An Animal Model of Hepatotoxicity Associated with Halothane Anesthesia

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Hepatic necrosis was induced in rats by a single exposure to 1 per cent halothane in oxygen following pretreatment with a single dose of Aroclor 1254, a polychlorinated biphenyl. The hepatic lesion was centrilobular and multifocal, and morphologically similar to that reported to occur in man. In-vitro incubation with 14C-halothane indicated an enhanced covalent binding of halothane metabolites to hepatic microsomal macromolecules, particularly lipids, following Aroclor 1254 pretreatment. Lipoperoxidation of microsomal unsaturated fatty acids was not observed with these animals. (Key words: Anesthetics, volatile, halothane: Toxicity, hepatic; Liver, hepatotoxicity; Biotransformation, enzyme induction; Pharmacology, Aroclor 1254.)

HALOTHANE-ASSOCIATED hepatic injury and halothane hepatitis are terms used to describe the rare, unpredictable, and sporadic cases of hepatic failure following anesthesia with halothane (2-bromo-2-chloro-1,1,1-trifluoromethane). The mechanism and even the existence of such injury are speculative, primarily because no animal model of extensive hepatic necrosis following halothane anesthesia has been developed. Histologic changes ascribed to this toxicity are primarily confined to the centrilobular area and include ballooning of cells, demarcation, cellular infiltration, fatty metamorphosis, and parenchymal cell necrosis.1-4 Major hypotheses for the mechanism of halothane-associated hepatic injury include: 1) formation of chemically reactive intermediates during the biotransformation of halothane; 2) hypersensitivity to the anesthetic or one of its degradative metabolites.5,6 Recent studies of the metabolism of halothane and the covalent binding of its metabolites to macromolecules of the liver lend support to the possibility of a reactive intermediate.7,5

Halothane undergoes biotransformation in man and laboratory animals, with the major urinary metabolites identified as trifluoroacetic acid (TFA), free or conjugated, and bromide ion.5,8,9 Studies by Van Dyke et al.5 indicate that TFA does not bind covalently to subcellular components of the liver, nor does this metabolite produce hepatic injury in rats. Since halothane and TFA do not bind covalently, other intermediates formed during the conversion of halothane to TFA have been implicated as the chemical species producing hepatic damage. The structures of these intermediates are unknown, but various free radicals and reactive products such as 2-bromo-2-chloro-1,1-difluoroethylene, trifluoroacetaldehyde, and/or trifluoroethanol have been suggested.8,9,12 Induction of the hepatic microsomal enzymes of rats with phenobarbital slightly enhances the binding of halothane metabolites to microsomes, but does not lead to massive centrilobular necrosis following halothane anesthesia.11 In this communication we report that halothane causes centrilobular hepatic necrosis in rats pretreated with Aroclor 1254 (a polychlorinated biphenyl containing 54 per cent Cl by weight). Studies to elucidate the mechanism of this action were performed.

Methods

Male Sprague-Dawley rats weighing 275-325 g were treated as follows: the polychlorodibiphenyl mixture, Aroclor 1254 (fig.1), 500 mg/kg (ip, in sesame oil), was administered to 30 rats four days prior to anesthesia for two hours with 1 per cent halothane in O₂; an additional 20 rats were treated with Aroclor 1254 but not anesthetized; a final group of ten rats was treated with sesame oil and anesthetized. No anesthetic death oc-
curred. Twenty-four hours after anesthesia the animals were sacrificed, and the livers perfused via the portal vein with cold physiologic saline solution prior to fixation in buffered formalin for histologic studies. Blood from several animals was collected for transaminase (SGPT) determination.

The covalent binding of halothane to microsomal protein and lipids was studied in vitro using microsomes isolated from rats pretreated with Aroclor 1254 or sesame oil. Conditions for the incubation were those previously reported, with the following modification: $^14$C-halothane (0.1 mM final concentration, specific activity 0.5 mCi/mmol) was added to an incubation volume of 2 ml containing hepatic microsomes adjusted to a protein concentration of 1 mg/ml with Tris KCl buffer, and an NADPH-generating system. Covalent binding has been employed as a biochemical variable paralleling hepatic morphologic necrosis. After 10 minutes of incubation at 37°C in air or N₂, the reactions were terminated by adding 5 ml of cold chloroform:methanol (3:1), which extracted the lipids from the aqueous phase. After an additional chloroform:methanol extraction, 5 ml of 10 per cent trichloroacetic acid (TCA) were added to the aqueous phase to precipitate protein. The chloroform:methanol layers were washed twice with 3 volumes of Tris-KCl buffer (pH 7.4), filtered to remove any interphase, and then repeatedly dried and redissolved in chloroform:methanol to constant radioactivity. The TCA-precipitated proteins were extensively extracted at 60°C with 5 per cent TCA (five washes), chloroform: methanol (six washes), and once each with 3 M sodium acetate and acetone. Since Van Dyke reported that lipid binding of $^{14}$C-halothane metabolites was associated primarily with phospholipids, results are expressed in terms of phosphorus content.

Concentrations of protein cytochrome P-450 and NADPH cytochrome c reductase activity were determined by routine methods previously described.

Since peroxidation of polyenoic fatty acids has been implicated in the hepatotoxicity produced by CCl₄ and CHCl₃, we measured the in-vitro production of malonaldehyde by previously described methods. Microsomes from control and Aroclor 1254-treated rats were incubated in air with an NADPH-

![](https://example.com/image1.png)

**Fig. 1.** Empirical structural formula of the poly-chlorobiphenyls (PCB). Aroclor 1254 contains 34 per cent chlorine by weight attached at random carbon atoms on the biphenyl rings.

generating system and Tris-KCl buffer (pH 7.4) saturated with halothane for 10 min at 37°C.

**Results**

Centrilobular necrosis was apparent 24 hours after exposure to 1 per cent halothane in 50 per cent of the animals pretreated with Aroclor 1254. This was evidenced by dissociation of the liver cell cords, nondiscernible nuclei, ballooning of liver cells, infiltration of inflammatory cells, and coagulation necrosis (figs. 2 and 3). Two pathologists who reviewed the slides stated that the overall morphologic changes were similar to those of viral hepatitis in man. The severities of necrosis in the centrilobular area that was damaged varied somewhat. In most animals more than 50 per cent of the lobule was coagulated and infiltrated with inflammatory cells. None of these changes was apparent either in the livers of rats pretreated with Aroclor 1254 without exposure to halothane or in the livers of control animals anesthetized with halothane. All rats pretreated with Aroclor 1254 and anesthetized with halothane that did not show massive centrilobular necrosis had focal areas of necrosis surrounding the central vein. The only histopathologic change observed in the animals given Aroclor 1254 but not exposed to halothane consisted of small sudanophilic vacuoles in the cytoplasm of the hepatocytes. SGPT levels were 179 ± 81 Karmen units/ml in five of the Aroclor 1254-halothane-treated rats, compared with 31 ± 4 units/ml for the five Aroclor 1254 controls and 29 ± 3 units/ml for six controls exposed to 1 per cent halothane and sacrificed at 24 hours. This difference is significant at the $P < 0.001$ level. Only one concentration-time was employed,
as it was not intended to study dose–response relationships.

As indicated in Table 1, the binding of $^{14}$C was increased in microsomes from animals pretreated with Aroclor 1254. Binding to protein was increased 64 per cent and binding to lipids 430 per cent. Incubation in an atmosphere of $X_2$ resulted in greater covalent binding to proteins and lipids than incubation in air. However, Aroclor 1254 pretreatment did
24 hours after anesthesia were characterized as developing from multiple foci, were extensive, and originated in the centrilobular area. The lesions showed dissociation of liver cords, loss of nuclei, ballooning of liver cells, infiltration of inflammatory cells, and coagulation necrosis. (×25).

not enhance this anaerobic binding, as the Aroclor 1254-pretreated and control microsomes showed similar enhancements of binding.

There was no evidence that the hepatotoxic effect of halothane and Aroclor 1254 was the result of lipoperoxidation. Concentrations of malonaldehyde (±SE) produced by control microsomes (2.75 ± 0.2 μm mol·min⁻¹ mg⁻¹) and the microsomes from treated rats
Table 1. Effects of Aroclor 1254 Pretreatment and Incubation Atmosphere on Binding to Microsomal Protein and Lipids of $^{3}$H from $^{3}$C-Halothane

<table>
<thead>
<tr>
<th>Source of Microsomes</th>
<th>Protein Binding Picomoles $\mu$g $^{-1}$</th>
<th>Lipid Binding Picomoles $\mu$mol $^{-1}$ P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (sesame oil)</td>
<td>594 ± 107 $^*$</td>
<td>1,934 ± 1,000</td>
</tr>
<tr>
<td>Aroclor 1254-treated rats</td>
<td>974 ± 88 $^*$</td>
<td>1,917 ± 531 $^*$</td>
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</table>

Each value is the mean ± SEM for incubation of microsomes from six control rats and six rats treated with Aroclor 1254 (500 mg/kg, ip). Incubations were at 37°C for 10 min in a 2-ml volume containing 2 mg microsomal protein, 0.5 mM NADP, 2 mM glucose-6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase, and 0.1 mM $^{3}$C-halothane (specific activity 0.5 Ci/mmol). Omission of NADP resulted in background binding to lipids of 60 picomoles/mole P and to protein of 160 picomoles/mg protein, identical for control and Aroclor 1254 microsomes and for incubations in air and in N$_2$. These values were subtracted from the above results.

$^*$ $P < .05$, $^1$ $P < .01$ compared with control.

<table>
<thead>
<tr>
<th>Source of Microsomes</th>
<th>Protein $^a$</th>
<th>Cytochrome P-450</th>
<th>Cytochrome c Reductase $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (sesame oil)</td>
<td>21.6 ± 0.7</td>
<td>0.84 ± .04</td>
<td>55.3 ± 4.4</td>
</tr>
<tr>
<td>Aroclor 1254-treated rats</td>
<td>33.1 ± 0.5 $^*$</td>
<td>2.67 ± .14 $^1$</td>
<td>145.5 ± 5.5 $^1$</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM for six rats. Microsomes were obtained four days after administration of sesame oil or Aroclor 1254 (500 mg/kg, ip).

$^a$ $P < .05$, $^1$ $P < .01$.

(3.07 ± 0.2 mgm $^\cdot$ min $^\cdot$mg $^{-1}$) were not significantly different.

Aroclor 1254 is known to promote enlargement of the liver and enhance the activity of the microsomal drug-metabolizing enzymes. Each study, pretreatment with Aroclor 1254 increased the liver weight/body weight ratio from 0.046 ± .002 to 0.07 ± .006. The effects on components of the microsomal electron-transport system are summarized in Table 2. Microsomal concentrations of protein and cytochrome P-450 (149), as well as activity of cytochrome c reductase, were significantly elevated four days after intraperitoneal administration of Aroclor 1254 (500 mg/kg). The difference between carbon monoxide spectra of hepatic microsomes from Aroclor 1254-treated rats and control rats indicated a maximum absorbance at 449 nm rather than the usual 450 nm for the former (fig. 4). To insure that this spectral shift from 450 to 449 nm was real, the CO-binding spectra of microsomes from control and 3-methylcholanthrene-treated rats were compared with those obtained in rats pretreated with Aroclor 1254. The difference in CO spectra indicated that the maximum absorbance of microsomes from rats five days following treatment with Aroclor 1254 was at 449 nm, while those of control and 3-methylcholanthrene-treated rats were at 450 and 448 nm, respectively.

Discussion

The controversy concerning the mechanism by which halothane anesthesia may produce rare and sporadic cases of hepatic damage is unresolved because administration of halothane to experimental animals has not resulted in hepatic damage histologically identical to that produced in man. In this study, pronounced centrilobular necrosis was evident in 80 per cent of the rats pretreated with Aroclor 1254 following exposure to 1 per cent halothane anesthesia with an ade-
quate supply of oxygen. The changes observed were those of nonspecific cellular injury similar to those seen after administration of carbon tetrachloride or chloroform. Lesions in halothane-anesthetized Aroclor 1254-pretreated animals developed in multiple lobules, were extensive, and originated from the central vein. The coagulation type of necrosis in this animal model was also similar to that observed in the reported halothane-induced human lesion.  

The increased covalent binding of reactive intermediates of halothane to microsomal lipids and proteins from Aroclor 1254-treated rats indicates an altered qualitative and/or quantitative biotransformation of halothane. This change in biotransformation results in a greater production of chemically reactive intermediates, which subsequently interact with macromolecules of the liver. The greater the extent of this interaction, the greater the chance for an irreversible alteration of cellular integrity and cell death. The increased binding in an atmosphere of N₂ suggests that at least part of the metabolism of halothane may proceed by a reductive pathway (non-oxygen-dependent) and, as suggested by Van Dyke et al., oxygen may inhibit this pathway of biotransformation and/or interfere with the binding of the halothane intermediates. Any role of lowered oxygen tension in the production of the lesion induced by halothane would be only speculative, however, especially since anaerobic incubation was not associated with a difference in the binding to microsomes from Aroclor 1254-treated or control rats.

Further evidence that Aroclor 1254 treatment enhances biotransformation was indicated by the elevated levels of microsomal protein and cytochrome P-450 (449) and increased activity of NADPH-cytochrome c reductase. The polychlorobiphenyls in Aroclor 1254 are potent inducers with the following characteristics: induction of long duration; orally, intraperitoneally and topically effective; induction of metabolism of a variety of substrates. In addition, the CO-binding pigment produced is an unusual variant of cytochrome P-450 with an absorption maximum of 449 nm. The increase in concentration of this pigment, plus its unusual absorption maximum, may be responsible for changes in biotransformation of halothane. This P-450 variant possibly alters the qualitative, as well as the quantitative, biotransformation of the anesthetic.

Pretreatment of rats with phenobarbital enhances the binding of halothane metabolites to microsomal lipids and protein, but not as much as does Aroclor 1254. Although patchy subcapsular necrosis has been reported to occur when phenobarbital-pretreated rats are anesthetized with halothane, this combination does not result in centrilobular necrosis. Although pretreatment with Aroclor 1254 followed by halothane anesthesia produces hepatic necrosis in rats, a relationship of this phenomenon to the human syndrome is unknown. It is not the intent of this study to speculate that Aroclor 1254 is in any way responsible for the so-called “halothane hepatitis” syndrome of man. Rather, Aroclor 1254 has been employed as a tool to demonstrate a phenomenon that may serve as a model for further insight into the relevant clinical problem of halogenated anesthetics.
and hepatic damage. However, some discussion of the polychlorobiphenyls such as are contained in Aroclor 1254 is of interest. Polychlorobiphenyls are widely distributed in the environment,13 They are found in numerous animal tissues, including human fat22 and human milk.17 Although their effects on human drug metabolism are unknown, their persistence in the body could result in a continuous exposure of the liver to their enzyme-inducing effects. Polychlorobiphenyls are also known to produce neoplastic changes in the livers of rats.21 Recently, Rogers et al.25 reported that Aroclor 1254 abolishes rat-liver lysosomal membranes. Therefore, the participation of Aroclor 1254 in production of the halothane-associated lesion may be twofold: 1) altered biotransformation of halothane, as discussed above, and 2) the presence of another xenobiotic with additive toxic manifestations in the liver.

References