Acute Liver Injury by Vinyl Chloride: Involvement of Endoplasmic Reticulum in Phenobarbital-Pretreated Rats

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A single 6-hr exposure to vinyl chloride monomer (5%) produces extensive vacuolization of centrolobular liver parenchyma and focal midzonal necrosis in the hepatic lobule in phenobarbital-pretreated rats. Ultrastructurally, vacuolization consists of dilation of cysternae of rough endoplasmic reticulum and in the same cells smooth endoplasmic reticulum coalesces into discreet aggregates resembling denatured membranes. The findings support the hypothesis that vinyl chloride is hepatotoxic because it is converted into a toxic metabolite by components of the mixed function oxidase system of liver endoplasmic reticulum.

Introduction

Acute liver injury after vinyl chloride (VCM) has been noted briefly in earlier reports (1,2). Mastromatteo et al. (1) reported the appearance of fatty infiltration but could not demonstrate fat on frozen sections in guinea pigs exposed to 30% VCM for 30 min. Lester et al. (2) described the livers of rats exposed to 5% VCM for 8 hr on 19 consecutive days as containing marked swelling of cells, large irregular "vacuoles," and compressed sinusoids either focal or widespread.

We recently reported that 6 hr exposure to 5% VCM produces acute, biochemical and histologic injury in phenobarbital (PBT)-pretreated rats, but not in nonpretreated rats (3). Animals were pretreated with PBT because PBT induces certain components of the mixed function oxidase system located within the membranes of the endoplasmic reticulum and is responsible for the hepatotoxic activation of other halogenated hydrocarbons (4,5). This report is the first detailed ultrastructural description of acute liver injury by VCM.

Materials and Methods

Male Holtzman rats (250–300 g), housed in suspension cages, were provided Purina Lab Chow ad libitum. One group of animals was given ad libitum access to drinking water containing 0.1% sodium phenobarbital, 7 days prior to their initial VCM exposure and thereafter until sacrifice. Others, nonpretreated rats, were given tap water alone. Daily consumption of PBT was approximately 10 mg/100 g rat, which produces striking increases in smooth endoplasmic reticulum and doubling of cytochrome P-450 content and oxidative-N-demethylase activity (6,7).

PBT-pretreated and non-PBT-pretreated animals in groups of four were exposed to 5% VCM for 6 hr once, or to 5% VCM for 6 hr each on five consecutive days. Exposures were
conducted from 10 A.M. to 4 P.M. in a dynamic inhalation chamber modeled after Leach (8). Concentrations of VCM were adjusted and monitored by gas chromatography (9).

For morphologic examination of the liver, animals were anesthetized with pentobarbital 24 hr after the beginning of a single exposure, or immediately after the fifth exposure, portal veins were cannulated, and the liver was briefly perfused with Ringer’s lactate containing Isuprel, 1 mg/l., at 37°C, followed by buffered 1% glutaraldehyde. Following fixation, the liver was sliced and tissues for electron microscopy post-fixed in OsO<sub>4</sub>, and uranyl acetate and embedded in Epon. Appropriately stained sections were examined by light and electron microscopy. Liver slices, embedded in paraffin, were also examined following sectioning and staining by conventional histologic techniques.

**Results and Discussion**

Twenty-four hours following the onset of a single exposure of 5% VCM, there is diffuse vacuolization of the cytoplasm of centrolobular liver parenchyma and focal areas of necrosis of midzonal parenchyma in PBT-pretreated rats (Fig. 1B). Vacuolization involves approximately two-thirds of the hepatic parenchyma, and midzonal necrosis is focal throughout most of the liver and becomes extensive and confluent toward the dorsal aspect. In contrast, livers of animals exposed to VCM who were not pretreated with PBT appeared normal (Fig. 1A).

Livers of PBT-pretreated animals exposed to 5% VCM on five consecutive days contained broad “tracts” of stroma depleted of parenchymal cells which correspond in distribution

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**Figure 1.** Micrographs of liver (c = central vein, p = portal vein), ×50. (A) Non-PBT-pretreated animals 24 hr following onset of a single 6 hr exposure to 5% VCM; liver is histologically normal. (B) PBT pretreated animals 24 hr following onset of a single 6-hr exposure to 5% VCM. The centrolobular two-thirds of the liver lobules show diffuse vacuolization; and area of focal midzonal necrosis is shown at the bottom right (arrows). (C) Liver of PBT-pretreated animals immediately following the fifth 6-hr exposure to 5% VCM at 24 hr intervals. Large area of stroma of hepatic lobule depleted of parenchyma is at top (arrows). Liver at bottom appears normal.
afforded by a small dose of CCl₄ against injury from a large dose of CCl₄ subsequently administered (10). In contrast, livers of animals exposed to VCM on five consecutive days and who were not pretreated with PBT appeared normal.

Vacuolated liver parenchymal cells in PBT-pretreated rats 24 hr after the onset of exposure to 5% VCM revealed extensive dilation of the cisternae of the rough endoplasmic reticulum and coalescence of the smooth into discrete aggregates of smooth-surfaced tubules conspicuously flecked with areas of increased electron opacity (Figs. 3 and 4). As such, these resemble the labyrinthine tubular aggregates of denatured endoplasmic reticulum which appear as a consequence of poisoning with CCl₄ and CHI₃ (6,11) toxins whose capacities for cellular injury are linked to their reactivity in free-radical reactions (11). Although neutral lipid droplets do not appear to be increased, relatively large numbers of chylomicra are seen in the dilated cisternae of the rough endoplasmic reticulum (Figs. 4 and 5). Mitochondria occasionally show “punched out” areas of electron lucency of the mitochondrial matrix (Fig. 4). Golgi are unrecognizable, and nuclei—aside from showing effects of external compression from vacuolar dililation of the endoplasmic reticulum—appear normal.

Electron-opaque material in aggregates of smooth endoplasmic reticulum 24 hr following VCM exposure appear to be applied to the outer surfaces of tubular profiles and as such, resemble those seen following CCl₄ (6). Similar changes may also be seen in pellets of microsomes allowed to undergo lipid peroxidation in vitro (12). Thus, these morphologic changes observed following VCM exposure may result from the initiation of lipid peroxidation by a homolytically cleavable toxin within the membranes of the endoplasmic reticulum. Although numerous ribosomes may be seen in relation to the walls of the vacuoles considered to be dilated cisternae of the rough endoplasmic reticulum (Fig. 5), their relationship to this membrane and their degree of aggregation remain unclear in the relatively thick sections obtained so far.

The ultrastructural observations that the acute liver lesion produced by VCM primarily involves components of the endoplasmic reticulum coupled with the fact that PBT (which is
FIGURE 3. Light (inset) and electron microscopic appearance of vacuolated liver parenchymal cells 24 hr following a single 6-hr 5% VCM exposure in a PB-T-pretreated animal. Membranes of rough endoplasmic reticulum (RER) form walls of large vacuoles which are essentially free of visible content. Smooth endoplasmic reticulum (SER) forms compact masses which are flecked with areas of increased electron opacity. Several lipid droplets (LI) are seen. ×8000. Inset: Nuclei appear normal. Condensed SER form discrete cytoplasmic masses (arrows). ×3000.

an inducer of mixed function oxidase activity in the endoplasmic reticulum) enhances injury suggests that this organelle is the primary site of generation of toxic metabolites from VCM. Toxic metabolites generated from VCM by the mixed function oxidase system may include epoxides and be responsible for both acute cellular injury and VCM's tumorogenic potential (13).

Although this morphological picture of acute injury is distinctive for VCM and indicative of primary injury to the membranes of the
endoplasmic reticulum, this mechanism of toxic action cannot be generalized to all members of the chloroethylene family. Morphologically, the lesion following 1,1-dichloroethylene is totally dissimilar; it seems to involve primarily nuclear chromatin and mitochondria and spare endoplasmic reticulum (14).

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Figure 5. Liver of PBT-treated animal; treatment same as in Figure 3. Abundant ribosomes are present in cytoplasmic matrix adjacent to membranes lining dilated cisternae of RER. In a focal area of increased electron opacity present in SER, opaque material appears applied to the outer surfaces of tubules of reduced diameters. ×20,000.
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