MODULATION OF HALOTHANE AND VINYL CHLORIDE INDUCED ACUTE INJURY TO LIVER ENDOPLASMIC RETICULUM

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SUMMARY

Acute liver injury in mature male rats following a single exposure to halothane (0.85% x 5 hr) or to vinyl chloride (5% x 6 hr) can be consistently produced when preceded by pretreatment for 7 days with Aroclor 1254 (15 μmoles/100 g/day) and to a lesser extent phenobarbital (PBT) (40 μmoles). Morphologically the injury is characterized by primary involvement of the endoplasmic reticulum with denaturation of the smooth membranes. While halothane produces focal damage in PBT animals, centrolobular necrosis following halothane is widespread in Aroclor 1254 animals and is accompanied by increases in serum transaminases. Injury following VCM in PBT animals is characterized by striking vacuolization of hepatic centrolobular parenchyma, while in Aroclor 1254 animals vacuolization is panlobular and focal midzonal necrosis is extensive. Both PBT and Aroclor 1254 induce components of the xenobiotic metabolizing mixed function oxidase system (MFOS) altering rates and pathways of halocarbon metabolism. The enhanceable hepatotoxicity of halothane and VCM may be related to induction of specific pathways of their activation (toxification) via free radical or epoxide intermediates.

INTRODUCTION

Within this half of the 20th century halogenated hydrocarbon products have become ubiquitous components of our environment. Billions of pounds are produced each year for use in industry, agriculture and medicine. A number of these chemicals have been found to cause injury to man, and to our misfortune, such discoveries usually were made after use had become widespread (1,2,3). While the linkage of occupational vinyl chloride exposure to angiosarcoma of the liver may be viewed as a milestone in occupational hygiene (4), scientists should be challenged to identify the potential hazards of other similar halocarbons also in use.

Screening all industrial chemicals for carcinogenicity by classic in vivo techniques is a task of herculean proportions. Mutagenic potential may be detectable by the Ames method or other in vitro methods such as those used by Pienta and DiPaolo. Although such screenings may indicate compounds capable of neoplastic potential, the understanding of how xenobiotics alone, and in combination, cause both acute and chronic injury requires careful inquiry into the molecular basis of their toxic action. Examination of the chemical-pathology of halocarbon-induced cell damage should provide insights into the toxicological mechanisms involved, and ergo form the basis of new predictive tests.

Activation of many halocarbons to proximate toxins involves the enzymes of the mixed function oxidase system (MFOS) which in the liver is localized in the endoplasmic reticulum. A variety of drugs and environmental conditions alter the level and types of MFOS activity and have been used as biochemical tools to modulate response of animals to a variety of halocarbon toxins. Pretreatment with phenobarbital (PBT), a classic inducer of MFOS components has been used to heighten the hepatotoxic potential of CCl_4, bromobenzene, halothane ($CF_3CHBrCl$) and vinyl chloride ($ClHC{:}CH_2$) (5-8). Aroclor 1254 (1254) a polychlorinated biphenyl, widely used in industry as an insulator or plasticizer, is a more potent inducer of certain MFOS components and also a more potent enhancer of the hepatotoxicity of some

halocarbons than PBT. This presentation will describe our recent studies on the acute hepatotoxicity of halothane and vinyl chloride in animals with enhanced MFOS activity and discuss possible mechanisms of halocarbon toxification.

MATERIALS & METHODS

Male Sprague-Dawley rats (180-200 g) were housed on wire floor cages over processed clay animal litter and allowed free access to food and water. To induce the components of the hepatic MFOS, the animals were given 40 μmoles/100 g PBT or 15 μmoles/100 g Aroclor 1254 by gavage once daily for seven days. Compounds were solubilized in water with tracers of Tween 80. Controls received vehicle alone.

On the eighth day after an overnight fast the animals were exposed to air, halothane (0.85% x 5 hr) or vinyl chloride (5% x 6 hr) in inhalation chambers previously described (7,8). Animals were refed briefly then fasted overnight for sacrifice at 24 hr from commencement of halocarbon exposure. Microsomal enzyme assays were performed concomitantly with the experiments to confirm induction of MFOS components. Previously reported procedures for the collection and isolation of halothane metabolites were followed (7,9). Methods and results of these assays are described fully elsewhere (10). Serum glutamic oxalacetic and glutamic pyruvic transaminase (SGOT and SGPT) were determined with reagent kits from Sigma. Reduced glutathione was determined with Ellman's reagent according to Jaeger et al (11).

Model System for Acute Halothane Injury:

Interest in the hepatotoxic potential of halothane, currently the most popular and extensively used inhalation anesthetic, is derived from association between clinical halothane anesthesia and subsequent liver injury in the occasional patient (1). In experimental animals, multiple manipulations of length or frequency of halothane exposure and pretreatment with drugs or surgery or prior depletion of endogenous antioxidants have met with limited success in producing widespread

hepatic damage in the many species studied (7,12-15). In prior studies focal hepatic injury was consistently produced in PBT pretreated animals exposed to 0.85% halothane for 5 hr together with decreased cytochrome P 450 and transient increases in diene conjugation (7). These biochemically determined indices of injury to the endoplasmic reticulum are supported by ultrastructural changes in membranes of smooth endoplasmic reticulum in areas of injury (Fig. I) consistent with its denaturation (16). Postulating that this injury was related to altered metabolism or binding of the halothane molecule, subsequent experiments were conducted with tracer doses of $^{14}CF_3CHBrCl$ which revealed that less label was exhaled into expired gases and more excreted into the urine in PBT pretreated animals as compared to controls (9). In contrast, labeled halothane incorporation into cell subfractions or chemical constituents other than acid soluble fractions was not enhanced by PBT.

Aroclor 1254 pretreated animals appear to be a better model system in which to study the mechanism of halothane-induced liver injury. Exposure of these animals to 0.85% halothane in air for 5 hr results in elevated serum transaminases apparent as early as 2 hr after cessation of anesthesia (Table I) and widespread centrolobular necrosis by 24 hr (Fig. I). Levels of reduced glutathione were unaffected by anesthesia.

In the presence of NADPH, halothane is metabolized *in vitro* by the MFOS (17) but the relationships between injury and the amounts metabolized, specific metabolites produced or pathways of metabolism remain obscure. Somewhat to our surprise, preliminary experiments have indicated that the Aroclor 1254 animals excrete no more of a tracer dose of $^{14}CF_3CHBrCl$ in urine by 24 hr and have similar amounts of recoverable label in liver fractions other than cell sap in comparison to H_2O pretreated animals (Table II).

It is now possible to postulate multiple pathways for the metabolism of halothane (Fig. III) following its initial conversion to a free radical by an electron capture reaction with subsequent displacement of bromine (18). One subsequent

pathway may involve reaction with molecular oxygen, eventually forming an aldehyd . This could be "safely" oxidized to trifluoracetic acid, the primary urinary metabolite of halothane. Or the aldehyde could add to electrophilic sites in proteins. Generation of an aldehyde within the endoplasmic reticulum may account for the relatively specific labeling of microsomal protein by $^{14}CF_3CHBrCl$ in vivo (9). Chlorine would tend to be lost in the formation of the aldehyde.

Alternatively the free radical generated could either add across double bonds of polyunsaturated fatty acids of phospholipids, or abstract labile hydrogen atoms from susceptible molecular species such as methylene bridges of polyunsaturated fatty acids or non protein or protein sulfhydryls. In both addition and abstraction reactions chlorine would remain with the halothane carbons. In vivo investigations in Van Dykes laboratory with ^{14}C or ^{36}Cl labeled halothane have indicated that the metabolite bound to the phospholipid retains the Cl atom while that metabolite involved in protein binding does not (19). Anaerobiosis in the in vitro systems does not quantitatively alter the rate of NADPH dependent metabolism of halothane but does increase the amount of metabolite bound (17).

Hydrogen extraction pathways could lead to injured endoplasmic reticulum since loss of hydrogen atoms from methylene bridges of polyunsaturated fatty acids renders lipids vunerable to attack by molecular oxygen and subsequent peroxidative compositions. Increased content of lipid conjugated dienes, an indicator of hydrogen abstration, has been transiently observed after halothane anesthesia only in PBT pretreated animals (20,7). The debrominated (and/or dechlorinated) reaction product would be exhaled. Thus measurement of increased halothane metabolism by this pathway would not be reflected in urine, liver, or total exhaled radioactivity, but would require relatively sophisticated chromatographic techniques.

Model System for Acute Vinyl Chloride Injury:

A single exposure to vinyl chloride monomer (VCM) (5% x 6 hr) produces acute

liver injury in PBT pretreated rats (8). Involvement of the endoplasmic reticulum is apparent (Fig. I). Pretreatment with Aroclor 1254 seems to render animals exquisitely sensitive to VCM (Table III and Fig. II). Exposure to lower levels of VCM (1.25% or 2.5% x 6 hr) produces both histologic injury and enhances leakage of hepatic enzymes into serum.

Although H_2O pretreated, VCM-exposed animals have no apparent hepatic injury either histologically or according to serum transaminase levels (Table III) their livers are heavier and have higher contents of reduced glutathione (GSH) when compared to H_2O-pretreated, air controls. The magnitude of these VCM-induced changes is greater in PBT or Aroclor 1254 pretreated animals (Table IV). In other VCM experimental studies, Hefner, Jaeger and their colleagues (21,22) have reported decreased GSH contents in animals sacrificed immediately after single or multiple VCM exposures. Hefner's group has reported finding a mercapturic acid conjugate of a VCM metabolite in the urine (21). Our finding of elevated GSH levels 24 hr after VCM would be consistent with a rebound phenomenon. Following bromobenzene GSH levels plummet and then rebound to above normal levels by 24 hr (23). If the activation of VCM to a hepatotoxin occurs during its metabolism, an initial reaction would likely occur via the multimolecular MFOS, the enzyme system responsible for the conversion of most xenobiotics to more readily excretable metabolites. Metabolism of xenobiotics by this pathway may lead to toxification or detoxification. Correlations between induction of specific MFOS components and the degree of VCM-induced liver injury as measured by increased serum transaminases at 24 hr following VCM, reveals significant relationships between injury and both increased NADPH cytochrome P 450 reductase activity (as measured by reduction of cytochrome C) and total cytochrome P 450 content (24,25).

A non symetrically chlorinated homologue of VCM, trichloroethylene (TCE), has been studied more extensively. TCE complexes with cytochrome P 450 (26) and is

metabolized by components of the MFOS *in vitro*.[27] In PBT and Aroclor 1254 animals, TCE's metabolism is increased and liver injury enhanced (10). Correlations between induction of MFOS components and degree of TCE-induced liver injury are similar to that found for VCM, i.e., there are significant correlations between injury and increased NADPH cytochrome P 450 reductase activity and total cytochrome P 450 content (10).

Because of these similarities in the acute hepatotoxicities of VCM and TCE, a common mechanism for their metabolism (toxification) is a reasonable proposal (Fig. IV). Initial oxidation by the MFOS could produce an epoxide as the primary metabolite. Subsequently the epoxide could rearrange to form a β chlorinated acetaldehyde or interact with epoxide hydrase or GSH epoxide transferase to form either a diol or a glutathione conjugate as a secondary metabolite. The existance of these two latter pathways has not been verified for TCE. Chlorinated acetaldehydes could be converted to a tertiary generation of metabolites through hydration, or enzymatic reduction or oxidation. Chloral hydrate, trichlorethanol and trichloroacetic acid have all been identified as products of TCE metabolism (27) and monochloroacetic acid has been found in the urine of VCM exposed rats (21). The epoxides, the β chlorinated acetaldehyde and β chlorinated alcohols must all be considered potential proximate toxins.

In conclusion it is hoped that studies such as these will provide insight into the biochemical mechanisms of halocarbon induced liver injury and will establish a framework for the detection of other potentially toxic halocarbons.

FIGURE LEGENDS

Figure I: Masses of smooth endoplasmic reticulum in injured liver parenchymal cells of PBT pretreated rats 24 hr following exposure to (A) 0.85% halothane x 5 hr, and (B) 5% vinyl chloride x 6 hr. Focally, outer surfaces of tubular profiles in these tightly tangled tubular networks are associated with amorphous electron-opaque deposits (black "flecks") similar to those seen in labyrinthine tubular aggregates of denatured endoplasmic reticulum following CCl_4 (16) and following peroxidation of microsomes in vitro (28). Contiguous rough endoplasmic reticulum is vacuolated. x 16,000.

Figure II: Effect of PBT and Aroclor 1254 pretreatment on the acute toxicity of halothane and vinyl chloride 24 hr following the commencement of exposure. No lesions are seen in control animals exposed to these halocarbons. Focal vacuolization of midzonal parenchymal cells (arrows) is seen in PBT animals exposed to halothane. Extensive centrolobular necrosis is present in Aroclor 1254 pretreated animals exposed to halothane. Extensive centrolobular vacuolization following vinyl chloride in PBT animals becomes panlobular in Aroclor 1254 animals. c = central vein; x 60.

Figure III: Proposed scheme for metabolism of halothane by multiple pathways. The first step is an electron capture reaction with the formation of the triflurochloroethyl radical and bromide ion. The radical formed can react with oxygen (eventually forming an aldehyde) or add onto electrophilic sites in lipid or protein molecules (not shown) or abstract hydrogen atoms to form a gaseous product.

Figure IV: Proposed scheme for the metabolism of chlorinated ethylenes: trichloroethylene is the example. Oxidation of TCE by MFOS produces an epoxide as the primary metabolite. Subsequently, the epoxide may either rearrange to form trichlor aceta-

aldehyde (chloral) as the main secondary metabolite or interact with the epoxide hydratase or GSH epoxide transferase to form a diol or a glutathione conjugate respectively. The existence of these latter two pathways for TCE has not been established (*). Trichloroacetaldehyde is in turn converted to a tertiary generation of metabolites through hydration, or enzymatic reduction or oxidation. Chloral hydrate, trichloroethanol and trichloroacetic acid have all been identified as in vivo products of TCE metabolism, and chloroacetic acid identified following vinyl chloride. Scheme adapted from Ikeda and Ohtsufi.(29).

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TABLE I

SGOT LEVELS AFTER HALOTHANE† IN AROCLOR 1254 ANIMALS

Time After Exposure	Exposure to	
	Air (control)	Halothane†
hrs	Karmen units ± SEM*	
0	153 ± 18	149 ± 21
2	- - -	375 ± 152
24	211 ± 21	603 ± 129††

† 0.85% x 5 hr in air.

†† $p<0.025$ compared to respective air (control) group.

* 4 to 6 animals in each experimental group.

TABLE II

RECOVERY AT 24 HR OF $^{14}CF_3CHBrCl$ METABOLITES IN URINE AND LIVER

MFOS Inducer	Urine	Liver	
		Total	Acid Soluble
	- % dose* ± SEM -		
First Series††			
H_2O (control) (8)**	8.6 ± 0.6	1.1 ± 0.1	0.5 ± 0.1
PBT (12)	15.3 ± 1.2	1.9 ± 0.1†	0.9 ± 0.1†
Second Series			
H_2O (control) (4)	15.5 ± 2.3	2.5 ± 0.2	0.8 ± 0.1
Aroclor 1254 (6)	12.7 ± 2.4	2.6 ± 0.3	1.5 ± 0.1†

* = 7.0 x 10^6 dpm ^{14}C; 10 μmoles/halothane/kg.

** (number of animals).

† Difference between experimental and control $p<0.05$

†† Reynolds and Moslen(9) .

TABLE III

EFFECT OF MFOS INDUCERS ON SGOT 24 HR

AFTER EXPOSURE TO VCM†

MFOS Inducer	Exposure to	
	Air (control)	VCM†
	Karmen units ± SEM*	
H_2O (control)	185 ± 11	196 ± 16
PBT	156 ± 6	572 ± 72††
Aroclor 1254	211 ± 20	1162 ± 376††

† 5% VCM x 6 hr.

* 4 to 9 animals in each experimental group.

†† $p<0.05$ between VCM and respective air (control) group.

TABLE IV

EFFECT OF MFOS INDUCERS ON LIVER WEIGHT AND REDUCED GLUTATHIONE CONTENT 24 HR FOLLOWING VCM†

MFOS Inducer	Liver Weight		Glutathione	
	Air (control)	VCM	Air (control)	VCM
	gm/100 gm animal*		mg/gm liver*	
H_2O (control)	4.3 ± 0.2	4.9 ± 0.2	1.2 ± 0.1	1.5 ± 0.1
PBT	5.9 ± 0.0	7.7 ± 0.6††	1.5 ± 0.2	3.0 ± 0.2††
Aroclor 1254	6.2 ± 0.2	8.7 ± 0.1††	1.1 ± 0.1	3.4 ± 0.2††

† 5% VCM x 6 hr.

* 4 to 8 animals in each experimental group; mean ± SEM.

†† $p<0.025$ between VCM and respective air (control) group.

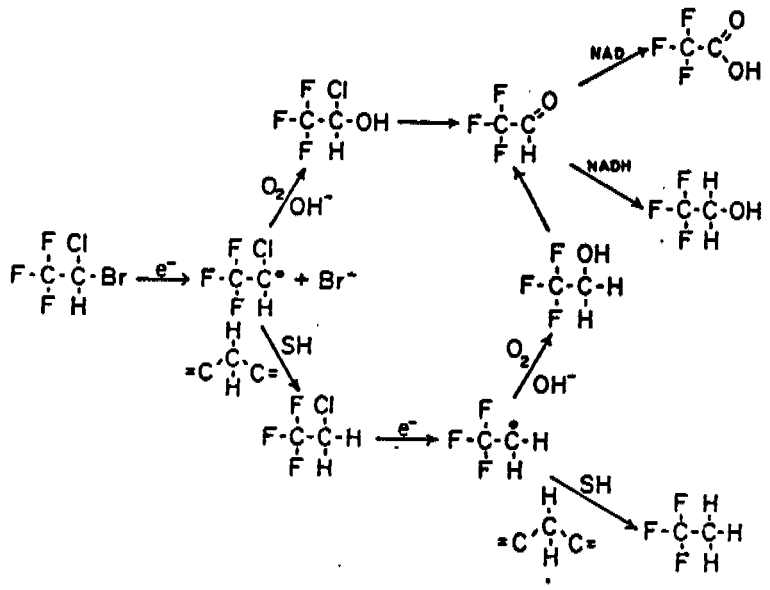

Fig 3

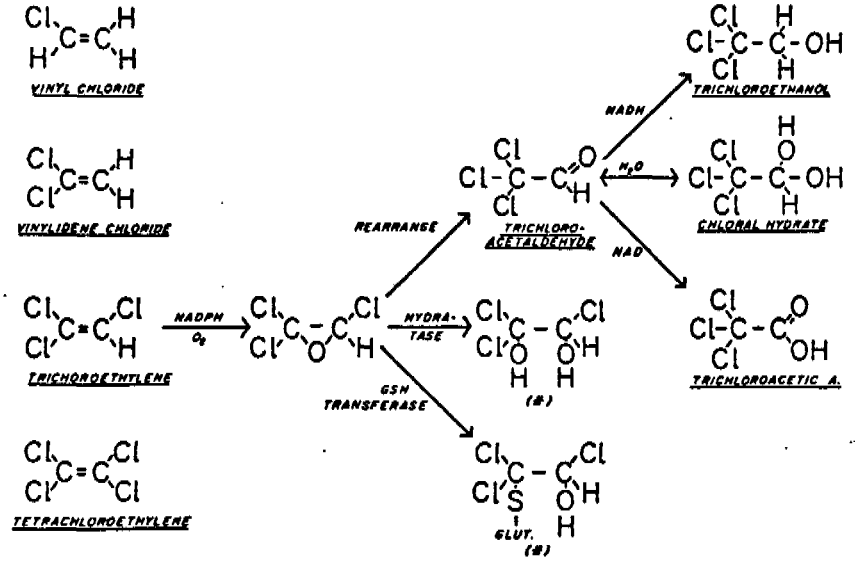

Fig 4

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