EFFECT OF ETHANOL ON THE FATE OF VINYL CHLORIDE IN RATS

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January 20, 1977

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R&S 021536

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ABSTRACT

Ethanol pretreatment is known to alter the metabolism of many chemicals. Since it has been demonstrated previously that a single dose of ethanol inhibits the biotransformation of vinyl chloride (VC), the objective of this study was to investigate the effect of repeated and acute administration of ethanol on the fate of VC in rats. One group of rats was given 3.2 g/kg ethanol 0.5 hr prior to exposure of VC and another group was maintained on drinking water providing a daily dose of 11.4 g/kg ethanol for 22 days before exposure to VC. Subsequently, these rats and an untreated control group were exposed to an atmosphere containing 100 ppm ^{14}C-VC for 6 hours.

The rats pretreated repeatedly with ethanol showed a slight reduction in the total amount of VC metabolized (6%) and the degree of binding to hepatic macromolecules (26%) when compared to the group receiving no ethanol. In contrast, those pretreated acutely with ethanol showed a marked reduction in total metabolism (72%) and hepatic macromolecular binding (81%) when compared to controls. Similarly, repeated ethanol treatment did not affect markedly the routes or rates of excretion of ^{14}C-activity. However, associated with the reduction in overall metabolism of VC the acute ethanol treated rat excreted a larger proportion of the recovered radioactivity as expired VC than the VC exposed control

(13 versus 3%). It was concluded that repeated administration of ethanol for 22 consecutive days has little effect on the fate of VC in rats. In contrast, acute administration of ethanol markedly inhibits the metabolism of VC and subsequent covalent binding to hepatic macromolecules.

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INTRODUCTION

Ethanol has been shown to accelerate or inhibit the biotransformation of other chemicals depending on the dosage and time interval between the administration of ethanol and another chemical (Mezey, 1976; Liu, et al., 1975; Mallov and Baesl, 1972; Rubin, et al., 1970). Pretreatment of rats with a single dose of ethanol inhibits the biotransformation of vinyl chloride (VC) (Hefner, et al., 1975). This is particularly important because the biotransformation of VC to a reactive metabolite is believed to be responsible for its carcinogenic activity (Hefner, et al., 1975; VanDuuren, 1975; Bolt, et al., 1975). Data indicate that the hepatic mixed function oxidase (MFO) enzymes and glutathione-S-transferase enzymes are involved in the intoxification and detoxification of VC in vivo (Hefner, et al., 1975; Watanabe, et al., 1976 a,b; Bolt, et al., 1976). Furthermore, the biotransformation of VC has been shown to be induced by DDT, clotrimazol (Bolt, et al., 1976), AROCHLOR 1254 (Reynolds, et al., 1975) and inhibited by 3-bromophenyl-4(5)-imidazole, 6-nitro-1,2,3-benzothiadiazole (Bolt, et al., 1976) as well as ethanol.

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It has been established that ethanol is capable of inducing or inhibiting biotransformations mediated by the P-450 dependent MFO system; therefore this prompted the present study to determine the effects of ethanol on the fate of VC in rats. Metabolites of VC have been shown to bind covalently with hepatic macromolecules (Bolt, et al., 1975; Kappus, et al., 1976). It is thought that such macromolecular binding may relate to the carcinogenic effect of VC; therefore, emphasis was placed on determining the covalent binding of radioactivity to hepatic macromolecules following a single inhalation exposure to ^{14}C-labeled VC in rats pretreated with single or repeated doses of ethanol.

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METHODS

Material. Vinyl chloride (Matheson Gas Products) of 99.9% minimum purity was used throughout the study. ^{14}C-labeled VC was synthesized from (1,2 ^{14}C) 1,2-dichloroethane (New England Nuclear, Lot #819-292, 4.8 mCi/mmole) immediately prior to use (Wagner and Muelder, 1975). ^{14}C-VC synthesized in this manner has been reported to have a typical radiochemical purity of 95-96% (Wagner, et al., 1975). Non-labeled VC (Matheson Gas Products) was mixed with the ^{14}C-material to obtain the desired specific activity.

Animals. Male Sprague-Dawley rats (Spartan Research Laboratory) initially weighing 150-165 g were used in the study. All animals were housed in rooms in which a constant humidity, temperature, and 12 hour light-dark cycle (7 AM-7 PM, EDT) were maintained.

Ethanol was administered in the drinking water (10% v/v) to a group of 4 rats for 22 consecutive days. The ethanol-water was removed and replaced with tap water 18 hours prior to exposure to VC. The average amount of ethanol consumed was 11.4 g/kg/day. Another group of 4 rats received 3.2 g/kg ethanol by intraperitoneal injection of a 30% aqueous solution one-half hour prior to exposure to VC. These groups

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will be referred to respectively as repeated and acute ethanol pretreated. An additional 4 rats not treated with ethanol but exposed to VC served as the exposure control group.

Exposure. All rats were exposed under dynamic conditions to a concentration of 100 ppm ^{14}C-VC in a 30 l glass inhalation chamber. The exposure was conducted from 2:15 to 8:15 AM. VC was metered into the chamber air flow (~6 l/min) from a Saran bag with a dual syringe pump. The concentration of VC was monitored continuously by recirculating a fraction of the chamber atmosphere through a gas cell of an infrared spectrophotometer (Wilks) calibrated at a wavelength of 10.6 µm. The chamber atmosphere was also analyzed at hourly intervals by gas chromatography. Samples for ^{14}C-activity determinations were taken hourly by bubbling 1 ml aliquots of the chamber atmosphere into a scintillation solution containing Concifluor (Mallinckrodt Chemical), 2-methoxy-ethanol, toluene (6:11:83) (Watanabe, et al., 1976a). Radioactivity was determined by liquid scintillation spectrometry. The mean analytical concentration of VC was 102 ± 1 ppm (SD). The specific activity was 897 dpm per microgram VC.

The inhalation chamber was operated in a laboratory fume hood to prevent contamination of the working environment. After transit through the inhalation chamber ^{14}C-VC was adsorbed on activated charcoal. These traps were disposed of as radioactive waste according to standard regulations.

Procedure. A total of twelve rats (4/ethanol treatment group and 4 not given ethanol) were exposed to 100 ppm ^{14}C-VC for 6 hours as described previously. Following the exposure one rat per group was placed in a glass Roth-type metabolism cage for the collection of urine, feces and expired air. Room air was drawn through the cages at 400-500 ml/min. The air leaving the chamber was passed first through a glass tube containing about 40 g of Drierite (W. A. Hammond Drierite Co.) to remove moisture. Subsequent transit through a series of two cold finger traps containing 50 ml of toluene, 2-methoxyethanol (80:20), and a single trap containing 120 ml of 5 M ethanolamine in 2-methoxyethanol enabled the collection of ^{14}C-VC and $^{14}CO_2$, respectively. The cold finger traps were immersed in 2-methoxyethanol, dry ice baths throughout the collection periods. The trap for CO_2 was maintained at room temperature.

Samples of excreta were collected for 48 hours after termination of exposure and analyzed for ^{14}C activity. Expired VC was collected at 0.5 hr intervals for 4 hr; the CO_2 trap and urine receptacle (immersed in dry ice bath) were changed at 12 hr intervals for 48 hr; and feces were collected every 24 hr. At the termination of the study (48 hr) the animals were killed by a blow to the head, and the liver was collected

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for analysis of ^{14}C activity. The remaining carcass was skinned, homogenized (50% w/v) in distilled water, and analyzed for ^{14}C activity. Samples of excreta and tissue (liver and skin) were prepared for scintillation counting as described previously (Watanabe, et al., 1976a).

Radioactivity was determined by counting in a Mark II or Mark III (Searle Analytic, Inc.) liquid scintillation spectrometer. External standard channel ratios were used to determine the counting efficiency. Counts per minute were converted to disintegrations per minute using a standard quench curve.

The remaining nine rats (3/group) were killed by a blow to the head immediately following exposure. A piece of liver tissue (1 g) was sampled and used for determining macromolecular binding of radioactivity by the method of Jollow, et al., (1974). The carcass, liver, and skin were analyzed for total radioactivity as described above.

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RESULTS

The total metabolism of VC and radioactivity bound to hepatic macromolecules following repeated and acute pretreatment with ethanol compared to non-pretreated exposed rats is summarized in Table 1. The rats pretreated repeatedly with ethanol showed a slight reduction in total metabolism and macromolecular binding, 6 and 26% respectively. However, those pretreated acutely with ethanol showed a marked reduction of 72% in total metabolism and 81% in covalent binding to hepatic macromolecules when compared to the non-pretreated VC exposed controls.

There was no change in the percentage of recovered radioactivity excreted by the various routes in rats which received ethanol repeatedly (Table 2). In contrast, there was an increase in the percentage of VC per se expired by the rat given an acute dose of ethanol which is consistent with the reduced metabolism observed previously. Since acute ethanol pretreatment inhibited metabolism of VC, there was also a reduction in the total amount of radioactivity recovered in excreta. The predominant route of excretion of the metabolized VC in all cases was the urine. Separation of radioactivity in the urine by high pressure liquid

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chromatography on a ZIPAX column (E.I. duPont Company) with a linear gradient of distilled water to 100% acetate buffer (10% acetic acid, 1% pyridine) showed no qualitative differences in the profile of radioactive urinary metabolites between any of the groups.

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DISCUSSION

The results clearly indicate that repeated treatment with ethanol for 22 days had little effect on the fate of VC in rats exposed to 100 ppm VC. The slight reduction in total metabolism and hepatic macromolecular covalent binding observed following repeated ethanol treatment was likely due to residual ethanol remaining in the body since acute ethanol administration markedly depressed these parameters. It could be suggested that 22 days of pretreatment was insufficient time to induce changes which might alter the metabolism of VC. However, Liu, et al., (1975) showed that as little as 10 days ethanol treatment was sufficient to cause an increase in liver P-450 dependent aniline hydroxylase and a decrease in benzphetamine N-demethylase activity. The duration of treatment in the present study is not long enough to cause marked changes in lipid metabolism which may lead to fatty infiltration and ultimately to cirrhois of the liver; grossly the livers of these animals appeared normal.

Presumably the reduction in hepatic macromolecular binding in the presence of ethanol would occur whether the intake were on a repeated or acute basis as long as ethanol remained in the body. If the reduction in total metabolism and covalent binding is a true index of activation, this suggests that ethanol consumption may inhibit the toxicity including carcinogenicity of VC. However, it is likely that

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if repeated ethanol consumption had led to chronic liver disease there may be an increase in the sensitivity of the liver to an additional insult by a hepatotoxic or carcinogenic chemical. A study is currently in progress designed to elucidate the synergistic or antagonistic effects of repeated ethanol treatment on the carcinogenicity of VC. Our data suggest that ethanol pretreatment immediately prior to or concurrent with exposure to VC may inhibit cancer induction.

The marked inhibition of VC biotransformation by acute ethanol treatment supports our previous observation of inhibition of the metabolic uptake of VC when exposed by inhalation (Hefner, et al., 1975). It was shown in these earlier studies that the rate of uptake of VC, presumed to be due to metabolism, was slower at atmospheric concentrations between 220-1000 ppm than at levels below 100 ppm. Since ethanol treatment inhibited the metabolic uptake to a greater extent at concentrations less than 100 ppm, it was concluded that several pathways were involved in the biotransformation of VC which were concentration dependent. At that time it was postulated that alcohol dehydrogenase and the MFO enzymes were involved in VC metabolism. Although both of these enzymes would be inhibited by ethanol, subsequent studies failed to confirm the involvement of alcohol dehydrogenase.

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After identification of cysteine conjugates as the major metabolites of VC the prevailing conclusion was that P-450 dependent MFO and glutathione transferase enzymes were the primary enzymes involved in VC metabolism (Watanabe, et al., 1976a).

Of particular importance is the present observation that the percent radioactivity covalently bound to macromolecules versus the total radioactivity in the liver was markedly reduced in the acute ethanol pretreated rats. The radioactivity in the liver primarily represents metabolites of VC. Therefore, if ethanol inhibited only the primary activation step, the percent covalently bound versus total metabolized should be the same for all treatments. The reduction in this parameter with acute ethanol pretreatment lends additional support to the previous conclusion that VC is metabolized by several pathways. The ethanol sensitive pathway may be mediated by the hepatic microsomes. This is consistent with the observed reduction in macromolecular binding following acute pretreatment with ethanol. The second pathway may invole the direct reaction of VC with glutathione via one of the glutathione transferases. This pathway would be influenced less by ethanol. The intracacies of the metabolism of VC are understood poorly and an important

aspect for future study will be to elucidate the relative rates of the metabolic pathways leading to intoxification and detoxification. However, the key to unraveling the mechanism of action of VC lies not only with elucidating its metabolic pathways but also with its reaction with critical macromolecules.

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TABLE 1

TOTAL METABOLISM AND HEPATIC MACROMOLECULAR BINDING FOLLOWING ETHANOL PRETREATMENT AND A SINGLE 6 HR EXPOSURE TO 100 ppm VINYL CHLORIDE[a]

Pretreatment	A µg VC equivalents metabolized	B µg VC equivalents bound per g protein	B/A x 100	Percent Bound[b]
None (exposed controls)	3069±304	66.6±8.1	2.17±.05	22±1
Chronic ethanol[d]	2872±70	49.4±2.9[c]	1.72±.12[c]	17±2[c]
Acute ethanol[e]	859±635[c]	15.5±5.0[c]	1.33±.07[c]	10±0[c]

[a]All values are means ± standard deviations, 3 rats/group except for acute ethanol group where only 2 observations were obtained for binding and related parameters because binding in one animal was below the detection limit.

[b]µg VC equiv. bound per g liver/total µg equiv. VC determined per g liver X 100.

[c]Statistically significantly different from exposed controls, Student t-test ($p < 0.05$).

[d]Ethanol administered in the drinking water (10% v/v) at an average dose of 11.4 g/kg/day for 22 consecutive days; ethanol was withdrawn 18 hours before exposure to VC.

[e]Ethanol administered by ip injection at a dose of 3.2 g/kg, 0.5 hour before exposure to VC.

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TABLE 2

PERCENTAGE ^{14}C-ACTIVITY ELIMINATED DURING 48 HOURS FOLLOWING A SINGLE 6 HOUR INHALATION EXPOSURE TO 100 ppm ^{14}C-VINYL CHLORIDE [a]

	Pre-Treatment					
	None		Chronic Ethanol[b]		Acute Ethanol[c]	
Expired:	(%)	(µg equiv. VC)	(%)	(µg equiv. VC)	(%)	(µg equiv. VC)
as VC	2.46	(86)[b]	2.78	(88)	13.10	(110)
as CO_2	11.46	(400)	12.15	(384)	14.88	(125)
Urine	59.21	(2067)	62.10	(1963)	53.69	(451)
Feces	7.68	(268)	5.35	(169)	5.71	(48)
Carcass & Tissue	19.19	(670)	17.62	(557)	12.62	(106)
Total µg equivalents VC recovered		(3491)		(3161)		(840)

[a] Expressed as percentage of the total radioactivity recovered, one rat per treatment.

[b] Ethanol was administered in the drinking water (10% v/v) at an average dose of 11.4 g/kg/day for 22 consecutive days; ethanol was withdrawn 18 hours before exposure to VC.

[c] Ethanol was administered by ip injection at a dose of 3.2 g/kg, 0.5 hour before exposure to VC.

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