Anesthesiology
67:660–664, 1987

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 noncirrhotic 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 (CCl_4) provide a model for the human condition.[1] Furthermore, rats are known to metabolize volatile anesthetics in much the same way as humans.[2] 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 ($CF_3CHBrCl$) is of particular interest in this regard because it has been associated with hepatotoxicity in humans.[3,4] 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.[5,6] Studies in rats in which reductive metabolism of halothane has been correlated with hepatic necrosis support such a hypothesis.[7] 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 CCl_4 to generate cirrhosis according to the method of Proctor and Chatamra.[8] In brief, CCl_4 in corn oil (total volume, 0.5 ml) was administered intragastrically once a week for 12 weeks. The initial dose of CCl_4 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 CCl_4, 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, TFAA). Rats were then placed in individual metabolic cages and, after a 3-day period to allow time for acclimitization 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[9]) halothane in 50% oxygen. Exposure to the anesthetic was performed in a 1000-L Plexiglas™ chamber. Anesthetic concentration was moni-

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Received from the Department of Anesthesia, Stanford University School of Medicine (SUSM), Stanford, California; and the Anesthesiology Service, Veterans Administration Medical Center (VAMC), Palo Alto, California. Accepted for publication June 3, 1987. Supported by the Department of Anesthesia, Stanford University School of Medicine, the Veterans Administration and the Anesthesia/Pharmacology Research Foundation.

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TABLE 1. Terminal Body and Selected Organ Weights (g; Mean ± SEM)

Group	Terminal Body	Liver	Spleen	Kidneys		Testes	
				R	L	R	L
Noncirrhotic (n = 12)	453 ± 9.5	17.1 ± 0.6	1.3 ± .05	1.8 ± .05	1.8 ± .05	1.9 ± .06	1.9 ± .06
Cirrhotic (n = 12)	436 ± 9.5	21.1 ± 1.3*	1.8 ± .16*	1.9 ± .06	2.0 ± .06	1.8 ± .05	1.8 ± .03

* Significantly greater than noncirrhotic; $P \leq 0.01$.

tored continuously with a Miran™ 1-F infrared gas analyzer, and kept within 5% of the desired value. Oxygen concentration was monitored continuously with an IL-402™ 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™ 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.[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.[12] Bromide and TFAA were measured by the gas chromatographic method of Maiorino *et al.*[13] One hundred microliters of either serum or urine were mixed with 500 μl of 84% sulphuric acid, and 100 μl of dimethyl sulphate, and incubated for 45 min in a capped vial at 37° C. Methylated TFAA and Br^- from 100 μl headspace were separated on a carbopack column at 100° C in a Hewlett-Packard™ 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 Br^- and TFAA increased after anesthesia, with peak levels occurring at about 24 h (fig. 1). A similar pattern of urinary excretion of 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 F^-, however, did not

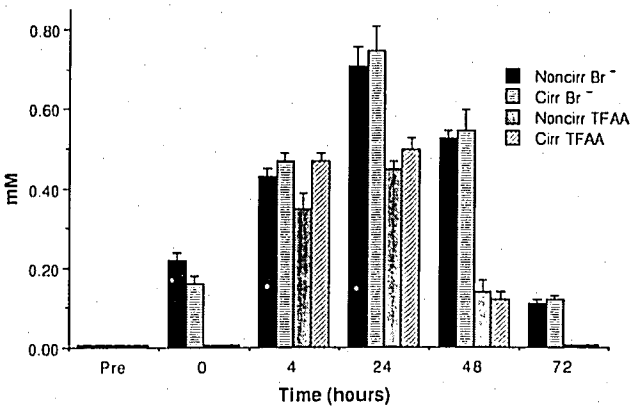

FIG. 1. Serum bromide (Br^-) and trifluoroacetic acid (TFAA) concentrations (mean ± SEM) from noncirrhotic (noncirr) and cirrhotic (cir) rats before and after 1.05% halothane for 3 h.

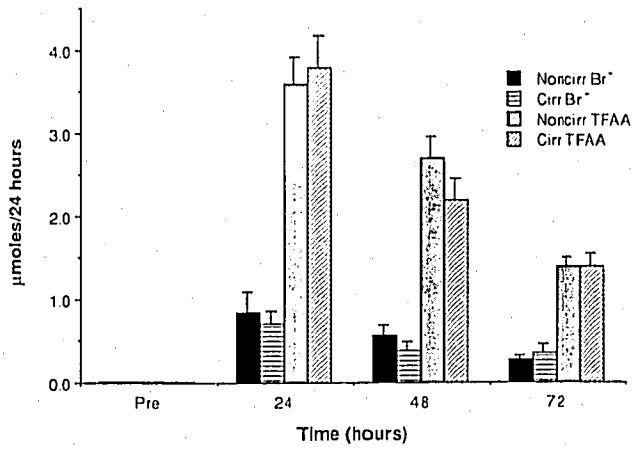

FIG. 2. Twenty-four-hour urinary excretions (mean ± SEM) of bromide (Br^-) and trifluoroacetic acid (TFAA) from noncirrhotic (noncirr) and cirrhotic (cir) rats before and after 1.05% halothane for 3 h.

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 CCl_4-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 CCl_4 to rats has been used as a model of cirrhosis for over 50 yr.[1] Although there are some important differences between CCl_4-induced cirrhosis in rats and cirrhosis in humans, many features are the same.[1] 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,[11] 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,[14] 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 CCl_4 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,[14,15] once the acute effects of CCl_4 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.[5] 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

TABLE 2. Serum Fluoride Concentrations Before and After Halothane Anesthesia (μM; Mean ± SEM)

Group	Pre	Post (H)				
		0	4	24	48	72
Noncirrhotic (n = 12)	2.7 ± .4	3.5 ± .7	3.8 ± 4.5	2.2 ± .4	2.1 ± .5	2.0 ± .3
Cirrhotic (n = 12)	2.2 ± .3	2.4 ± .2	2.6 ± .34	2.0 ± .5	2.3 ± .2	1.8 ± .1

TABLE 3. Urinary Fluoride Excretion Before and After Halothane Anesthesia (μmoles/24 H; Mean ± SEM)

	Pre	Post (Days) 1	2	3
Noncirrhotic (n = 12)	2.6 ± .3	3.8 ± .2	2.7 ± .1	2.3 ± .2
Cirrhotic (n = 12)	3.5 ± .3	4.1 ± .4	2.8 ± .2	2.5 ± .2

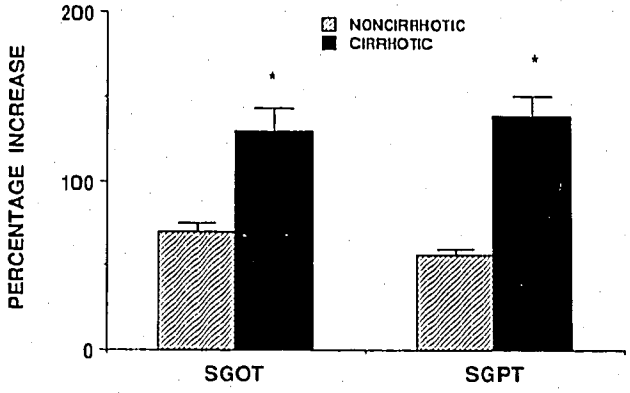

FIG. 3. Percentage increase (mean ± SEM) of SGOT and SGPT values in noncirrhotic and cirrhotic rats after 1.05% halothane for 3 h. * Indicates that the increase is significantly greater in the cirrhotic compared with noncirrhotic rats at the $P \leq 0.05$ level.

of reactive intermediates which are capable of binding to tissue macromolecules.[5] In particular, many investigators believe that the reductive pathway is a key factor in the production of "halothane-associated hepatitis." Certainly, in the hypoxic rat model of halothane hepatitis, 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 halothane 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 hypoxia.[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 metabolism 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 anesthetics 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 positively 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 comparatively 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 halothane anesthesia results in acute damage to cirrhotic

TABLE 4. Selected Serum Chemistry Values Before and After Halothane Anesthesia (Mean ± SEM)

Group	A/G Ratios* Pre	A/G Ratios* Post	SGOT† Pre	SGOT† Post	SGPT† Pre	SGPT† Post	Total Bilirubin‡ Pre	Total Bilirubin‡ Post
Noncirrhotic (n = 12)	1.3 ± .06	1.1 ± .01	50 ± 7	85 ± 7¶	36 ± 3	56 ± 4¶	0.2 ± .1	0.2 ± .1
Cirrhotic (n = 12)	0.9 ± .02§	0.9 ± .02§	65 ± 6	149 ± 16§¶	41 ± 5	98 ± 11§¶	1.0 ± .1§	1.3 ± .3§

* Albumin/globulin ratio.
† In IU/L.
‡ In mg/dl.
§ Significantly different from noncirrhotic values ($P \leq 0.01$).
¶ Significantly greater than preanesthetic values ($P \leq 0.01$).

livers. The same hypothesis has been proposed to account for halothane-induced liver injury seen in rats with normal livers.[19–21] The hypoxia may be due to reduced liver blood flow which is known to occur in normal liver during halothane anesthesia.[22]

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