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Hepatic Effects of Repeated Halothane Anesthetics in the Hypoxic Rat Model

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The hepatic effects of repeated anesthesia of phenobarbital-induced Fischer 344 rats with 1% halothane/14% oxygen were investigated, after anesthetics were administered at either 1-day or 5-day intervals. Urinary excretion of fluoride, a product of reductive halothane metabolism, was increased in the 24-h period following anesthesia, but was the same after the first, second, and third anesthetics. Rats killed 24 h after a single anesthetic all had centrilobular hepatocellular necrosis. All animals killed 24 h after the second or third anesthetic also had centrilobular necrosis, but, in most animals, this was no more extensive than that following a single anesthetic, regardless of whether the interval between anesthetics was one or five days. (Key words: Anesthetics, volatile: halothane. Liver: hepatotoxicity.)

REPEATED EXPOSURE to halothane over a short period is widely accepted to be a risk factor for halothane hepatitis.[1] Although the mechanism by which repeated administrations predispose to halothane hepatotoxicity is unknown, it has been suggested that the first exposure in some way sensitizes hepatocytes, such that a subsequent exposure initiates injury.[1] Another possibility is that a first exposure leads to induction of drug metabolizing enzymes in the liver, so that metabolism of halothane to potentially toxic metabolites is increased after a second exposure.[2]

The present study investigated the effects of repeated halothane anesthetics in the hypoxic rat model. In this model, phenobarbital-induced rats are anesthetized for 2 h with 1% halothane in oxygen:nitrogen, 14:86. Although the response varies among different rat strains, with Fischer 344 rats, all animals develop centrilobular hepatocellular necrosis within 24 h.[3] Liver injury is associated with formation of toxic halothane metabolites by the reductive pathway,[4,5] a minor pathway of halothane metabolism in humans and rats. Halothane metabolism and liver injury were assessed during repeated (from zero to three) halothane anesthetics at intervals of 1 or 5 days. These time intervals were chosen so that re-exposure would occur when the previous injury was near maximal or essentially completely repaired.¶

Methods

ANIMALS

Male Fischer 344 rats were used in all experiments. Animals ages ranged from 83–117 (mean, 95) days at the commencement of the experiments. Enzyme induction was brought about by addition of sodium phenobarbital, 0.1 g/l, to the drinking water 7 days prior to the first scheduled exposure (to halothane/hypoxia or hypoxia). To minimize the CNS depressant effects of phenobarbital during the period of anesthesia, the phenobarbital solution was withdrawn from all rats (including controls) 2 h prior to each exposure, but otherwise was continuous until the last exposure of the series. After the last exposure, rats were given plain tap water to drink until they were killed, which, unless otherwise stated, was 24 h after the beginning of the last exposure. Animals were allowed free access to food (M and V Cubes, Milling Industries, South Australia), except during the exposure periods.

TIME-COURSE OF FLUORIDE EXCRETION AFTER HALOTHANE ANESTHESIA

Twenty-two phenobarbital-induced rats were randomly assigned to receive 2 h of 1% halothane in oxygen:nitrogen 14:86 (halothane/hypoxia) (n = 13) or 2 h of oxygen:nitrogen 14:86 (hypoxia) (n = 9). Animals were placed into individual plastic metabolic cages for collection of urine for 1 day prior to exposure and 3 days after exposure. The metabolic cages were replaced with clean ones each day to prevent carryover contamination of the urine.

REPEATED HALOTHANE EXPOSURES

Sixty phenobarbital-induced rats were randomly divided into six groups of ten. These received either three

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¶ Hall P, Cousins MJ, Knights KM, Gourlay GK: Halothane hepatitis in an animal model: Time course of hepatic damage (abstract). Hepatology 2:131, 1982

TABLE 1. Treatment Protocol for Repeated Anesthetics—1-day Interval

Group	Treatment		
	Day 1	Day 2	Day 3
HHH	+	+	+
CHH	−	+	+
CCH	−	−	+
CCC	−	−	−
CHC	−	+	−
HCC	+	−	−

Treatment Codes: + = 2 h of halothane/hypoxia; − = 2 h of hypoxia.

Group Name Codes: H = Halothane/hypoxia exposure; C = Control (i.e., hypoxia exposure).

exposures to halothane/hypoxia, three exposures to hypoxia, or some combination of these, at 1-day intervals, as shown in table 1. Rats were placed in clean plastic metabolic cages for 22 h after the end of each exposure, for collection of urine. Animals were killed 22 h after the end of the last exposure. Blood was collected for measurement of serum bromide concentration (see below) and serum alanine aminotransferase (ALT) activity (Cobas Autoanalyser, Hoffman-La Roche, Basle, Switzerland), and liver tissue was taken from the median lobe. Groups CHC and HCC were included only to establish the appearance of the liver 2 or 3 days after a single anesthetic. Halothane metabolite data are not presented for these groups.

A similar experiment was carried out with a 5-day interval between each exposure. Treatments were again similar to those in table 1, except that the interval between exposures was longer and groups CHC and HCC were not included.

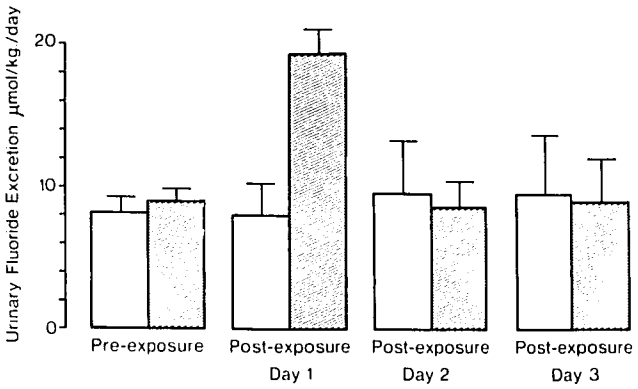

FIG. 1. Effect of halothane anesthesia on fluoride excretion. At the beginning of day 2, animals were exposed for 2 h to hypoxia alone (open bars) or halothane/hypoxia (hatched bars). Mean and 95% confidence limit of mean, n = 9 (hypoxia) or 13 (halothane/hypoxia).

MEASUREMENT OF HALOTHANE METABOLITES

Serum bromide concentration[6] and urinary fluoride concentration[7] were measured with ion selective electrodes (Orion Research Inc., Cambridge, MA). Urinary fluoride excretion was expressed as micromoles excreted per day per kg body weight, as, in our experience, this is more consistent among rats than unadjusted fluoride excretion.

HISTOPATHOLOGY

Liver tissue was fixed in 10% buffered formalin (*p*H 7.0), dehydrated through graded acetones and infiltrated and embedded in epoxy resin. Two-micron sections were stained with hematoxylin and eosin and coded. Liver sections were examined by a pathologist (PH) who was unaware of the treatment groups to which the animals belonged. Sections were categorized according to whether liver injury was present or absent, and, if present, the types of changes and their severity were noted.

STATISTICAL ANALYSIS

Body weight changes and serum bromide concentrations following one, two, or three anesthetics were analyzed by a non-parametric test for trend.[8]

Urinary fluoride excretion during repeated anesthetics was analysed using the regression approach to repeated measures analysis of variance.[9] Initially, a full model accounting for variation in fluoride excretion among and within rats, as well as variation due to number of anesthetics, was fitted. Any effect on flouride excretion due to the number of anesthetics administered (one, two, or three) was assessed by testing the significance of the difference in residual sums of squares between the full model and a reduced model, in which number of halothane administrations was dichotomized (*i.e.*, no anesthetics *vs.* one or more anesthetics). The significance of the sums of squares change on dropping this dichotomized variable indicated whether fluoride excretion was increased following halothane anesthesia (regardless of the number of anesthetics).

Results

FLUORIDE EXCRETION FOLLOWING HALOTHANE ANESTHESIA

Daily fluoride excretion from rats exposed to hypoxia only was constant over the 4 days of urine collection. On the day following exposure to halothane/hypoxia, fluoride excretion was more than doubled, but returned to baseline values on the second day (fig. 1),

demonstrating that excretion of fluoride formed by metabolism of halothane does not continue for more than 1 day.

Changes in Body Weight during Repeated Anesthetics

Rats exposed to halothane at 1-day intervals lost an average of 35 ± 4 (mean ± 95% confidence limits of mean) grams of body weight during the experiment. There was no significant trend toward greater weight loss as more anesthetics were administered ($P > 0.2$), suggesting that phenobarbital administration was a more important factor affecting weight loss than was anesthesia.

Animals which were exposed at 5-day intervals lost an average of 25 ± 4 grams of body weight during the experiment. Again, there was no significant evidence of a trend toward greater weight loss as more anesthetics were administered ($P > 0.05$).

Halothane Metabolism during Repeated Anesthetics

Serum bromide concentrations of all animals which had not received halothane were below the detectable limit (0.2 mM). When anesthetics were administered at 1-day intervals, serum bromide concentrations showed a significant ($P < 0.01$) increase almost in direct proportion to the number of anesthetics administered (fig. 2). Fluoride excretion was significantly increased following halothane anesthesia ($P < 0.01$), but this increase was independent of the number of anesthetics previously administered ($P > 0.5$) (fig. 3), indicating that similar amounts of fluoride were formed during each anesthetic.

When the interval between exposures was increased to 5 days, serum bromide concentrations again increased significantly ($P < 0.01$) as further anesthetics were given, but the extent of accumulation (from 3.02 ± 0.44 mM, mean ± 95% confidence interval, after a single anesthetic, to 5.15 ± 0.28 mM after a third anesthetic) was lower than when anesthetics were given at 1-day intervals. Fluoride excretion was essentially the same as in the 1-day interval experiment, with no evidence of altered fluoride formation after a second or third anesthetic ($P > 0.1$) (fig. 4).

Effects of Repeated Halothane Anesthetics on the Liver

In the rats exposed at 1-day intervals, serum alanine aminotransferase (ALT) activities were 68 ± 9 (mean, 95% confidence limits of mean) IU/L in animals which had not received any anesthetics (group CCC), and 390 ± 223 after one anesthetic (group, CCH). After two anesthetics, ALT had risen to 3200 ± 900 (group CHH), and, after three, fell somewhat to 1800 ± 700 (group HHH). These latter figures may not reflect the

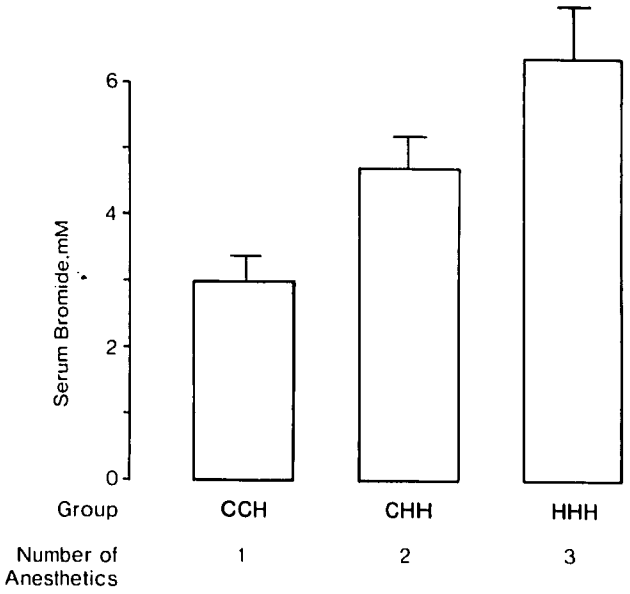

FIG. 2. Effect of repeated halothane anesthetics at 1-day intervals on serum bromide concentration. Mean and 95% confidence limits of mean, n = 10.

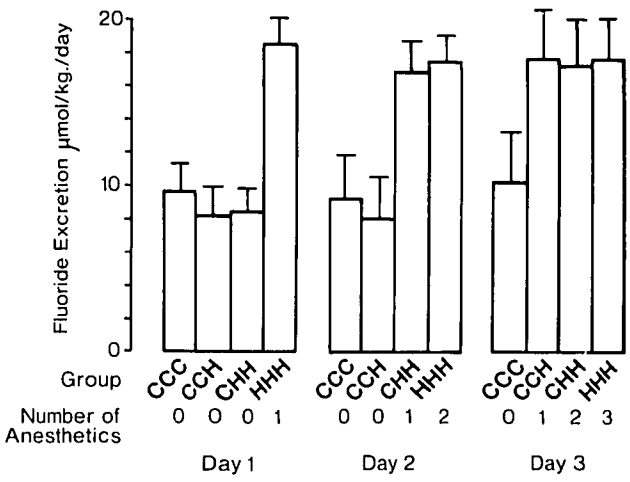

FIG. 3. Urinary fluoride excretion by four groups of rats undergoing repeated exposures at 1-day intervals. Group CCC received hypoxia only on days 1, 2, and 3. Group CCH received hypoxia on days 1 and 2 and halothane/hypoxia on day 3. Group CHH received hypoxia on day 1 and halothane/hypoxia on days 2 and 3, while group HHH received halothane/hypoxia on days 1, 2, and 3. Mean and 95% confidence limits of mean, n = 10, except for group CHH, for which n = 8.

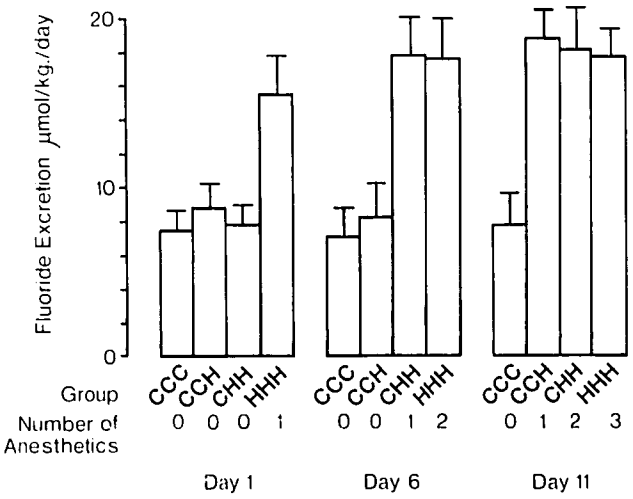

FIG. 4. Urinary fluoride excretion by four groups of rats undergoing repeated exposure at 5-day intervals. Group CCC received hypoxia only on days 1, 6, and 11. Group CCH received hypoxia on days 1 and 6, and halothane/hypoxia on day 11. Group CHH received hypoxia on day 1, and halothane/hypoxia on days 6 and 11. Mean and 95% confidence interval of mean, n = 10.

extent of liver injury due to the repeated anesthetics, however, as serum ALT continues to increase for more than 24 h after one anesthetic.[10]

When a 5-day interval was allowed between exposures, serum ALT of the rats which received no anesthetics (57 ± 8) and those which had received one anesthetic (330 ± 90) were similar to those of the 1-day interval experiment. Serum ALT activities after two anesthetics (420 ± 400), however, were now similar to those following a single anesthetic, but had again fallen somewhat after three anesthetics (220 ± 110).

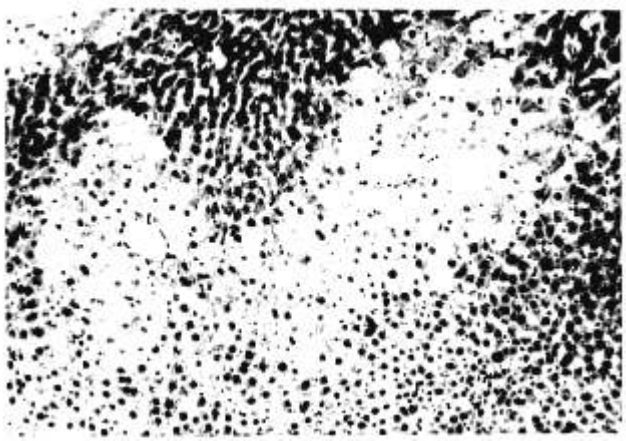

FIG. 5. Liver from a rat killed 1 day after a single halothane anesthetic (group CCH). Centrilobular necrosis is apparent; occasional liver cells at the periphery of the necrotic zone show ballooning degeneration. H&E × 145.

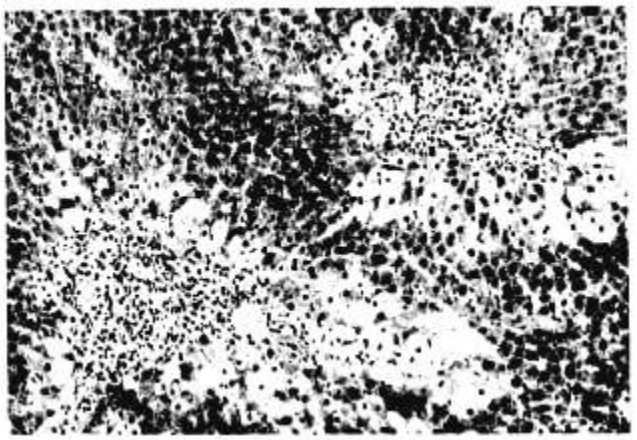

FIG. 6. Liver from a rat killed 1 day after three halothane anesthetics given at 1-day intervals (group HHH). Two areas of hepatocyte necrosis and lysis with an associated infiltrate of lymphocytes and macrophages are apparent. Hepatocytes at the periphery of these areas show ballooning degeneration. H&E × 145.

LIVER PATHOLOGY

Anesthetics Administered at 1-day Intervals. None of the livers from rats which had not received halothane (group CCC) showed hepatocellular degeneration or necrosis. Livers of rats killed 1 day after a single anesthetic (group CCH) all had confluent hepatocellular necrosis around the terminal hepatic venules, and focal ballooning degeneration of hepatocytes was seen at the periphery of the necrotic zones (fig. 5). The inflammatory response to liver injury was either minimal or mild and focal. The livers of animals killed 2 days after a single anesthetic (group CHC) all showed centrilobular necrosis with lysis of some necrotic liver cells and a moderately heavy infiltrate of lymphocytes and macrophages. Mitoses were frequent, indicating liver cell regeneration. The livers of animals killed 3 days after a single anesthetic (group HCC) showed similar appearances to group CHC, but had widespread lysis of damaged cells, a more marked inflammatory response, and more numerous mitoses. The inflammatory response and repair processes complicated the comparison of the single anesthetic groups with the repeated anesthetic groups. However, it was clear that, with the exception of two animals in group CHH described below, hepatic injury following a second (group CHH) or third (group HHH) anesthetic was no more extensive than after the first anesthetic. In fact, some animals in group HHH showed only focal ballooning degeneration at the periphery of the zone of injury related to previous anesthetics (fig. 6).

Two rats (in group CHH) developed more extensive necrosis after a second anesthetic; the liver injury ex-

tended into the mid regions of the liver lobules, but spared the periportal regions. Occasional neutrophil polymorphs were scattered through the necrotic tissue. One of these rats was found to be ill on the morning following its second anesthetic; consequently, it was killed approximately 1 h before the scheduled time. The serum bromide concentration in this rat was 4.03 mM, somewhat below the mean value (4.70, S.D. 0.63) for group CHH. This animal had not excreted any urine after its second anesthetic, and, therefore, fluoride excretion data are not available. The second rat showed no outward signs of illness, and was killed at the scheduled time. It had a serum bromide concentration of 4.14 mM; again, below the group mean. This animal did excrete urine after its second anesthetic, but the amount of fluoride excreted was only 0.7 micromol/kg/day. This is greatly below the amount normally excreted by control rats, which is usually in the range 6–10 micromol/kg/day. This suggests that this animal was unable to excrete fluoride. Fluoride excretion by both of these animals is excluded from figure 3.

Anesthetics Administered at 5-day Intervals. None of the rats which had two or three anesthetics at 5-day intervals had massive liver injury, and, in many animals, the injury was less severe than seen in those which had a single anesthetic. In many of the livers from group HHH animals, hepatocytes around the terminal hepatic venules showed prominent ballooning degeneration, and only occasional liver cells showed frank necrosis (fig. 7). There was little or no residual evidence of liver injury associated with the previous anesthetics.

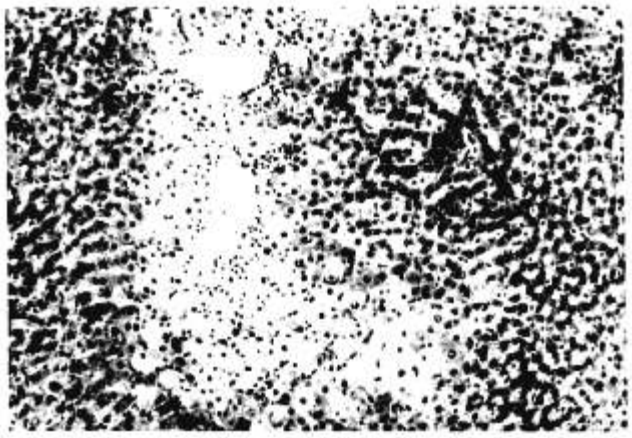

FIG. 7. Liver from a rat killed 1 day after three halothane anesthetics given at 5-day intervals (group HHH). Hepatocytes around the terminal hepatic venules show ballooning degeneration, and there is a mild infiltrate of lymphocytes. H&E × 145.

Discussion

In many reported cases of halothane hepatitis, patients have been anesthetized with halothane on more than one occasion over a short period, giving rise to the suggestion that a first exposure renders the liver more susceptible to injury from a subsequent exposure.[1] This study shows that the usual response of the hypoxic rat model is different, with similar injury occurring after the first, second, and third anesthetics, regardless of whether re-exposure occurs before or after recovery from the previous injury. In accord with this finding, halothane metabolism is also unaffected by repeated exposures. In the hypoxic rat model, liver injury is associated with intermediates formed during the reductive metabolism of halothane.[4,5] Excretion of fluoride, a product of this pathway, was the same after one, two, or three anesthetics. Our data are insufficient to allow a precise comparison of the extent of oxidative metabolism during repeated anesthetics. Bromide, a product of this pathway, has a long half-life in the body, and accumulates when repeated halothane anesthetics are administered. Our data are consistent with little change in bromide formation.

Earlier studies have shown that concentrations of cytochrome P-450 in liver microsomes are depressed when phenobarbital-induced rats are anesthetized with halothane under hypoxic conditions.[10] This depression lasts for over 24 h. It might be anticipated that this would be reflected in the drug metabolizing capacity of the liver, but we saw no evidence of this. It may be that the forms of cytochrome P-450 which are destroyed are not involved in halothane metabolism, or that the *in vitro* measurements of cytochrome P-450 do not always represent the pool of metabolically active enzyme.

We have been unable to identify any factors which caused two rats given two anesthetics at 1-day intervals to respond with more severe liver injury than other animals in the same group. Observation during anesthesia did not reveal any evidence of respiratory obstruction. However, many possible causes of hepatic injury, such as hypotension or impaired liver perfusion, were not monitored in our study.

Results of this study show that, in the hypoxic rat model, the extent of reductive metabolism of halothane is similar after a first, second, or third anesthetic, indicating that, in this model, halothane does not induce its own metabolism. A recent study in children also found reductive metabolism to be similar as repeated halothane anesthetics were administered.[11] It appears that other mechanisms must be sought to explain why halothane hepatitis is more common after multiple than after single anesthetics.

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