Metabolism of Halothane in Obese Fischer 344 Rats

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Halothane is metabolized by an oxidative pathway to stable, non-volatile end products, trifluoroacetic acid (TFAA; CHF₂COOH) and bromide (Br⁻). There is evidence that both oxidatively and reductively formed intermediates may produce hepatotoxicity, although the exact etiology of the fulminant hepatic necrosis seen in humans is unproven. Obese patients receiving volatile anesthetics exhibit higher serum anesthetic metabolite concentrations than do normal-weight patients, and thus might be at greater risk of hepatotoxicity because of higher concentrations of reactive intermediates from halothane metabolism. To eliminate the variables inherent in human clinical studies leading to confounding interpretation of data, this study determined the contributions of oxidative and reductive pathways to halothane metabolism in an animal model of human hypertrophic obesity, the most common form of human obesity. Eight pairs of obese (high-fat diet) and normal-weight (standard chow), male Fischer 344 rats were anesthetized with halothane for 4 h at an inspired concentration of 0.78%. Serum and urinary concentrations of TFAA, Br⁻, and F⁻ were measured. Thirty-six hours following halothane anesthesia, mean serum TFAA concentrations peaked at 7.3 ± 1.1 mm in obese rats and 4.7 ± 0.7 mm in nonobese rats. TFAA urinary excretions during the 180-h period postanesthesia were 519 ± 69 and 336 ± 22 μmol, respectively. Peak serum Br⁻ concentrations were 9.1 ± 1.0 and 6.9 ± 0.6 mm for obese and nonobese rats, respectively, and Br⁻ urinary excretions were 127 ± 30 and 79 ± 14 μmol, respectively. Peak serum F⁻ concentrations of 4.1 ± 1.0 and 4.9 ± 0.6 μM for obese and nonobese rats were not significantly different; F⁻ excretions were not elevated. These data demonstrate that halothane metabolite production via the oxidative pathway is significantly increased in obese compared with nonobese rats. In contrast, there is no evidence for metabolite production via reductive routes in obese rats above that seen in nonobese rats. (Key words: Anesthetics, volatile; halothane. Biotransformation: fluoromethylbromides. Diet: high fat. Ions: fluoride, bromide. Metabolism, halothane; trifluoroacetic acid. Obesity.)

HALOTHANE METABOLISM has been studied extensively in humans and animals because of its possible role in the rare occurrence of a severe and frequently fatal hepatic dysfunction following halothane anesthesia. Both humans and animals metabolize halothane (CF₃CHBrCl) by several metabolic pathways to yield primarily three stable, nonvolatile end products.¹ The oxidative pathway yields tri-

fluoroacetic acid (TFAA; CF₃COOH) and bromide (Br⁻). The nonoxidative (reductive) pathways yield Br⁻ and fluoride (F⁻).

The early animal models of halothane-induced hepatitis focused on the involvement of reductive halothane metabolism induced by hypoxic exposure of enzyme-induced rats for the production of hepatic injury.¹² Relatively recently, an alternative model of halothane hepatotoxicity was proposed by Lunam et al.¹³ in which the extent and incidence of liver damage following halothane exposure in the IMVS (Institute of Medical and Veterinary Science, Adelaide, Australia) guinea pig was not dependent on an hypoxic concentration of inspired oxygen during anesthesia. They suggested that hepatotoxicity may be related to the generation of a trifluorinated acylhalide intermediate via the oxidative pathway.

Studies of obese patients⁴⁻⁶ and animals⁵ have demonstrated significantly enhanced serum metabolite concentrations compared with that in normal-weight controls following administration of volatile anesthetic agents. If one or more reactive intermediates of halothane metabolism are essential in the etiology of hepatotoxicity, it then follows that obese subjects would be at increased risk secondary to enhanced anesthetic metabolism. Indeed, obesity has been implicated as a contributing factor to the halothane hepatotoxicity observed in humans.⁶⁻¹⁰

A number of clinical studies have examined serum metabolite concentrations in obese and nonobese humans after halothane anesthesia. Generally, an increase in products of both oxidative and reductive metabolism has been observed.¹¹ Bentley et al.¹² studied both obese surgical patients undergoing gastric stapling and nonobese surgical patients undergoing lower abdominal surgery. Increased serum F⁻ concentrations were observed in obese patients (approximate peak mean value, 3 μM), whereas normal-weight patients exhibited no change from baseline concentrations (approximate mean, 1 μM). It thus appeared as if only obese patients metabolized halothane via both oxidative and reductive pathways, whereas normal-weight patients exhibited exclusively oxidative metabolism. The authors also observed that serum Br⁻ but not serum TFAA concentrations were elevated in obese compared with nonobese patients. This implied that the rate of oxidative metabolism was the same in both obese and nonobese patients.

Young et al.⁴ also found marked elevations in serum F⁻ concentrations (mean peak value, 10.4 μM) of obese patients after halothane anesthesia, but unfortunately, the control group with which they were compared was historic¹² and not contemporary. The majority of studies
of normal-weight humans\textsuperscript{1,11,13} and animals,\textsuperscript{1,5,14} however, in contrast to those studies showing no change, have documented elevations in serum F\textsuperscript{−} concentrations following halothane anesthesia. These results indicate that reductive metabolism occurs to some extent even in normal-weight subjects.

This study was designed to determine in an animal model of moderate human obesity the quantitative and qualitative differences in halothane metabolism \textit{via} both oxidative and reductive pathways, thus eliminating many of the confounding factors contributing to inherent differences among clinical studies (\textit{e.g.}, type of surgery, duration and concentration, genetics, age, sex). This animal model, as has been previously described,\textsuperscript{7} corresponds closely with human hypertrophic obesity, which is both the most prevalent form and usual extent of obesity\textsuperscript{15} encountered in patients presenting for surgery. The increase in fat mass in rats following sexual maturity occurs by hypertrophy of the central fat globule.\textsuperscript{16} These animals exhibit gross increases in abdominal girth and fat in subcutaneous, omental, and abdominal areas as well as weights approximately 120\% of normal.

\textbf{Materials and Methods}

Eighty male Fischer 344 rats (Hilltop Lab Animals, Inc., Scottsdale, Pennsylvania) were housed in light and temperature controlled conditions. Following 1 week of acclimatization they were ranked and paired according to weight. One member of each pair was randomly assigned to a high-fat diet. [Potter’s high-fat diet (ICN Nutritional Biochemicals, Cleveland, Ohio) was modified to contain (g/100 g diet): peanut butter (Adams Old Fashioned Unsalted Creamy Peanut Butter, Adams Foods, Tacoma, Washington), 20; Crisco, 48; casein 20; DL-methionine, 0.2; sucrose, 2.8; cellulose, 1.6; salt mix W, 4.0; Vitamin Diet Fortification Mixture, 1.76; and liver powder, 1.6. The major nutrient composition was (g/100 g): protein, 26; carbohydrate, 8; and fat, 58. The other member was assigned to a standard rat chow diet [Wayne Lab Blox (Allied Mills, Inc., Chicago, Illinois) with a composition in g/100 g of crude protein 24.6, crude fat 4.4, crude fiber 3.9 and ash 8.0]. Food and drinking water were provided \textit{ad libitum}. After 16 weeks, eight pairs nine months of age and showing the largest weight differences (mean difference = 20\%) were used for this study (obese, 438 ± 20 g; nonobese, 365 ± 18 g). Animals were housed in metabolic cages for one week prior to and for one week after anesthesia. For baseline biochemical measurements, 24-h urine collections were made for four days prior to anesthesia and blood from clipping the tail was obtained two days prior to anesthesia. Animal studies were approved by the animal care and use committees at both Stanford University and the Palo Alto Veterans Administration.

Animals were anesthetized for 4 h with an inspired concentration of 0.78\% halothane (equivalent to approximately 0.75 of MAC) in a 1,000-l plexiglass chamber. Anesthetic chamber concentration was monitored by a Miran 1FF\textsuperscript{®} infrared monitor and was maintained within 5\% of the desired concentration. Inspired oxygen concentration was maintained at 25\%. Rectal temperatures were monitored in representative rats, and a heated water mattress was used to maintain the rats in the normothermic range. In two nonobese and two obese rats evaluated between 3 and 4 h of halothane anesthesia under these same conditions, the following average arterial blood gases were obtained for nonobese and obese rats, respectively: pH, 7.36 and 7.42; pO\textsubscript{2}, 84 and 87; pCO\textsubscript{2}, 50 and 47; SaO\textsubscript{2}, 96\% and 97\%.

Immediately after anesthesia (0 h) and at 12, 16, 20, 36, 60, and 84 h postanesthesia blood was obtained from the tail. Following blood sampling at time 0, rats were returned to metabolic cages where they received drinking water and their regular chow or high fat diet \textit{ad libitum}. A 12-h urine collection was made immediately following anesthesia; thereafter, 24-h urine collections were made. Rats were decapitated following CO\textsubscript{2} sedation 180 h after anesthesia. Blood was collected from the neck; the kidneys, liver, and heart were removed and weighed.

Serum and urine were analyzed for TFAA, Br\textsuperscript{−}, and F\textsuperscript{−}. TFAA and Br\textsuperscript{−} levels were measured by a modified method of Maiorino et al.\textsuperscript{18} as described by Rice et al.\textsuperscript{14} Briefly, 100 \mu l of either serum or urine were mixed with 500 \mu l of 84\% sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) and 100 \mu l of dimethyl sulfate [(CH\textsubscript{3})\textsubscript{2}SO\textsubscript{4}], and incubated for 45 min in a capped Reactivia\textsuperscript{®} (Supelco) at 37° C. Methylated TFAA and Br\textsuperscript{−} from 100 \mu l of headspace gas were separated on a column of 60/80 mesh carbopack B, 1% SP-1000 at 100° C in a Hewlett-Packard 5890A GC with F.I.D. and integrator. Inorganic F\textsuperscript{−} was measured by ion-specific electrode.\textsuperscript{19}

Metabolite excretions per 24 h and for the 180-h period postanesthesia were calculated from urine concentrations and volumes. Serum also was analyzed for GOT and GPT (Sigma Transaminase Kit, for Procedure No. 505) two days prior to anesthesia and at 20 and 36 h postanesthesia.

Data were analyzed by Student’s $t$ test or analysis of variance (ANOVA) with time of sampling as the repeated measure, as appropriate. When differences were identified by ANOVA, specific comparisons were made utilizing Student’s $t$ test for \textit{post hoc} evaluations.

\textbf{Results}

Mean serum TFAA concentrations from obese and nonobese rats peaked 36 h postanesthesia (7.8 ± 1.1 and 4.7 ± 0.7 nM, respectively; fig. 1). Obese rats had significantly higher concentrations compared with nonobese...
Fig. 1. Serum TFAA concentrations (mean ± SD) in obese versus nonobese rats following halothane exposure. TFAA was absent from serum samples prior to anesthesia. All time points following halothane exposure were significantly different between groups (P < 0.001).

Fig. 2. Urinary TFAA excretions (mean ± SD) in obese versus nonobese rats following halothane exposure. TFAA was absent from urine samples prior to anesthesia. Significant differences between groups were present at 60, 84, 132, 156, and 180 h following halothane exposure (P < 0.001).
180-h postanesthetic period was 127 ± 30 μmol for obese rats compared with 79 ± 14 μmol for nonobese rats (P < 0.002; table 1).

Mean serum F⁻ concentrations were higher in nonobese than obese animals before anesthesia reflecting dietary differences (fig. 5). Unlike the other metabolites, serum F⁻ concentrations peaked early, immediately at the end of anesthesia (0 h): 4.9 ± 1.0 and 4.1 ± 0.6 μM for nonobese and obese rats, respectively. Overall, the two groups maintained their preanesthetic differences and peak levels were not significantly different. Urinary F⁻ excretions both prior to and following halothane anesthesia were significantly less for obese rats (P < 0.001; table 1), but there was no significant change in F⁻ excretions from preanesthetic values following halothane exposure.

There were no differences in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) between groups before or after anesthesia; no elevation in serum enzymes was seen postanesthesia in either group. Heart and kidney weights were not significantly different between obese and nonobese rats. Livers of obese rats were significantly heavier than livers of nonobese rats (11.0 ± 0.8 g vs. 8.6 ± 0.5 g; P < 0.002).

**Discussion**

In this study, elevations in serum TFAA and Br⁻ concentrations after halothane anesthesia were significantly greater in obese than in normal-weight Fischer 344 rats. Reflecting serum concentrations, urinary excretion of TFAA and Br⁻ was significantly elevated in obese compared with that from nonobese rats. Urinary TFAA excretion was virtually complete by 180 h after anesthesia. In contrast, urinary Br⁻ excretion was not complete within

**TABLE 1. Urinary Excretion of TFAA, Br⁻, and F⁻ (mean ± SD; n = 8)**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Preanesthesia*</th>
<th>180-h Urinary Excretions (μmol)</th>
<th>Postanesthesia†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obese</td>
<td>Nonobese</td>
<td>Obese</td>
</tr>
<tr>
<td>TFAA</td>
<td>—</td>
<td>—</td>
<td>510 ± 69</td>
</tr>
<tr>
<td>Br⁻</td>
<td>—</td>
<td>—</td>
<td>127 ± 30</td>
</tr>
<tr>
<td>F⁻</td>
<td>12.3 ± 1.7</td>
<td>22.3 ± 5.1‡</td>
<td>11.2 ± 1.7</td>
</tr>
</tbody>
</table>

* For comparative purposes, we calculated 180-h excretion data based on four days of control urinary collections. TFAA and Br⁻ were not detected in urinary samples prior to anesthesia.
† 180-h excretions are sums of the measured excretions during the postanesthetic period.
‡ Obese versus nonobese, P < 0.001.
§ Obese versus nonobese, P < 0.002.
Fig. 4. Urinary bromide excretion rates (mean ± SD) in obese versus nonobese rats following halothane exposure. Br⁻ was not detected in urine samples prior to anesthesia. Significant differences between groups were present at 12, 36, 60, and 156 h following halothane exposure ($P < 0.05$).

The time period of this study (the elimination half-life is approximately 300 h). Serum F⁻ concentrations increased slightly in both groups, but there were no differences between groups in the magnitude of this elevation. Thus, there were quantitative differences in oxidative but not reductive metabolism between the two groups. There are several possible explanations for the increased production of halothane metabolites in obese rats.

Fig. 5. Serum F⁻ concentrations (mean ± SD) in obese versus nonobese rats following halothane exposure. Significant differences between groups were present prior to halothane exposure. These differences were apparent ($P < 0.001$) following halothane exposure until the 60-h collection, which was not different between groups.
compared with normal-weight subjects. Storage of halothane in a much larger fat depot (as is present in this rat model of human obesity) may result in sustained halothane release in the postanesthetic period and thus prolonged availability of halothane for biotransformation. There may also be increased hepatic extraction of halothane in the obese subject, as has been suggested to explain the increased metabolism of enflurane in obese patients. Alternatively, the total amount of drug metabolizing enzymes in the liver of the obese patient may be increased or enzyme affinity may somehow be altered. Studies of liver from obese and nonobese Fischer 344 rats, however, have not demonstrated increased microsomal enzyme concentrations or activities that would result in enhanced halothane metabolism.

The significantly higher serum TFAA concentrations seen in obese compared with nonobese rats are not surprising. Other studies comparing the metabolism of volatile anesthetics in both normal-weight and obese subjects have shown that obese subjects usually exhibit higher anesthetic metabolite concentrations. In contrast, in a clinical study of halothane metabolism, Bentley et al. did not observe significant differences in serum TFAA concentrations between morbidly obese and nonobese patients. Significant differences in oxidative metabolism between moderately obese and nonobese rats in our study of halothane may reflect the more uniform nature of the animal model compared with the heterogeneous nature of the surgical patient population. It is also possible that the significant differences in anesthetic metabolism seen in the clinical study by Bentley et al. were secondary to differences in the surgical site (i.e., upper vs. lower abdominal) and the resulting differences in hepatic blood flow. Perhaps the most significant finding of our animal study is the similarity of increases in serum F- concentrations following halothane exposures suggesting that reductive halothane metabolism in the two groups was equivalent. Until the specific halothane-metabolizing cytochrome P-450 isozymes have been examined in both the obese rat and human, it is impossible to know the absolute parallels in metabolism between these species.

Bromide, Br-, and TFAA all have the potential to produce toxicity. High serum Br- concentrations in the range of 6–12 mM in humans are associated with clinical signs and symptoms of bromidism; no obvious behavioral differences were observed in this rat study. Bromide concentrations in this toxic range have not been reported after halothane anesthesia in patients, obese or otherwise. Increases in serum F- concentrations reflect the extent of reductive metabolism and the production of hepatic reactive intermediates; hepatotoxicity was not present as evidenced by unchanged serum transaminase concentrations in the postanesthetic period. Inorganic F- produces a vasopressin-resistant polyuric renal insufficiency at relatively high serum concentrations (>50 μM); these concentrations are not achieved following halothane administration. Millimolar concentrations of TFAA (as low as 0.4 to 4 mM) can displace drugs from plasma binding sites and thus effectively raise the plasma concentration of free drug. More important, TFAA concentrations may reflect the extent to which oxidative halothane metabolism produces a reactive trifluoroacetyl halide that binds with protein. The trifluoroacetylated protein adducts, recognized by specific antitrifluoroacetyl antibodies, may be important in the etiology of halothane hepatotoxicity. Recent work by Kenna et al. has demonstrated that the serum from patients with halothane hepatitis recognizes hepatic neoantigens containing the trifluoroacetyl moiety derived from halothane.

This study clearly demonstrated enhanced oxidative metabolism of halothane in obese rats. In contrast, there is no evidence for metabolite production via reductive routes in obese rats to a greater extent than that seen in nonobese rats.

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References

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