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VINYL CHLORIDE

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ABSTRACT

Covalent binding of radioactivity to hepatic macromolecules in rats exposed to ^{14}C -labeled vinyl chloride (VC) was studied to determine if VC induced carcinogenesis may be related to electrophilic alkylation of macromolecules in vivo. Male Sprague-Dawley rats were exposed to 1, 10, 25, 50, 100, 250, 500, 1000 or 5000 ppm ^{14}C -VC for 6 hours. Following exposure radioactivity covalently bound to hepatic macromolecules and purified nucleic acids (RNA, DNA) were determined. The total amount of ^{14}C -VC metabolized and hepatic glutathione (GSH) content was also determined. The total amount of radioactivity bound to macromolecules in the liver did not increase proportionately with the increase in the exposure concentration of VC. A disproportionate decrease in macromolecular binding was observed as the concentration of VC increased. The covalent binding to hepatic macromolecules was related to the amount of VC metabolized. At exposures greater than 50 ppm, the amount of ^{14}C bound to macromolecules in the liver correlates with induction of hepatic angiosarcoma. There was no preferential binding of radioactivity to either DNA or RNA in the liver. Hepatic glutathione content was significantly depressed only at exposure concentrations greater than 100 ppm.

INTRODUCTION

Considerable effort has been devoted to research on vinyl chloride (VC) since it was demonstrated to be carcinogenic in man and animals (Creech and Johnson, 1974; Maltoni and Lefemine; 1975). The concept of a reactive metabolite of VC being responsible for the carcinogenic activity (Hefner, et al., 1975; Van Duuren, 1975) is supported by evidence of enhanced mutagenic activity of VC to bacteria in the presence of microsomal enzyme activating systems (Bartsh, et al., 1975; Malavielle, et al., 1975; Rannug, et al., 1974). Metabolites of VC have been identified in the urine of rats as conjugates of cysteine (Green and Hathway, 1975; Watanabe, et al., 1976a), suggesting that VC is biotransformed to electrophilic metabolites and that the primary detoxification mechanism for these metabolites is conjugation with hepatic glutathione prior to excretion. Complimentary to these data was elucidation of a dose-related reduction of hepatic glutathione in rats exposed to 50-2000 ppm VC for 7 hours (Watanabe, et al., 1976b). It was therefore hypothesized that reactive metabolites formed during exposure to low levels of VC (less than 50 ppm) will be readily detoxified by reaction with glutathione. However, as the exposure is increased, detoxification will be impaired by the reduction of hepatic glutathione. This will lead to an increase in the level of reactive metabolites and result in an increased reaction of these metabolites with intracellular macromolecules.

METHODS

Material. ^{14}C -labeled VC was synthesized from (1,2- ^{14}C) 1,2-dichloroethane (New England Nuclear, Lot #819-221 and 819-292, 5.0 and 4.8 mCi/mmmole, respectively) immediately prior to use (Wagner and Muelder, 1975). The synthesized ^{14}C -VC has been reported to be 95-96% radiochemically pure (Wagner, et al., 1975). Non-labeled VC (Matheson Gas Products) of 99.9% minimum purity was mixed with the ^{14}C -material to obtain the desired specific activity. Typically, 40 ml of the ^{14}C -VC, helium gas mixture was injected into a 5-10 liter Saran bag (Ansbec, Inc.) containing the desired quantity of non-labeled VC.

Animals. Male Sprague-Dawley rats (Spartan Research Laboratory) weighing 220-250 g were used throughout the study. All animals were housed in rooms in which a constant humidity, temperature and a 12 hour light-dark cycle (7 AM - 7 PM, EST) were maintained. Food and water were provided ad libitum except during exposure. Exposures were conducted between 9:00 AM and 4:00 PM. Groups of rats (3-6) were exposed to 1, 10, 25, 50, 100, 250, 500, 1000, or 5000 ppm ^{14}C -VC for 6 hours. Control animals used for hepatic nonprotein sulfhydryl determinations (5/group) were exposed concomitantly to room air. An additional group of 5 rats pretreated with phenobarbital (80 mg/kg/day, ip, 3 days prior to exposure) were exposed at the 100 ppm level.

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

Procedure. Following the 6 hour exposure to varying concentrations of ^{14}C -VC (1-5000 ppm) the rats were killed immediately by a blow to the head. An aliquot of liver was sampled and used for determining hepatic nonprotein sulfhydryl content by a modification of the method of Sedlak and Lindsay (1968). The remaining liver was frozen immediately on dry ice and stored at -20°C until analyzed. The carcass was analyzed for total radioactivity as described previously (Watanabe, et al., 1976a). The radioactivity determined in the tissue and carcass was non-volatile, therefore this radioactivity represented the total amount of metabolized VC. Protein binding of ^{14}C -labeled VC to hepatic tissue was determined by the method of Jollow, et al., (1973). Hepatic nucleic acids (DNA and RNA) were isolated from the 1, 100, 250, and 1000 ppm exposure groups and radioactivity was determined by direct counting of the aqueous fractions by liquid scintillation spectrometry (see below).

Isolation of Nucleic Acids. RNA and DNA were isolated from rat liver by modification of the techniques described by

precipitated by addition of sodium acetate (4 g/100 ml) and 2 volumes 2-ethoxyethanol. The precipitate was redissolved in 10 ml 0.015M sodium chloride-0.0015M sodium citrate at 2°C. The final DNA precipitation was accomplished using 5 ml ice cold isopropanol. RNA and DNA pellets were dried at 2°C under reduced pressure and weighed. RNA and DNA were quantified by the orcinol and diphenylamine reactions respectively (Keleti and Lederer, 1974).

Hepatic non-protein sulfhydryl content (primarily GSH) was not depressed significantly at 1, 10, 25 or 50 ppm. Only at concentrations of 100 ppm or greater was a dose-related depression of hepatic GSH evident. Metabolism of VC was not increased in rats exposed to 100 ppm VC after pretreatment with phenobarbital. Macromolecular binding, however, was increased markedly when compared to non-pretreated animals.

Isolation of RNA and DNA by a non-digestive procedure from the liver of animals exposed to 1, 100, 250, and 1000 ppm VC failed to reveal any detectable radioactivity. The sensitivity was such that if the hepatic radioactivity was distributed uniformly by weight throughout the tissue, measureable radioactivity would have been detected in the isolated nucleic acids.

macromolecules (Miller and Miller, 1971). The covalent binding to hepatic macromolecules reflects the activation of VC to reactive metabolites. Therefore, both total metabolism of VC and covalent binding appear to correlate with the induction of hepatic angiosarcoma in rats exposed to concentrations greater than 50 ppm.

The toxicologic significance of the metabolism and covalent binding of VC at levels below 50 ppm is not clear. Deviation in these parameters from the log linear relationship at higher levels indicates that the carcinogenic response of the population may be changed at lower level exposures. However, this hypothesis can not be validated until the results of carcinogenesis bioassays currently being conducted at 25, 10, and 1 ppm become available.

The reactive metabolites of VC are detoxified presumably by reaction with GSH (Watanabe, et al., 1976b). Therefore, it is important that significant depression of hepatic GSH was dose-related only at levels of 100 ppm and greater. This is consistent with previous studies which showed that a single 50 ppm exposure to VC was in the threshold zone for depression of hepatic GSH (Watanabe, et al., 1976b). This suggests that carcinogenicity of VC is related to the decreased ability to detoxify the reactive metabolites of VC.

Both our results and that of Bolt, et al., (1976a) confirm the conclusion that VC does not preferentially react with hepatic nucleic acids. It is important to emphasize that the methodologies employed only detect covalent binding and exclude any other more subtle interactions. Therefore, a very small degree of covalent binding to nucleic acids does not exclude the possibility of other interactions which result in the loss of the ability to control cellular replication.

Pretreatment with phenobarbital did not increase the total metabolism of VC. Bolt, et al., (1976b) has reported similar data showing that phenobarbital pretreatment failed to stimulate the uptake of VC following a static inhalation exposure. Although total metabolism was not affected by phenobarbital, the protein binding was increased markedly. The true significance of the increased protein binding is difficult to interpret since phenobarbital increases total protein in the liver, and the increase in binding may reflect a nonspecific interaction merely due to an increase in the available protein binding sites.

In summary, the results of the studies reported herein do not associate the carcinogenic effect of VC with a disproportionate increase in binding of electrophilic metabolites of

The hepatocyte may constitute primarily a means for detoxification since they are not particularly susceptible to VC induced toxicity. Toxicity may be induced in tissues with smaller capacity to detoxify the reactive metabolites of VC. The induction of tumors of the nervous system by ethylnitrosourea has been correlated with the persistence of O-6-ethylguanine in the nervous system (Goth and Rajewsky, 1974). Although other metabolizing organs such as the liver produce O-6-ethylguanine the turnover rate of DNA mediated by repair mechanisms is sufficient to prevent induction of cancer. Likewise, the mechanism of carcinogenesis of VC may be due to an inability of the target tissue, in this case endothelium, to repair lesions whether the reaction be with nucleic acids or critical proteins.

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TABLE 1

Total Metabolism, Hepatic Macromolecular Binding and Hepatic Glutathione (GSH) Levels
Following Inhalation Exposure (6 Hours) To Vinyl Chloride (VC)^a

Nominal Conc.	A	B	B/A x 100	Hepatic GSH (% Control)	Percent of Total ¹⁴ C-Activity in Liver Bound to Macromolecules
	µg VC Equivalents Metabolized	µg VC Equivalents Bound Per g Protein			
1	29.8±3.2	0.5±0.08	1.78±0.48	104	20±3
10	242±26	3.3±0.2	1.38±0.36	89	21±2
25	557±42	12.2±4.0	2.15±0.60	93	21±2
50	1,181±93	23.3±3.4	1.99±0.36	94	20±2
100	2,406±173	47.6±4.8	1.99±0.26	81 ^b	25±2
250	3,826±345	89.6±12.3	2.35±0.28	70 ^b	22±2
500	6,263±355	98.8±5.0	1.58±0.20	60 ^b	25±3
1000	4,257±765	106.8±22.2	2.55±0.58	51 ^b	22±2
5000	9,255±1,467	113.5±10.4	1.12±0.13	39 ^b	22±3
pretreatment with phenobarbital ^c					
100	2,160±166	80.0±23.9	3.70±0.82		39±3

^a Means ± standard deviation

^b Statistically different from controls, Student t-test (p < 0.05)

^c Rats were injected ip with sodium phenobarbital, 80 mg/kg for 3 days prior to exposure

LEGEND

Figure 1 Hepatic protein binding and glutathione (GSH) depression expressed as a function of the logarithm of the exposure concentration to vinyl chloride (VC). Protein binding (●) μg equivalents VC bound per g protein (mean $\pm$ S.D.) GSH (▲) percent of control.

FIGURE 1

