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VINYL CHLORIDE-INDUCED DEPRESSION OF HEPATIC NON-PROTEIN SULFHYDRYL CONTENT AND EFFECTS ON BROMOSULPHALEIN (BSP) CLEARANCE IN RATS

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SUMMARY

Rats were exposed to atmospheres of 2000, 1000, 250, 150, 50 and 10 ppm vinyl chloride (VC) for 1—7 h to determine the effect of VC on the hepatic non-protein sulfhydryl content. Exposure to 2000, 1000, 250 and 150 ppm VC caused a progressive depression of the hepatic non-protein sulfhydryl content. Following exposure to 50 ppm VC for 7 h the depression was inconsistent, and no depression was observed after 10 ppm VC for 7 h. Also, exposure to 1000 ppm VC did not alter the serum clearance of bromosulphalein (BSP).

INTRODUCTION

In 1971, Viola et al. [1] reported that tumors of the skin, lung and bone developed in rats exposed to an atmosphere containing 30 000 ppm VC for 4 h per day, 5 days per week for 12 months. More recently, Maltoni and Lefemine [2] reported angiosarcomas of the liver in rats exposed to 10 000, 6000, 2500, 500, 250 and 50 ppm VC, 4 h per day, 5 days per week for 12 months and subsequently maintained and observed until death. VC has also been associated with the development of angiosarcoma and portal cirrhosis of the liver as well as other untoward effects in workers exposed to unknown but undoubtedly high concentrations of VC [3,4].

Reduction in the level of hepatic non-protein sulfhydryl content (glutathione and cysteine) have been observed in rats exposed to 50 ppm VC for 7

Abbreviations: BSP, bromosulphalein; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); VC, vinyl chloride.

h, 500 and 5000 ppm for 1 and 3 weeks, and 15 000 ppm for 1 week [5]. No definitive association between exposure concentration and degree of depression was found. Protein-bound hepatic sulfhydryl content of rats was unaffected by exposure to VC.

Numerous studies have been reported which relate an increase in toxicity to a reduction in the non-protein sulfhydryl content of the liver. Hayes et al. [6] reported a correlation between the single dose lethality of monochloroacetic acid and depression of the non-protein sulfhydryl content of the liver and kidney of rats. Jaeger et al. [7] reported a depression of the non-protein sulfhydryl content in rats exposed to 1,1-dichloroethylene. Also demonstrated was an increased lethality in rats whose hepatic non-protein sulfhydryl content had been reduced by starvation for 16 h prior to exposure to 1,1-dichloroethylene.

Other studies demonstrate a direct correlation between the in vivo concentration of non-protein sulfhydryl and the degree of whole body protection from various alkylating agents and radiation. For example, administration of glutathione or cysteine provides protection against the untoward effects of various aliphatic and aromatic mustards, triethylenemelamine, X-rays and ionizing radiation [8—12]. Therefore, it is likely that glutathione and cysteine may provide a natural defense against reactive agents generated within the body, as well as synthetic or naturally occurring alkylating agents which are absorbed into the body. Therefore, reduction of the hepatic non-protein sulfhydryl content in animals exposed to VC may constitute predisposition to toxicity and carcinogenicity mediated either by VC per se, or its metabolites.

Kramer and Mutchler [13] reported the decreased clearance of BSP in workers exposed to VC. Since BSP clearance involves conjugation with glutathione, a decrease in glutathione due to exposure to VC may have been responsible for the altered clearance of BSP.

The experiments described herein were undertaken to evaluate the effect of acute inhalation exposure to VC on non-protein sulfhydryl content of the livers of rats.

METHODS

Animals. Male Sprague-Dawley rats weighing from 200—350 g purchased from Spartan Research Inc., were used throughout the studies. The rats were acclimated in rooms maintained at constant temperature, humidity, with a 12 h light/dark cycle (8 a.m. to 8 p.m., Eastern Standard Time). Food and water were withheld during exposure, otherwise they were available ad libitum.

Exposure techniques. Groups (n = 5) of rats were exposed to mean analytical concentrations (± standard deviation) of 2000 (± 100), 1000 (± 100), 250 (± 5), 150 (± 5), 50 (± 2) or 10 (± 1) ppm VC for 1 to 7 h. Groups (n = 5) of control rats were exposed simultaneously to ambient room air. Dynamic inhalation exposures were carried out in a glass-walled 160-liter chamber.

The desired VC concentration in the chamber was achieved by metering VC gas (99.9% purity Matheson Gas) at a controlled rate into the chamber airstream (30 l/min). The chamber concentration of VC was continuously monitored by recirculating through a Miran I infra-red spectrophotometer (Wilks) set at 10.6 or 10.9 μ. The infra-red spectrophotometer was calibrated before and after each exposure with standards prepared in 100-liter Saran bags.

Variation of hepatic non-protein sulfhydryl levels. Groups (n = 5) of control male rats were sacrificed between 9:30 a.m. and 2:00 p.m. Eastern Standard Time. Livers were removed and assayed for non-protein sulfhydryl content as described below. A plot of the non-protein sulfhydryl content versus time established the extent of variation in rats (Fig. 1). Based on the variation curve, exposures to VC were conducted such that all control and exposed rats were sacrificed between 9:30 and 10:30 a.m. Eastern Standard time.

Non-protein sulfhydryl assay. The method used for the non-protein sulfhydryl assay was a modification of that described by Sedlak and Lindsay [14]. Throughout the duration of the assay, all samples were chilled in an ice bath. A sample of liver (300 mg) was homogenized in 8.0 ml of 0.02 M disodium EDTA for 1 min using a Brinkman Polytron tissue homogenizer. The non-protein sulfhydryl content was determined after precipitating the protein by addition of 1 ml of 50% trichloroacetic acid to a 5-ml sample of liver homogenate. Each sample was diluted with 4 ml of distilled water and after 15 min centrifuged at 4000 *g*. A 2-ml aliquot of each supernatant was mixed with 4 ml of 0.4 M Tris—HCl buffer (pH 8.9). Immediately before reading the absorbance (412 nm), 0.1 ml of 0.01 M DTNB was added. The molar concentration of non-protein sulfhydryl groups in the sample was calculated using a molar extinction coefficient determined from standards of

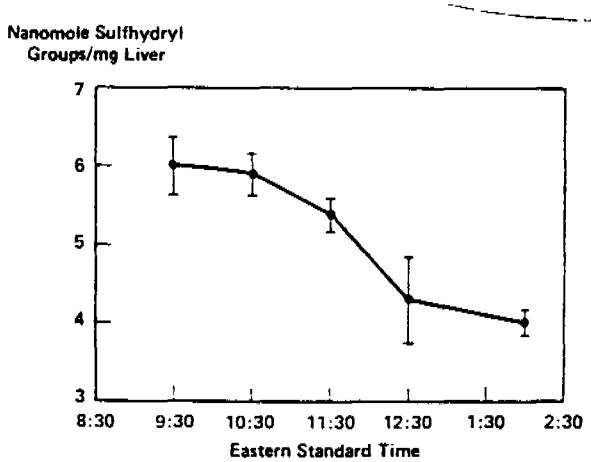

Fig. 1. Variation in hepatic non-protein sulfhydryl content versus time of day (9:30 a.m. — 2:30 p.m. Eastern Standard Time). Each point represents the mean ± standard error of 5 animals.

known concentrations of glutathione and cysteine. A plot of absorbance versus concentration of glutathione or cysteine coincided with that reported by Sedlak and Lindsay [14].

BSP retention time. Groups (n = 5) of male rats were exposed for 7 h to 1000 ppm VC. Control rats were exposed simultaneously to ambient room air. Immediately after exposure, each exposed and control rat was injected with 20 mg/kg BSP solution in the caudal vein. Whole blood was collected from the orbital sinus at 5 and 30 min following BSP injection. A 0.25-ml aliquot of serum was prepared and added to 0.1 ml of 2.5 N NaOH plus 4.65 ml physiological saline. The absorbance of each sample was read at 575 nm against a respective reagent blank. The molar concentration of BSP in each sample was calculated using a molar extinction coefficient determined from standards of known concentrations of BSP.

RESULTS

Effect of VC exposure on rat liver non-protein sulfhydryl content

The percent depression of hepatic non-protein sulfhydryl content as a function of time for rats exposed to 2000, 1000, 250, 150, 50 or 10 ppm VC is shown in Fig. 2. Percent depression was calculated as follows:

$$\text{Percent Depression} = 100\left(1 - \frac{\text{Treated}}{\text{Control}}\right)$$

Statistically significant differences between treated and control groups were

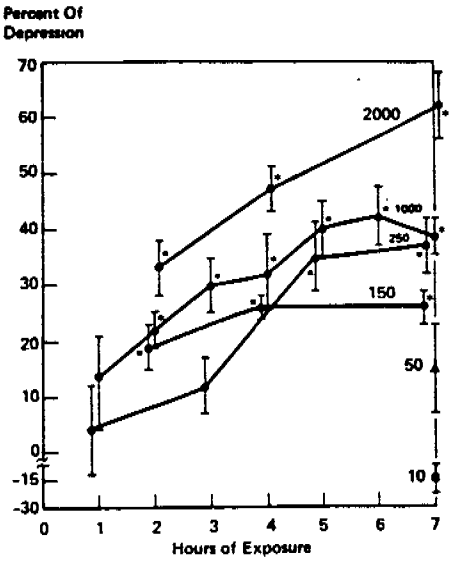

Fig. 2. Percent depression of hepatic non-protein sulfhydryl content versus duration of exposure to 2000, 1000, 250, 150, 50 and 10 ppm VC. Each point represents the mean ± standard error of 5 animals except the point for 50 ppm which represents 25 animals. *Statistically different from controls ($p < 0.05$).

TABLE I

SERUM BSP CLEARANCE FOLLOWING EXPOSURE TO VINYL CHLORIDE

Rats (3—5 per group) were exposed to 1000 ppm VC for 7 h. BSP was administered intravenously immediately following exposure, and the serum concentration was determined after 5 and 30 min. The values represent the mean ± standard deviation.

	Serum BSP Concentration (mg %)	
	5 min	30 min
Control	29.0 ± 4.3	2.3 ± 0.1
Vinyl chloride exposed	26.7 ± 1.3	2.2 ± 0.2

determined by using the Student t test ($p < 0.05$). Exposure to 2000 ppm VC resulted in a progressive depression of hepatic non-protein sulfhydryl content reaching 33% within 2 h, 47% after 4 h, and 62% after 7 h. Following 1000, 250 and 150 ppm VC an apparent maximum depression for each level was observed after 4—5 h of exposure. The depression after 7 h of exposure to 50 ppm VC was inconsistent. This is reflected in the large standard error (refer to Fig. 2). The 50 ppm exposure was run 5 times, and only once the values of the treated rats were found to be statistically significantly depressed from the controls. No depression of the hepatic non-protein sulfhydryl content was observed in rats exposed to 10 ppm VC for 7 h.

Effects of VC exposure on BSP retention time

Concentrations of serum BSP determined at 5 and 30 min after intravenous injection in rats exposed for 7 h to an analytical concentration of 1000 ppm VC are shown in Table I. Clearance of BSP was not altered by exposure to 1000 ppm VC for up to 7 h.

DISCUSSION

In the study reported herein, the depression of hepatic non-protein sulfhydryl content in rats exposed to various concentrations of VC has been characterized. Exposure to 2000 ppm VC for 2—7 h resulted in a progressive depression attaining a maximum value of 62% at 7 h. Following exposure to 1000, 250 or 150 ppm VC the hepatic non-protein sulfhydryl content was progressively depleted through 4—5 h. However, further depression was not evident with continued exposure from 5—7 h. This plateau effect is very likely due to a compensatory synthesis of glutathione (the predominant non-protein sulfhydryl compound in the liver) which has a rapid half-life of 1.75—4 h [15—17]. Indications of a compensatory synthesis of hepatic non-protein sulfhydryl groups as a result of exposure to VC has been observed previously [5]. These results indicate that reactive metabolites of

VC are formed which react with hepatic non-protein sulfhydryl groups. The inconsistent depression following 7-h exposures to 50 ppm VC, and the absence of any depression in rats exposed for 7 h to 10 ppm VC, indicates that a threshold for depression must exist between 10 and 50 ppm VC. Of particular significance is the relationship of these findings to the carcinogenesis of VC. In the studies of Maltoni and Lefemine [2], the reported incidence of angiosarcoma of the liver in rats exposed 4 h per day, 5 days per week to 6000 and 2500 ppm VC was 22%. After exposure to 500, 250 and 50 ppm VC tumor incidence decreased with respective values of 12, 7 and 5%. Reference to Fig. 2 suggests that after 4 h of exposure the depression of the hepatic non-protein sulfhydryl content was dose-related. The lower incidence of angiosarcomas in rats exposed to 500, 250 or 50 ppm VC coincides with a reduced degree of depression in the hepatic non-protein sulfhydryl content. The absence of depression in rats exposed to 10 ppm even after 7 h suggests that this level of exposure may not induce tumors. Additional experiments have been initiated to determine whether rats exposed to 25, 10 or 5 ppm VC develop tumors [18]. At least until the results of these latter experiments become available, it appears that there is a very good correlation between the depression of the non-protein sulfhydryl content of the liver and the induction of hepatic tumors.

Gillette [19] has recently summarized the role of chemically reactive metabolites of foreign compounds in toxicity. He and his coworkers have demonstrated that halogenated benzenes, in particular bromobenzene, as well as some other hepatotoxins do not cause hepatic necrosis until the doses administered are sufficiently large to overwhelm the detoxification pathways for the reactive metabolites of these compounds. Conjugation of the reactive alkylating metabolites with glutathione is one of the primary mechanisms of detoxification. Necrosis is induced only when the glutathione levels of the liver have been depressed sufficiently to allow the reactive metabolites to combine with different kinds of intracellular macromolecules — proteins, lipids, glycogen, DNA and RNA.

In a subsequent paper Gillette [20] presents theoretical pharmacokinetics for the covalent binding of the reactive metabolites of bromobenzene and other compounds to glutathione and macromolecules. In this paper, he illustrates how the rate of formation and detoxification of the reactive metabolites of foreign compounds may play a key role in toxicity. Depletion of glutathione, if involved in the detoxification process, would not only allow the reactive metabolites to combine with macromolecules but would very likely induce a change in the overall pharmacokinetics of the foreign compound. Although Gilette and coworkers have not yet attempted to correlate the covalent binding of the reactive metabolites to DNA with carcinogenesis, such a reaction has been speculated as a potential mechanism for carcinogenesis [21].

In previous studies, it has been demonstrated that VC is readily metabolized in rats exposed via inhalation [5] and ingestion [22]. In the former study, it was also found that repeated exposure of rats to atmospheres con-

taining VC caused a reduction in the hepatic non-protein sulfhydryl content. Recently, two of the three major urinary metabolites of VC have been identified as *N*-acetyl-*S*(2-hydroxyethyl)cysteine and thiodiglycolic acid [23]. Thus, it appears that reactive metabolites of VC are formed which bind covalently to glutathione and are subsequently hydrolyzed and excreted in the urine as conjugates or catabolic products of cysteine. Furthermore, the present study indicates that in rats exposed to 50, 150, 250, 1000 or 2000 ppm VC, the formation of these metabolites and their subsequent conjugates exceeds the capacity of the liver to replenish the non-protein sulfhydryl groups.

A decreased clearance of BSP in workers exposed to VC has been reported [13]. Previous studies have demonstrated that conjugation of BSP in the liver with glutathione is essential for its subsequent excretion in bile [24,25]. Therefore, it was conceivable that a reduction of the hepatic non-protein sulfhydryl content of rats exposed to VC may delay the clearance of BSP. The absence of a decreased clearance of BSP from the plasma of rats exposed to 1000 ppm is not, as it may seem, an inconsistency. The clearance of BSP occurs in three steps: (*1*) uptake by the liver and storage, (*2*) conjugation with glutathione and (*3*) excretion in the bile. Overall the transport of BSP from plasma to bile is limited by conjugation with glutathione. Our experiment measured only the uptake of BSP by the liver as determined by its clearance from plasma; therefore, only a minimal if any effect on BSP clearance was expected. Nonetheless, the report of Kramer and Mutchler [13] indicated the need for the experiment, particularly in light of the need to develop a clinical test for ascertaining overexposure to VC in workers. Although additional experiments may show that exposure to VC inhibits the conjugation of BSP with glutathione and its subsequent excretion in bile, such information has little practical clinical significance.

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