Genetic Differences in Reductive Metabolism and Hepatotoxicity of Halothane in Three Rat Strains


The relationship between the reductive metabolism of halothane and hepatotoxicity was examined in three rat strains (Fischer 344, Sprague-Dawley, and black hooded Wistar) to determine if there were genetic differences in 1) the reductive metabolism of halothane under identical exposure conditions, and 2) the susceptibility to the hepatotoxic effects of halothane. Halothane hepatotoxicity was produced in all rat strains by exposing phenobarbital-pretreated rats to 1% per cent halothane under mild hypoxia (14% per cent oxygen, inspired) for 2 h. Generally the levels of both 2-chloro-1,1,1-trifluoroethane (CTF) and 2-chloro-1,1-difluoroethylen (CDE), two volatile metabolites of halothane, increased from the onset of anesthetic exposure and reached a plateau after approximately 60 min. The exception to this trend were phenobarbital-pretreated Wistar rats (exposed to 1% per cent halothane with 14% per cent oxygen) where the levels of either CDF or CTF were high initially (10 min sample) and decreased in subsequent samples to reach a plateau after 80 min. The plateau levels of both CDF (approximately 6 ppm) and CTF (approximately 20 ppm) were not consistently different among the three rat strains exposed to halothane (1% per cent) and hypoxia with prior enzyme induction. There were, however, significant differences in both biochemical and pathological changes among the three strains exposed under the above identical conditions when the rats were killed 24 h after anesthetic exposure. For example, serum alanine aminotransferase (ALT) was increased fourfold in the Fischer strain but only doubled for the other two strains. Moreover, while all three strains had various amounts of hepatocyte damage in the vicinity of the central veins when the rats were exposed to halothane, hypoxia, and enzyme induction, only the Fischer strain showed hepatocyte damage under the exposure conditions of halothane (1% per cent) and normoxia (21% per cent oxygen, inspired) with prior enzyme induction. The results support the role of reductive metabolism of halothane in the etiology of halothane hepatotoxicity. Furthermore, they suggest that genetic variations in the susceptibility of the liver to the reactive intermediates or metabolites formed during reductive metabolism of halothane may be a significant factor in halothane hepatotoxicity. (Key words: Anesthetics, volatile: halothane. Bio-transformation (drug); enzyme induction; fluorometabolites; genetic factors. Complications: hepatitis. Metabolism; enzyme induction; genetic factors. Toxicity: hepatic; metabolites.)

Although halothane is considered to be a safe and effective volatile anesthetic agent, one problem continues to be the possibility of hepatitis following halothane anesthesia. Based upon retrospective epidemiological data, it was initially thought that such patients exhibited a hypersensitivity reaction to the halothane molecule, although subsequent evidence has not fully supported this concept. Over the last few years, an alternative hypothesis has been suggested, based upon animal studies that halothane may be metabolized in the liver via a reductive pathway to produce reactive intermediates or metabolites which bind to macromolecules such as lipids and proteins, producing either direct toxicity or a delayed immunological response. All of the oxidative metabolites, trifluoroacetoc acid (TFA), TFA-ethanolamine, and bromide are relatively nontoxic. Recently, Mukai et al.² identified two new volatile metabolites, 2-chloro-1,1,1-trifluoroethane (CTF) and 2-chloro-1,1-difluoroethylen (CDE), in the end-expired breath of rabbits. These compounds have subsequently been identified in the expired breath of humans.⁴ Sharpe, Trudell, and Cohen⁴ have suggested that both CDF and CTF are formed via reductive metabolism of the halothane molecule.

Brown, Sipes, and Baker⁵ have suggested that CTF may be the metabolite that initiates liver damage. However, earlier studies by Ravenos and Lemon⁶ showed that CTF was nontoxic when administered by inhalation. It remains possible that the postulated free radical or carbonion⁷ may initiate cellular damage.⁸ Recent in vitro evidence supports the possibility that the reductive metabolism of halothane is involved in halothane hepatotoxicity. For example, there is quantitatively far greater covalent binding of either ¹⁴C- or ³²⁵Cl-labeled halothane to both microsomal lipid and protein when halothane is administered under hypoxic conditions compared to that observed under normoxia.⁹ This finding indicates that there is irreversible binding during halothane biotransformation and the bound species retains the chlorine within its molecular structure.

When halothane is administered under mild hy-
POXIA (14 per cent inspired oxygen) to rats that have been previously treated with phenobarbital, marked elevation in serum alanine aminotransferase (ALT) and hepatic necrosis is observed in the vicinity of the central veins 24 h following the termination of the halothane anesthetic; both the phenobarbital induction and intraanesthetic hypoxia would be expected to increase the extent of the reductive metabolism of halothane. According to retrospective epidemiological data, it appears that these two factors are not directly implicated clinically. However, it is possible that patients who manifest the signs of halothane hepatotoxicity have a genetic predisposition for high rates of reductive halothane metabolism or may have increased susceptibility to hepatotoxic effects initiated by reductive metabolism.

Previous communications from this laboratory have reported that Fischer 344 rats metabolize halothane in both a qualitatively and quantitatively manner similar to humans. The present communication extends these studies to other rat strains (Wistar and Sprague-Dawley) to determine: 1) if genetic differences in the reductive metabolism of halothane occur under identical halothane exposure conditions, and 2) if there are genetic differences in susceptibility to hepatotoxic effects of halothane.

Materials and Methods

Three different inbred strains of male rats were used in this study: Fischer 344, black hooded Wistar, and Sprague-Dawley. All animals were housed under identical controlled conditions of light and temperature as previously described for at least one week prior to their inclusion in the study. Animals weighed between 200–280 g at the time of the experiments.

Those animals receiving phenobarbital had a daily intraperitoneal injection of sodium phenobarbital (75 mg/kg body weight) for four consecutive days, the last dose given 24 h prior to anesthetic exposure.

The animals were randomly allocated to one of seven treatment groups: Group 1—untreated control breathing room air; Group II—untreated, exposed to hypoxia (14 per cent oxygen, inspired) with nitrogen balance for 2 h; Group III—phenobarbital-pretreated, breathing room air; Group IV—phenobarbital-pretreated, exposed to hypoxia for 2 h; Group V—untreated, exposed to hypoxia and 1 per cent halothane with a nitrogen balance for 2 h; Group VI—phenobarbital-pretreated, exposed to hypoxia and 1 per cent halothane for 2 h; and Group VII—phenobarbital-pretreated, breathing room air with 1 per cent halothane for 2 h.

The day before the exposure, rats were bled (100 μl) via the tail and serum ALT (alanine amino transferase) estimated using an Enzyme TR (True Rate) Analyzer (Beckman Instruments Inc.) operated in accordance with the manufacturers recommendation and incorporating appropriate quality control samples.

During anesthetic exposure, food and water were removed from all animals. Animals in Groups I to VII were exposed under the stated conditions over three days for each strain. Groups I, II, and V were exposed on the first day; Groups III, IV, and VI on the second day, and Group VII on the third day with another set of Group III rats as controls on this day (i.e., day 3). All exposure conditions (i.e., Groups 1–VII) were reproduced on at least two, but usually three, separate occasions (using four rats for each exposure) for each rat strain. Anesthetic exposure was initiated at the same time of the day for all rat strains.

Group I animals were kept in a separate cage in the laboratory while Groups II and V animals were exposed. Group II animals were placed in a perspex chamber (volume ~150 l) with controlled temperature and exposed to 14 per cent O₂ with nitrogen balance for 2 h. Oxygen concentration was checked every 5–10 min via a Centronic® 200 MGA quadrupole mass spectrometer. Group V animals were placed in a perspex anesthetic chamber (volume ~150 l) containing 2 per cent halothane in 98 per cent oxygen with temperature control via an electric blanket. Within 1–2 min, the rats were individually moved into a glass multiport rat mask assembly as previously described. Each port accommodates a rat head up to the level of the ears and a seal was obtained with surgical rubber latex without obstructing respiration. Halothane was delivered to the glass chamber from a previously calibrated Fluotec MK® III vaporizer and the concentration adjusted to 1 ± 0.05 per cent and the oxygen to 14 ± 0.5 per cent using the mass spectrometer; the animals were exposed to these conditions for 2 h. Continuous measurements of the O₂, CO₂, and halothane levels were made using the mass spectrometer. No rebreathing occurred with a fresh gas flow through the mask of 2 l/min (unpublished results). Other exposure conditions were as previously described. Groups III, IV, and VI were exposed in a manner identical to Groups I, II, and V, respectively, except that they were pretreated with phenobarbital as previously described. Group VII animals were exposed on the third day in a manner similar to Group VI animals except that the oxygen concentration was adjusted to 21 ± 0.5 per cent. Another set of Group III rats served as controls for this day.
Animals exposed to halothane in the glass mask assembly had arterialized tail blood samples collected every half hour. This procedure involved warming the tail for 2 min in water at 40°C prior to bleeding.** Blood gas analysis (P\textsubscript{O}, P\textsubscript{CO}, and pH) was carried out using a IL 213 blood gas analyzer; halothane concentration was determined by direct injection and 1 μl blood into a Varian 1440\textsuperscript{a} gas chromatograph as previously described.\textsuperscript{3} All control and treated animals were killed by stunning and decapitation 24 h after termination of the anesthetic treatment and serum collected for ALT levels; liver tissue was taken immediately for histological examination by light and electron microscopy.

The liver tissue was fixed in 10 per cent neutral buffered formalin, then dehydrated through graded alcohols, cleared in acetone, infiltrated and embedded in Taab\textsuperscript{a} araldite. Two-μm sections were stained with hematoxylin and eosin. The liver tissue samples were coded and the pathologist (PH) examined the sections without prior knowledge of the strain of rat or the type of treatment. Hepatocyte damage was defined as cell degeneration (ballooning of the hepatocyte which is possibly reversible) and cell death (acidophilic necrosis now frequently referred to as apoptosis). In addition, the presence of any fat droplets and/or glycogen depletion (confirmed with Periodic Acid Schiff stain) were assessed. Hepatocyte damage, if present, was subjectively graded as mild (focal hepatocyte damage in the region of the central veins), moderate (hepatocyte damage essentially surrounding the central veins), or severe (hepatocyte damage surrounding the central veins and extending further into the lobules). In addition, the presence of an inflammatory response in the areas of damage was noted.

The volatile metabolites, 1-chloro-2,2,-2, trifluoroethane (CTF) and 1-chloro-2,2-difluoro ethylene (CDF), in expired breath of rats were collected using the glass mask assembly as previously described.\textsuperscript{3} Samples were collected at 10-min intervals from the start of halothane administration until 40 min, and then at 20-min intervals until 120 min. The levels of both CDF and CTF in end-expired breath were quantitated by gas chromatography as previously described.\textsuperscript{3}

The paired Student's t test was used to compare the results for paired variables from experiments with a level of significance of at least P < 0.05. An analysis of variance was used for comparisons among various treatment groups of different rat strains.

** Separate studies have shown that blood gas results collected using this technique were comparable to samples collected from the carotid artery (via catheter).
Results

Extent of Reductive Metabolism Under Various Conditions

The time course of formation of CDF and CTF under various conditions using Fischer 344 rats is shown in figure 1. For both volatile metabolites, the levels in end-expired breath were higher when halothane (1 per cent) was administered under mild hypoxia than in air. Phenobarbital treatment of the animals prior to halothane exposure resulted in the expected increase in metabolite levels compared to untreated animals exposed under similar conditions. The levels of CDF in end-expired breath were always less than CTF under similar exposure conditions.

The levels of both volatile metabolites increased soon after commencement of halothane administration and reached a plateau level usually by 60–80 min. These plateau levels (mean ± SD of 60- to 120-min samples) under various exposure conditions are shown in figure 2. The time course of formation for both CDF and CTF in Sprague-Dawley rats was similar to that already shown for Fischer 344 rats. The mean plateau levels (60- to 120-min samples) (fig. 2) of volatile metabolites were similar for Fischer 344 and Sprague-Dawley rats when halothane (1 per cent) was administered under hypoxic condition (table 1). However, the levels of CTF in end-expired breath was significantly higher (table 1) in the Sprague-Dawley strain compared to Fischer rats when halothane was administered in air (with prior phenobarbital pretreatment).

In the Wistar strain, the time course of both CDF and CTF formation (fig. 1) contrasted with the above when halothane (1 per cent) was administered with 14 per cent O₂ to rats pretreated with phenobarbital. Under these conditions, the level of either CDF or CTF was five times higher than in Fischer 344 or Sprague-Dawley rats in the first sample and then decreased progressively in subsequent samples to reach a plateau (mean ± SD of 80- to 120-min samples) not significantly different from the other two strains under similar exposure conditions (fig. 2 and table 1, B vs. E vs. H).

The arterialized tail blood samples collected during halothane exposure had halothane blood concentrations (mean ± SD) of 182 ± 32 mg/l (Sprague-Dawley), 203 ± 27 mg/l (Wistar), and 174 ± 27 mg/l (Fischer 344). Blood-gas analysis of samples from Fischer 344 rats for halothane (1 per cent) administered with 14 per cent O₂, 14 per cent O₂ (phenobarbital pretreatment), and 21 per cent O₂ (phenobarbital pretreatment), respectively, were as follows: Pₐₐ, 52.8 ± 8.0, 47.3 ± 2.9, and 73.8 ± 2.5 torr; Pᵢₐₐ, 46.1 ± 3.8, 46.8 ± 3.4, and 48.8 ± 2.7 torr; and pH 7.47 ± 0.05, 7.46 ± 0.06, and 7.42 ± 0.02. Similar blood-gas results were obtained for Sprague-Dawley and hooded Wistar strains.

Biochemical Changes Resulting from Various Halothane Exposure Conditions

The preexposure serum ALT values for the three different strains were of a similar magnitude, between
Sprague-Dawley and Wistar strains, the postexposure ALT value was approximately double when compared to the preexposure values, but was increased over fourfold for the Fischer 344 strain. When exposure to halothane (1 per cent) in air was combined with phenobarbital pretreatment, only the Fischer 344 strain had a significant increase in postexposure ALT values.

**Pathological Changes Associated with Various Halothane Exposure Conditions**

In all three strains, the animals exposed to halothane (1 per cent) in 14 per cent oxygen (for 2 h) with prior treatment with phenobarbital (Group VI) all showed hepatocyte damage. All of the Fischer 344 strain (fig. 3) and 75 per cent of the Sprague-Dawley and hooded Wistar strains exposed under the above conditions were either moderately or severely damaged (table 3) in the immediate vicinity of the central veins. Mild hepatocyte damage was apparent in the remaining animals of this treatment group. Fine fatty droplets were observed in hepatocytes of many animals; however, gross fatty changes (large lipid droplets) were only observed in the halothane, hypoxia, phenobarbital-induced group. This group (hypoxia exposed) also had the most severe incidence of glycogen depletion but nonspecific glycogen depletion was apparent in most groups. None of the control animals showed any evidence of liver damage. Under normoxic conditions with prior phenobarbital

### Table 2. Pre- and Postexposure Serum ALT (IU/l) Values* for the Three Rat Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sprague-Dawley</th>
<th>Wistar</th>
<th>Fischer 344</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Preexposure</td>
<td>Postexposure</td>
<td>Preexposure</td>
</tr>
<tr>
<td>21 per cent O₂</td>
<td>53.0 ± 13.5</td>
<td>57.7 ± 8.3</td>
<td>59.5 ± 16.0</td>
</tr>
<tr>
<td>21 per cent O₂</td>
<td>68.0 ± 8.2</td>
<td>66.6 ± 2.6</td>
<td>49.5 ± 5.8</td>
</tr>
<tr>
<td>PB pretreated</td>
<td>50.3 ± 4.7</td>
<td>63.5 ± 8.1</td>
<td>50.3 ± 8.1</td>
</tr>
<tr>
<td>14 per cent O₂</td>
<td>89.3 ± 19.3</td>
<td>68.3 ± 5.7</td>
<td>58.9 ± 9.8</td>
</tr>
<tr>
<td>14 per cent O₂</td>
<td>53.3 ± 6.4</td>
<td>63.0 ± 14.5</td>
<td>51.8 ± 5.0</td>
</tr>
<tr>
<td>14 per cent O₂</td>
<td>68.5 ± 10.5</td>
<td>106.9 ± 34.0</td>
<td>52.3 ± 9.9</td>
</tr>
<tr>
<td>14 per cent O₂</td>
<td>68.5 ± 10.5</td>
<td>106.9 ± 34.0</td>
<td>52.3 ± 9.9</td>
</tr>
<tr>
<td>14 per cent O₂</td>
<td>90.3 ± 10.0</td>
<td>86.7 ± 22.8</td>
<td>53.0 ± 11.8</td>
</tr>
</tbody>
</table>

* Serum ALT values are means ± SD.
† Number of determinations for each exposure condition and rat strain.
‡ Preexposure and postexposure values are significantly different from each other at P < 0.025 using a paired Student's t test.
pretreatment, only the Fischer 344 rats exposed to halothane (1 per cent) in air for 2 h had mild hepatocyte damage.

Discussion

There is increasing evidence from investigations in various strains of rats in support of the concept that reductive metabolism of halothane is the central factor in the etiology of halothane hepatotoxicity. Of the various reductive metabolites identified to date, the volatile compounds, CTF and CDF provide a more sensitive index than inorganic fluoride. However, there have been no data concerning strain differences in reductive halothane metabolism or differences in susceptibility to halothane hepatotoxicity.

Studies originating from this laboratory and elsewhere have shown that rats pretreated with phenobarbital and exposed to halothane under hypoxic conditions develop elevated serum transaminase levels and hepatic necrosis in the vicinity of the central veins. Both of these treatments would increase the extent of reductive metabolism of halothane. More recently, Ross et al. have reported that the depletion of hepatic glutathione levels (with diethylmaleate) in Wistar rats prior to exposure to halothane (0.6 per cent) under hypoxic conditions (8 per cent oxygen, inspired) did not result in hepatic pathological damage. McLain et al. have reported that the levels of hepatic glutathione did not change.

<table>
<thead>
<tr>
<th>Extent of Damage</th>
<th>Fischer 344</th>
<th>Sprague-Dawley</th>
<th>Wistar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mild</td>
<td>—</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total Number of Animals with Severe–Moderate Damage</td>
<td>7</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Total Number of Rats Examined</td>
<td>7</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

* The numbers in the table represent the number of animals with either mild, moderate, or severe hepatocyte damage.
from appropriate control values at 3 h or 24 h following exposure to halothane (1 per cent) and hypoxia (14 per cent oxygen, inspired) with prior enzyme induction, implying that glutathione may not exert any protective effect by detoxifying any reactive intermediates formed during halothane biotransformation. However, the levels of cytochrome P450 were not cytochrome b5 were reduced at 24 h following exposure to halothane under hypoxic conditions with prior enzyme induction, suggesting that the reactive intermediates or metabolites formed during the reductive metabolism of halothane exert a specific rather than a generalized effect on hepatic enzymes.

While such factors as enzyme induction and hypoxic inspired oxygen mixtures have not been identified as direct etiologic factors in retrospective clinical epidemiological studies, it is possible that more subtle forms of hypoxia occur in the liver and that susceptible individuals have a genetic predisposition to high rates of reductive halothane metabolism. Moreover, Gorsky and Cascorbi have shown that male Swiss-Webster mice pretreated with phenobarbital and exposed to halothane (1 per cent) under hypoxic conditions (7 per cent oxygen, inspired) failed to develop hepatic necrosis, and this was associated with low levels of halothane reductive metabolism as shown by serum fluoride levels measured at the termination of the anesthetic. These findings support the concept that genetic variations in reductive halothane biotransformations and/or hepatic susceptibility to toxic halothane metabolites may be related to the potential to develop hepatotoxicity following halothane exposure.

Previous studies have shown that the Fischer 344 rat metabolized halothane in a qualitatively similar manner to humans. Further, there were similar amounts of both oxidative and volatile metabolites in Fischer 344 rats and humans at various times following identical halothane exposure conditions. However, the present study shows that there are differences in halothane metabolism when the Wistar rat is compared to the Fischer 344 and Sprague-Dawley rats, particularly under the conditions previously reported to induce halothane hepatotoxicity. Generally, the levels of CDF and CTF in end-expired breath increased from the onset of halothane administration and reached a plateau after approximately 60 min in all three strains under various exposure conditions. The exception to this trend was the Wistar strain exposed to halothane under hypoxic conditions with prior enzyme induction where the levels of CDF and CTF were sharply elevated in the first sample and subsequently declined as the anesthetic progressed (fig. 1) to reach a plateau at 80 min not significantly different from the other two strains. One possible explanation for this apparently anomalous result is that the high initial level of reactive intermediates formed, binds to lipids and proteins in the hepatocyte, thus inhibiting the subsequent metabolism of halothane in the Wistar strain. However, this treatment regime in the Wistar strain does not result in serum ALT or pathological damage in excess of that observed with the other two strains.

Somewhat surprisingly, all three strains had steady-state levels of either CDF or CTF in end-expired breath that were not significantly different following halothane, hypoxia and prior enzyme induction. However, this treatment regime resulted in a four-fold increase in serum ALT in the Fischer 344 strain 24 h following the termination of the anesthetic but only a doubling of serum ALT for the other two strains at identical times. Moreover, there was both a biochemical abnormality (doubling of postexposure serum ALT) and minor pathological damage following halothane with normoxia (21 per cent oxygen, inspired) with prior enzyme induction in the Fischer 344 strain but not in the other two strains. Indeed, the exposure of Sprague-Dawley rats to halothane under normoxic conditions resulted in higher levels of CTF but no evidence of hepatic damage, suggesting that Fischer 344 rats may be more susceptible to halothane’s hepatotoxic effects.

The corresponding levels of either CTF or CDF in the Fischer strain at normoxia with enzyme induction were not significantly different than observed under the exposure condition of halothane and hypoxia alone although the former exposure conditions resulted in minor pathological and biochemical damage, but the latter was not associated with any damage. Halothane, hypoxia with prior enzyme induction was the only treatment condition that resulted in substantial pathological damage, the extent of which by “blind” analysis was comparable between the three strains. All of the Fischer 344 strain and 75 per cent of the Wistar and Sprague-Dawley strains had hepatic damage classified as either severe or moderate.

The results obtained in the present study provide further general experimental evidence in support of reductive metabolism of halothane as the central mechanism in the etiology of halothane hepatotoxicity. The study also indicates that there are strain differences among rats in the reductive metabolism of halothane and susceptibility of the liver to the hepatotoxic intermediates or metabolites formed during halothane metabolism. Some degree of inconsistency between the levels of volatile metabolites (i.e., CDF and CTF) and the presence or absence of biochemical and pathological damage following var-
arious exposure conditions suggests that the routine measurement of these volatile metabolites may be only an indirect index of halothane hepatotoxicity. It is more likely that damage is caused by reactive intermediates and that increase in cellular binding of reactive intermediates may not always be accompanied by parallel increases in CDF and CTF. Support for pharmacogenetics as a factor in human halothane hepatitis is provided by a recent report of halothane hepatitis in three pairs of closely related women.18

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References