Halothane Metabolism in Cirrhotic Rats

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A rat model was used to determine whether the metabolism of halothane is changed in the presence of cirrhosis and whether exacerbation of liver dysfunction is correlated with such a change. Cirrhosis was produced by gavaging enzyme-induced male Wistar rats with carbon tetrachloride in corn oil once weekly for 12 weeks. Control rats received corn oil only. After a 3-week period without treatment, blood and urine were collected from each rat for determination of background levels of inorganic fluoride, bromide, and trifluoroacetic acid (halothane metabolites) and for assessment of liver function. Rats were then anesthetized with 1.05% halothane in 50% oxygen for 3 h. Following anesthesia, serial blood and urine samples were taken to monitor halothane metabolism and liver function. No differences were observed between cirrhotic and non-cirrhotic rats in serum levels and urinary excretion of halothane metabolites. However, serum levels of SGOT and SGPT were significantly increased about 1.5-fold in the noncirrhotic group and about 2.5-fold in the cirrhotic group after anesthesia. The increased levels observed in the cirrhotic group were significantly greater than in the noncirrhotic group. The results imply that the exacerbation of liver dysfunction after halothane anesthesia is most likely related to an indirect effect, such as change in liver blood flow, rather than to toxic metabolites. (Key words: Anesthetics, volatile: halothane. Metabolism: oxidative; reductive. Species: rat. Toxicity: acute hepatotoxicity; cirrhosis; liver.)

IN LIEU of human subjects, rats made cirrhotic with carbon tetrachloride (CCL₄) provide a model for the human condition. Furthermore, rats are known to metabolize volatile anesthetics in much the same way as humans. Thus, they are acceptable animals for studying changes of anesthetic metabolism in the presence of cirrhosis and for determining the effects of these changes on liver function.

Halothane (CF₃CHBrCl) is of particular interest in this regard because it has been associated with hepatotoxicity in humans. A commonly proposed hypothesis to account for the hepatotoxicity is that hepatocyte damage is caused by reactive intermediate metabolites generated by halothane’s reductive pathway of metabolism. Studies in rats in which reductive metabolism of halothane has been correlated with hepatic necrosis support such a hypothesis. Thus, increased reductive metabolism of halothane in patients could lead to an increased incidence of hepatic necrosis. Our primary aim in the present study was to determine, in an animal model, whether the reductive pathway of halothane metabolism is enhanced in the presence of cirrhosis, and whether any exacerbation of liver dysfunction is correlated with such a change.

Methods

Twenty-four male Wistar rats, 200–230 g, were individually ear-tagged and bedded on wood-chips in plastic polycarbonate cages covered with stainless steel lids. They were allowed tap water and small animal chow at all times, except during anesthetic exposure, when both were removed. After 2 weeks quarantine, 0.05% sodium phenobarbital was added to their drinking water to produce enzyme induction. One week later, they were divided randomly into two groups, each containing 12 rats. The first group (cirrhotic) was administered CCL₄ to generate cirrhosis according to the method of Proctor and Chatamra. In brief, CCL₄ in corn oil (total volume, 0.5 ml) was administered intragastrically once a week for 12 weeks. The initial dose of CCL₄ for all animals was 0.04 ml, but, thereafter, doses were individualized by basing the new dose on the degree of weight loss following the previous dose. By the twelfth week, doses averaged 0.20 ml (range; 0.12–0.28). After the last dose, phenobarbital was removed from the drinking water. The other group of rats (non-cirrhotic) were handled similarly, but were gavaged with 0.5 ml corn oil only.

Four weeks after the last dose of CCL₄, internal jugular vein catheters were placed in all rats using im ketamine anesthesia (70 mg/kg). Blood, 2.5 ml, was taken from the catheter for determinations of background serum chemistries (SMAC 20) and serum metabolites (Inorganic fluoride, F⁻; Inorganic bromide Br⁻; and Trifluoroacetic acid, TFFA). Rats were then placed in individual metabolic cages and, after a 3-day period to allow time for acclimatization and restoration of blood volume, a 24-h urine collection was made. The next day, both groups were exposed for 3 h to 1.05% (1 MAC in this species) halothane in 50% oxygen. Exposure to the anesthetic was performed in a 1000-L Plexiglas™ chamber. Anesthetic concentration was moni-
TABLE 1. Terminal Body and Selected Organ Weights (g; Mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Terminal Body</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidneys R</th>
<th>Kidneys L</th>
<th>Testes R</th>
<th>Testes L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncirrhotic</td>
<td>455 ± 9.5</td>
<td>17.1 ± 0.6</td>
<td>1.3 ± 0.05</td>
<td>1.8 ± 0.05</td>
<td>1.8 ± 0.05</td>
<td>1.9 ± 0.06</td>
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<td>(n = 12)</td>
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<tr>
<td>Cirrhotic</td>
<td>436 ± 9.5</td>
<td>21.1 ± 1.3*</td>
<td>1.8 ± 0.16*</td>
<td>1.9 ± 0.06</td>
<td>2.0 ± 0.06</td>
<td>1.8 ± 0.05</td>
<td>1.8 ± 0.03</td>
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<tr>
<td>(n = 12)</td>
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* Significantly greater than noncirrhotic; P ≤ 0.01.

tored continuously with a Miran\textsuperscript{TM} 1-F infrared gas analyzer, and kept within 5% of the desired value. Oxygen concentration was monitored continuously with an 1L-402\textsuperscript{TM} oxygen analyzer and maintained between 48 and 52%. The balance of gas in the chamber was nitrogen. Carbon dioxide concentration was intermittently monitored with a Beckman\textsuperscript{TM} LB-2 analyzer, and was kept less than 0.1%. Body temperature was measured continuously in two representative rats from each group and maintained at 37 ± 1°C by means of a warming blanket.

At the end of anesthesia, 1 ml of blood was taken for determination of serum metabolites and rats were returned to their metabolic cages. At 4 and 24 h after anesthesia, a further 1 ml of blood was taken for serum metabolites, and, at 48 h, 2.5 ml of blood was taken for serum metabolites and chemistries. Thus, 4.5 ml of blood was collected over a 48-h period, a comparatively small volume for rats weighing over 400 g. Twenty-four-hour urine collections were made for 3 days.

Seventy-two hours after anesthesia, 1 ml of blood was taken for a final determination of serum metabolites; then, rats were killed by carbon dioxide overdose and autopsied. Tissues were examined in situ, then dissected from the carcass and reexamined. Liver, spleen, kidneys, and testes were weighed and fixed in 10% neutral buffered formalin. Histologic sections were prepared and stained with hematoxylin and eosin. Liver sections were examined and graded for features of cirrhosis and superimposed acute hepatotoxic damage according to accepted criteria.\textsuperscript{10,11} Other tissues were screened for acute and chronic toxic damage by standard histopathological procedures. All examinations were done without knowledge of treatment.

Inorganic fluoride in blood and urine was measured with an ion-specific electrode.\textsuperscript{12} Bromide and TFAA were measured by the gas chromatographic method of Maiorino \textit{et al.}\textsuperscript{13} One hundred microliters of either serum or urine were mixed with 500 \( \mu \)l of 84% sulphuric acid, and 100 \( \mu \)l of dimethyl sulphate, and incubated for 45 min in a capped vial at 37°C. Methylated TFAA and \( \text{Br}^- \) from 100 \( \mu \)l headspace were separated on a carbopack column at 100°C in a Hewlett-Packard\textsuperscript{TM} 5830A GC with integrator. Metabolite excretions per 24 h were calculated from urine volumes.

Student's paired or unpaired \( t \) tests with the Bonferroni correction for multiple comparisons were used to determine differences between the groups; \( P < 0.05 \) was considered statistically significant.

**Results**

All rats survived exposure to 1 MAC halothane for 3 h and appeared to regain normal activity and health within several hours of anesthesia.

Body weight of cirrhotic rats was slightly less at death than that of noncirrhotic rats (table 1). Weights of liver and spleen of cirrhotic rats were significantly higher than those of noncirrhotic rats, in keeping with the diagnosis of cirrhosis and portal hypertension (table 1). In addition, ascites greater than 10 ml was present in six rats with cirrhosis. There were no group differences in the weights of the kidneys and testes (table 1).

Serum levels of \( \text{Br}^- \) and TFAA increased after anesthesia, with peak levels occurring at about 24 h (fig. 1). A similar pattern of urinary excretion of \( \text{Br}^- \) and TFAA was seen after anesthesia, with the highest rate of excretion occurring in the first 24 h (fig. 2). Serum levels and urinary excretion of \( \text{F}^- \), however, did not

![Fig. 1. Serum bromide (Br\textsuperscript{−}) and trifluoroacetic acid (TFAA) concentrations (mean ± SEM) from noncirrhotic (noncir) and cirrhotic (cir) rats before and after 1.05% halothane for 3 h.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931382/ on 06/22/2017)
increase significantly above control levels (tables 2, 3). No differences were seen at any sample point between noncirrhotic and cirrhotic groups in serum levels and urinary excretion of these metabolites.

The albumin/globulin ratio in cirrhotic rats was significantly decreased and the total bilirubin significantly increased before anesthesia compared with values in the noncirrhotic rats. The values did not change significantly after anesthesia for either group. In contrast, SGOT and SGPT were not different between groups before anesthesia, but increased significantly in both groups after anesthesia (table 4). Moreover, the percentage increase in values was significantly greater in the cirrhotic group than in the noncirrhotic group (fig. 3).

Histological examinations of livers from CCl₄-treated rats revealed moderate degrees of cirrhosis in all animals. Neither livers from cirrhotic rats nor those from noncirrhotic rats showed evidence of acute hepatotoxicity. Sections from spleen, kidneys, and testes were normal.

Discussion

Repeated administration of CCl₄ to rats has been used as a model of cirrhosis for over 50 yr. Although there are some important differences between CCl₄-induced cirrhosis in rats and cirrhosis in humans, many features are the same. In particular, the histological appearance of advanced cirrhosis in rats is the same as micronodular or alcoholic cirrhosis in humans; namely, there are regenerative nodules, less than 3 mm in diameter, separated by thick plates of collagen. The histological progression of cirrhosis in rats is well described, and can be used as a measure of severity. In mild cases, sections show prominence of Kupffer cells, a few proliferating bile ducts, and a few bands of fibrous tissue dividing otherwise normal lobules. In moderate cases, the fibrosis is more marked and divides the lobules into small groups of cells; very little normal tissue remains. In the most severe form, the fibrous plates are thicker and there is extensive fibrosis of portal tracts, with pronounced cellular infiltration and proliferation of the bile ducts. By these histological criteria, most of our rats had moderate degrees of cirrhosis. When sections from these rats were compared blindly with those from our previous study, the average severity of cirrhosis was much greater. The most likely reason for the difference between the studies was our increased experience in judging the appropriate dose of CCl₄ required for generating cirrhosis.

Although cirrhosis is strictly a histological diagnosis, its presence can be inferred from such clinical signs as hepatomegaly, splenomegaly, and ascites, and such biochemical markers as a low serum albumin/globulin ratio and an elevated serum bilirubin level. These features were all present in our rats with cirrhosis. Levels of the serum transaminases, SGOT and SGPT, are not usually elevated in cirrhosis unless an active process is occurring. Thus, in the present study and in our previous studies, once the acute effects of CCl₄ had subsided in rats with cirrhosis, levels of SGOT and SGPT returned to normal.

Halothane is metabolized in humans and animals both oxidatively and reductively. Metabolic end-products of the oxidative pathway are Br⁻ and TFAA, whereas those of the reductive pathway are Br⁻ and F⁻. Although quantitatively less important than the oxidative pathway, the reductive pathway may have greater implications for toxicity, since it involves the production
of reactive intermediates which are capable of binding
to tissue macromolecules. In particular, many investiga-
tors believe that the reductive pathway is a key factor
in the production of "halothane-associated hepatitis." 
Certainly, in the hypoxic rat model of halothane hepa-
titis, binding of reactive intermediates to liver tissue
and production of free radicals can be correlated with
the degree of liver injury.7,16,17

However, although the reductive pathway of hal-
thane metabolism in normal liver seldom, if ever,
generates sufficient toxic metabolites to produce significant
liver damage, the situation in diseased liver could
be different. In particular, cirrhotic livers have abnormal
and often reduced blood flow with areas of relative hy-
poxia.18 Thus, one could hypothesize that the reductive
pathway would be favored with increased production of
F⁻ and reactive intermediates which could covalently
bind to liver tissue and, hence, cause acute liver injury.
Results of the present study with moderately cirrhotic
rats do not support this hypothesis, since there were no
qualitative or quantitative changes in halothane metab-
olism and no morphologic evidence of acute liver
damage.

Nevertheless, in the present study, the detrimental
effects of halothane on liver function were greater in
the cirrhotic than in the noncirrhotic rats, as evidenced
by higher levels of SGOT and SGPT. This finding was
somewhat at variance from those of previous studies, in
which we found that halothane and other volatile anes-
thesics worsened liver function, but to the same extent
in cirrhotic and noncirrhotic rats.14,15 The more likely
explanation for the different results is that greater
degrees of cirrhosis were achieved in the present study.
If this explanation is correct, it raises the possibility that
the degree of post-anesthetic liver dysfunction is posi-
tively correlated with the degree of preexisting liver
disease. A less likely reason for the difference in results
could lie in the method of generating the cirrhosis; in
our first two studies, the inhalational route of exposure
to carbon tetrachloride was used, whereas, in the
present study, the gastric route was used. We could not
test either hypothesis in the present study because of the
uniformity of liver cirrhosis achieved and the compara-
tively small group sizes used.

The mechanism whereby halothane exacerbated liver
dysfunction in cirrhotic rats in the present study is yet to
be determined. Failure of the moderately cirrhotic liver
to alter the pattern of metabolism of halothane and, in
particular, to enhance its reductive pathway suggests
that direct toxicity from metabolites is not involved. An
alternative hypothesis is that hypoxia itself during hal-
thane anesthesia results in acute damage to cirrhotic

<table>
<thead>
<tr>
<th>Group</th>
<th>A/G Ratio</th>
<th>SGOT</th>
<th>SGPT</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Noncirrhotic (n = 12)</td>
<td>1.3 ± .06</td>
<td>1.1 ± .01</td>
<td>50 ± 7</td>
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<tr>
<td>Cirrhotic (n = 12)</td>
<td>0.9 ± .02$§</td>
<td>0.9 ± .02$§</td>
<td>65 ± 6</td>
</tr>
</tbody>
</table>

* Albumin/globulin ratio.
† in IU/L.
§ Significantly different from noncirrhotic values (P ≤ 0.01).
¶ Significantly greater than preanesthetic values (P ≤ 0.01).
livers. The same hypothesis has been proposed to account for halothane-induced liver injury seen in rats with normal livers.10–21 The hypoxia may be due to reduced liver blood flow which is known to occur in normal liver during halothane anesthesia.22

References