrelated to microsomal enzyme induction is not apparent from the data available at this time.

Summary

We have reviewed the literature related to the effects of ethanol on halogenated hydrocarbon solvent hepatotoxicity with an emphasis on trichloroethylene exposures. Both phenobarbital and ethanol have been shown to increase the toxic response to carbon tetrachloride, chloroform, and trichloroethylene. In addition, a large number of other alcohols have the ability to potentiate the hepatotoxic response to carbon tetrachloride. Phenobarbital apparently exerts its effect by induction of the microsomal mixed function oxidase system with the resulting increase in the rate of production of "toxic metabolites." Although increased activity of the microsomal oxidase system may result from chronic ethanol ingestion, the potentiation of solvent toxicity seen following a single dose of ethanol apparently cannot be fully accounted for on the basis of the known effects of ethanol on the microsomal mixed function oxidase system.

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