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Halothane Hepatotoxicity and Fluoride Production in Mice and Rats

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Other investigators have demonstrated halothane-induced hepatic injury in rats anesthetized in hypoxic environments. The authors examined this phenomenon in mice and investigated plasma fluoride levels in mice and rats anesthetized with halothane in 40, 21, and 7 per cent oxygen with or without pretreatment with phenobarbital or carbon tetrachloride. They found no hepatic necrosis in mice. Mice produced less fluoride than rats. This difference in halothane metabolism between Sprague-Dawley rats and Swiss-Webster mice may explain the failure to observe hepatic necrosis in mice. (Key words: Anesthetics, volatile: halothane. Biotransformation (drug): halothane. Ions: fluoride. Liver, hepatotoxicity.)

HALOTHANE HEPATOTOXICITY remains a clinical concern, and there is current interest in identifying mechanisms that might be responsible for halothane-induced hepatic injury in man. Sipes and Brown investigated the debromination of halothane in polychlorobiphenyl-pretreated rats.[1] They observed that such pretreatment unmasked a reductive metabolic pathway for halothane, and that bromine and presumably various reactive intermediates were produced. They further observed that carbon disulfide, a known antagonist of the mixed-function oxidase system, did not interfere with the debromination pathway.

Widger and colleagues have also investigated hepatotoxicity in rats.[2] They studied fluoride production and fixation of halothane metabolites in liver cells of rats pretreated with phenobarbital and anesthetized in a hypoxic environment. They observed that hypoxia enhanced fluoride production and increased the binding of reactive intermediates to tissue.

Because it is known that species vary in their abilities to metabolize xenobiotics, we wished to evaluate the universality of the model of Widger *et al.* Therefore, we investigated hepatotoxicity caused by halothane in mice anesthetized in a hypoxic environment, and we considered the relationship between inspired oxygen tension and fluoride production in mice pretreated with enzyme inducers and inhibitors.

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Methods

For the hepatic toxicity study, 90 male Swiss-Webster mice, weights 20–30 g, obtained from Carworth Farms were divided into six groups. All were maintained on tap water and Purina Mouse Chow® *ad libitum* and lived in metal cages on pine shavings. Two groups continued on this regimen until the day of anesthesia. Two other groups had phenobarbital sodium, 35 mg/100 ml, added to their drinking water for five days prior to anesthesia. Each animal in the last two groups received a 0.2-ml subcutaneous injection of carbon tetrachloride, 20 per cent solution in Wesson Oil®, 24 hours prior to anesthesia. The animals were anesthetized in glass desiccators using a high-flow system. Halothane was vaporized in oxygen, either 21 or 7 per cent, and the concentrations were adjusted in response to clinical signs. When an animal appeared to be having respiratory or circulatory distress, as evidenced by ocular or tail cyanosis, the halothane concentration was decreased slightly. Throughout most of the 135 min of anesthesia the halothane concentration remained 1 ± 0.1 vol per cent. Gas samples were withdrawn from the chamber to verify halothane concentration using gas chromatography and oxygen concentration with a Radiometer blood-gas machine. The oxygen concentration was monitored with a Harris Lake fuel-cell oxygen analyzer. After anesthesia the animals were returned to their cages and again received food and water *ad libitum*. Two days after anesthesia, the animals were killed and their livers removed and prepared for microscopic examination. Slides were examined by the authors and a pathologist familiar with the histologic characteristics of rodent livers.

For the fluoride study, 88 male Swiss-Webster mice, weights 20–30 g, and 32 male Sprague-Dawley rats, weights 200–300 g, from the same supplier, were pretreated with no medication or with phenobarbital or carbon tetrachloride in the same fashion as were those animals in the hepatic toxicity study. The animals were anesthetized for 135 min using halothane delivered in oxygen, 40, 21, or 7 per cent. An additional group of animals of each species was anesthetized with sodium thiopental, 50 mg/kg, intraperitoneally. These animals received no pretreatment and had not been exposed to any volatile anesthetic agent. At the end

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TABLE 1. Plasma Fluoride Levels in Mice and Rats According to Pretreatment and Inspired O_2 Fraction

	Animal	n	Pretreatment	Inspired Oxygen (Per Cent)	Anesthetic	Plasma Fluoride (μM ± SE)
A	Mouse	11	None	—	Thiopental	3.9 ± 1.1
B	Rat	11	None	—	Thiopental	4.2 ± 0.3
C	Mouse	9	None	40	Halothane	4.9 ± 0.3
D	Mouse	5	None	21	Halothane	7.3 ± 1.0
E	Mouse	9	None	7	Halothane	7.7 ± 0.9
F	Mouse	9	Phenobarbital	40	Halothane	10.0 ± 1.0
G	Mouse	5	Phenobarbital	21	Halothane	9.7 ± 1.5
H	Mouse	12	Phenobarbital	7	Halothane	12.4 ± 1.0
I	Mouse	8	CCl_4	40	Halothane	4.3 ± 0.37
J	Mouse	9	CCl_4	21	Halothane	6.1 ± 0.76
K	Mouse	11	CCl_4	7	Halothane	7.6 ± 0.5
L	Rat	6	None	40	Halothane	7.5 ± 0.9
M	Rat	6	Phenobarbital	40	Halothane	9.8 ± 1.5
N	Rat	4	Phenobarbital	21	Halothane	23 ± 4
O	Rat	5	Phenobarbital	7	Halothane	41 ± 8

L *vs.* B, $P = 0.001$.
N *vs.* M, $P = 0.01$.
O *vs.* M, $P = 0.005$.
An analysis of variance for all data obtained in mice showed significant effects of pretreatment with phenobarbital and carbon tetrachloride (CCl_4) ($P \ll 0.001$) and inspired oxygen ($P < 0.001$).

of anesthesia all animals were exsanguinated and blood was collected in heparinized syringes. The blood was centrifuged and the plasma fluoride concentration was measured using an Orion fluoride-specific electrode. Fluoride data for mice were analyzed using a two-factor analysis of variance. The Student two-tailed independent sample test was used for data obtained in rats.

Results

Examination of hematoxylin and eosin-stained specimens of livers from mice in the hepatic toxicity study showed various minor abnormalities such as vacuolization and cloudy swelling. However, necrosis was not found in any animal, nor was any difference among the groups observed.

The plasma fluoride level in unpretreated mice anesthetized with halothane in oxygen, 40 per cent, was 4.9 ± 0.3 μM, while values observed for mice anesthetized with halothane in oxygen, 21 and 7 per cent, were 7.3 ± 1.0 and 7.7 ± 0.9 μM, respectively. Mice pretreated with phenobarbital had fluoride levels near 10 μM, regardless of the oxygen environment. For these phenobarbital-pretreated mice, fluoride levels were higher than those observed in unpretreated animals. For animals pretreated with carbon tetrachloride before anesthesia with halothane in oxygen the following fluoride values were observed: oxygen, 40 per cent, 4.3 ± 0.37 μM; oxygen, 21 per cent, 6.1 ± 0.76 μM; oxygen, 7 per cent, 7.6 ± 0.5 μM.

A two-factor analysis of variance for data from all mice anesthetized with halothane indicates a highly significant effect of drug treatment on plasma fluoride (table 1). The inspired oxygen concentration also had a significant effect on plasma fluoride. There was no significant interaction between drug effects and oxygen effects. A similar analysis of the no-pretreatment and carbon tetrachloride-pretreatment data shows no significant drug effect.

Rats pretreated with phenobarbital did not tolerate 135 min of anesthesia in an oxygen, 7 per cent, environment. A few died during the first hour and most during the second hour of anesthesia. Animals were removed from the chamber when they appeared moribund. Fluoride levels were significantly higher in animals anesthetized with halothane in either 21 or 7 per cent oxygen (23 ± 4.0 and 41 ± 8.0 μM), compared with levels in animals anesthetized in 40 per cent oxygen (9.8 ± 1.5 μM). When rats were anesthetized in 40 per cent oxygen, pretreatment with phenobarbital had no significant effect on plasma fluoride level, compared with unpretreated animals.

Plasma fluoride levels were similar in rats and mice anesthetized with halothane in oxygen, 40 per cent; however, rats had higher fluoride levels with oxygen, 21 and 7 per cent.

Discussion

Although others have demonstrated hepatic injury in rats exposed to halothane in a hypoxic environment,[2] we were unable to produce hepatic damage in mice. While there is no evidence linking increased plasma fluoride levels directly to hepatic toxicity, the fluoride levels may serve as an indicator of the extent of halothane metabolism, and thus reflect the availability of reactive intermediates, which may cause cellular damage. Thus, the species difference observed in the toxicity study may be related to the fluoride data. Fluoride values were obtained at the end of anesthesia to afford comparison with the data of Widger *et al.*,[2] and in rats we found fluoride levels similar to those they obtained, although their animals, anesthetized with a higher concentration of halothane, had slightly higher fluoride levels. Our mice had significantly lower values for plasma fluoride at low oxygen concentrations than did our rats. This was true even though our mice had been subjected to enzyme induction. In previous studies,[3,4] it was shown that Swiss-Webster mice sustain maximal enzyme induction with the regimen we employed for this study. It is possible, then, that although the mice may metab-

olize halothane in a reductive pathway, the quantity of reactive intermediates generated is not sufficient to overwhelm intracellular defenses, so cellular death does not occur.

The fluoride data for mice are also qualitatively different from fluoride data obtained with rats. The role of hypoxia in rats has been emphasized by Widger *et al.*,[2] who commented that while their studies were done with phenobarbital-pretreated rats the phenobarbital was presumably not necessary but merely enhanced hypoxically induced fluoride production. Our data indicate that either hypoxia or phenobarbital will enhance fluoride production in mice. In fact, all phenobarbital-pretreated animals had similar fluoride levels, regardless of oxygen concentrations. However, when mice were anesthetized with halothane in oxygen, 7 per cent, those pretreated with phenobarbital had higher fluoride levels than did the others. It seems, then, that induction of the enzyme system is more important than hypoxia for fluoride production. It is, of course, possible that an even lower inspired oxygen tension would drive the system sufficiently that phenobarbital could not induce a further increase. However, a further decrease in inspired oxygen tension is not likely to be compatible with survival during halothane anesthesia.

Carbon tetrachloride had no significant effect on fluoride production. This may be analogous to the observation of Sipes and Brown that carbon disulfide did not interfere with bromide production in rats,[1] and may imply that a system other than P-450 is primarily responsible for this reaction.

The main purpose of this study was to compare fluoride production after halothane anesthesia in mice and rats. We have shown that, regardless of baseline, male Swiss-Webster mice produce much less fluoride than do male Sprague-Dawley rats. In addition, it appears that these mice are much less likely to show visible hepatic lesions than are Sprague-Dawley rats.

While the rat model for halothane-induced hepatotoxicity is intriguing, we have demonstrated that it is not universally applicable. Further investigation will have to show whether data obtained in either animal model of halothane biotransformation are applicable to man.

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