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Hepatic Injury Following Halothane, Enflurane, and Isoflurane Anesthesia in Rats

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Halothane anesthesia administered to enzyme-induced animals in a hypoxic atmosphere consistently produces hepatic necrosis. Rats pretreated with phenobarbital were exposed to hypoxia at varying intervals after administration of halothane, enflurane, or isoflurane anesthesia. Anesthetics were administered at 1 MAC for 2 h. For each agent, hypoxia consisting of 8 per cent oxygen-balance nitrogen for 1 h was imposed at the end of anesthesia. In other groups of rats, we also used a 15-, 30-, 60-, and 120-min interval of 100 per cent oxygen between 2 h of halothane anesthesia and the imposition of hypoxia. Controls included enzyme-induced animals with and without hypoxia, hypoxia alone, and cage controls. Hepatic injury was graded by histologic examination of the livers. Injury was greater when hypoxia followed halothane anesthesia than when it followed enflurane, isoflurane, or enzyme-induction alone. A difference in injury score existed between control animals and those anesthetized with halothane who received a 15-min interval of oxygen before hypoxia. Combined results from the 15- and 30-min delay groups also were different from control. There was no difference between control and halothane groups when the oxygen interval was 60 or 120 min. The injury score of the enflurane and isoflurane groups were comparable to that of controls. We conclude that a potential for hypoxia-induced liver injury during recovery exists after halothane anesthesia. Neither enflurane nor isoflurane anesthesia produced significant hepatic injury in this model. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Biotransformation: enzyme induction. Hypoxia. Liver: hepatotoxicity. Oxygen, consumption: liver.)

THE USE OF HALOTHANE results in a small but consistent incidence of postanesthetic hepatic dysfunction. Data derived from a recently developed animal model suggest that cellular damage may result from reactive intermediates formed during the reductive metabolism of halothane. Induction of the hepatic biotransformation enzyme system and a reduced FI_{O_2} are the critical variables necessary to initiate the events leading to halothane-induced liver injury.[1] The applicability of this model to humans may be limited. Although patients frequently receive drugs that are known to induce enzyme systems, it is unusual for severe arterial hypoxia to persist during anesthesia. However, hypoxia is an occasional complication of the postanesthetic recovery period, during which ventilation and inspired oxygen levels may be less rigorously controlled.

Recovery from volatile anesthetics is characterized by an initial rapid decrease in alveolar concentration during the first few minutes, followed by a 10- to 15-min period of slower removal from the lungs.[2] Smaller amounts are eliminated for several days.[2] Biotransformation proceeds throughout this period. Hypoxia occurring during recovery may therefore lead to significant reductive metabolism. The present study was designed to determine whether or not the hypoxic model is valid under conditions that simulate recovery from anesthesia. We examined hepatic injury produced by hypoxia imposed immediately after halothane, enflurane, or isoflurane anesthesia. We also studied four time intervals after halothane anesthesia to determine how long after anesthesia hypoxic injury could be demonstrated.

Methods

One hundred thirty-nine adult male Sprague-Dawley rats (Charles River) weighing approximately 350 g were given Purina Laboratory Chow® *ad libitum*. Microsomal enzymes were induced by adding phenobarbital (1 mg/ml) to the drinking water for 4 days. Two animals were eliminated from the study because of insufficient intake of phenobarbital solution. All other animals consumed 16 to 43 ml phenobarbital solution daily. Phenobarbital was discontinued 24 h before each study.[3]¶

Rats were exposed to anesthesia and hypoxia in individual plastic chambers. Nitrogen and oxygen were delivered via Matheson 603 and 602 Flowmeters®. The nitrogen flow rate was maintained at 5 l/min, and the oxygen flow was adjusted to achieve chamber concentrations appropriate for each group. Calibrated Ohio Vaporizers® were used for administration of halothane, enflurane, or isoflurane. We determined anesthetic concentrations in the chambers using infrared analysis (Beckman® LBII) or gas chromatography. Oxygen concentrations, which were monitored with a Beckman® E2

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¶ McLain GE, Brown BR Jr, Sipes IG: Inhibition of halothane hepatotoxicity in animal models. Abstracts of Scientific Papers, annual meeting of the American Society of Anesthesiologists, 1978, p 219.

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oxygen analyzer, were kept constant as indicated for each group. Carbon dioxide concentrations were measured with a Beckman® LBI analyzer. Heat lamps were used to maintain the rectal temperature of all anesthetized animals between 37.0° C and 38.5° C. Using CO_2 asphyxia, we killed rats 24 h after anesthesia/hypoxia and removed the livers for microscopic examination. Hepatic injury was graded in a "blind" manner according to the criteria of McLain *et al.*[1]: 0 = normal; 1 = slight cellular disruption; 2 = some staining variations and occasional vacuoles or balloon cells; 3 = occasional centrilobular necrosis; 4 = many centrilobular necrotic areas; and 5 = confluent centrilobular necrosis.

Four control groups and one halothane washout group were studied in addition to the three experiments that were performed. All experimental groups were pretreated with phenobarbital, and an anesthetic concentration of 1 MAC was used for each agent.[4]

Experiment 1: A comparison was made between halothane and enflurane administered in a hypoxic anesthetic atmosphere. Group 1 (n = 9) received 1 per cent halothane in 12 per cent oxygen for one hour. Group 2 (n = 10) received 2 per cent enflurane in 12 per cent oxygen for one hour.

Experiment 2: A comparison was made between halothane, enflurane, and isoflurane with hypoxia imposed at the end of anesthesia. Group 3 (n = 19) received 1 per cent halothane in 99 per cent oxygen. Group 4 (n = 19) received 2 per cent enflurane in 98 per cent oxygen. Group 5 (n = 15) received 1.4 per cent isoflurane in 99 per cent oxygen. Each anesthetic exposure lasted 2 h and was followed immediately by 8 per cent oxygen-balance nitrogen for 2 h.

Experiment 3: The amount of damage resulting from hypoxia imposed at varying intervals after halothane was determined. Groups 6–9 (n = 8 to 10) received 1 per cent halothane in 99 per cent oxygen and were given a 15-, 30-, 60-, or 120-min interval of 100 per cent oxygen before exposure to 2 h of 8 per cent oxygen-balance nitrogen.

Control Groups: Unanesthetized animals were studied to determine the effect of phenobarbital and hypoxia on liver damage. Control Group 1 (n = 20) was pretreated with phenobarbital and exposed to 8 per cent oxygen-balance nitrogen for 2 h. Control Group 2 (n = 10) was exposed to two hours of hypoxia without phenobarbital pretreatment. Control Group 3 (n = 10) received only phenobarbital. Control Group 4 (n = 10) were cage controls.

TABLE 1. Effect of Enzyme Induction and Hypoxia on Hepatic Injury with Halothane and Enflurane Anesthesia

Group	n	Agent	Histologic Score*	
			$F_{I_{O_2}}$	(Mean ± SE)
1	9	1 per cent halothane	0.12	3.8 ± 0.6
2	10	2 per cent enflurane	0.12	1.6 ± 0.6
C1	20	None (enzyme-induced)	0.08	1.3 ± 0.3

* Scores are based on a scale of 0 (no injury) to 5 (confluent centrilobular necrosis).

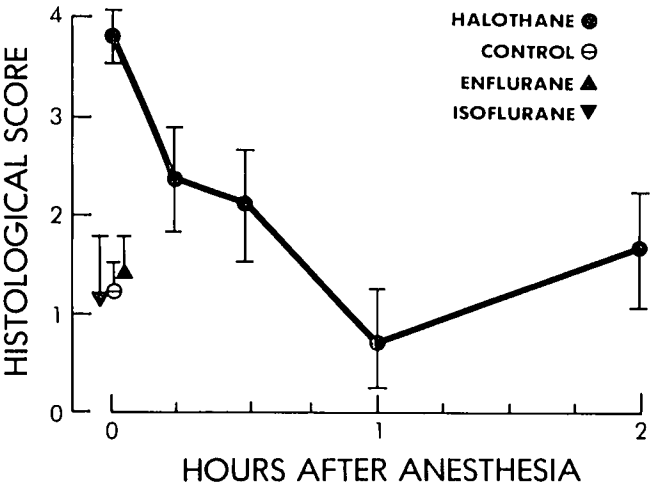

FIG. 1. Effects of enzyme induction and hypoxia ($F_{I_{O_2}}$ = 0.08) on hepatic injury following halothane, enflurane, and isoflurane anesthesia.

Washout Group: The time course of halothane washout was measured. This group (n = 5) received phenobarbital for enzyme induction as previously described. Animals were anesthetized with pentobarbital, 1.9 mg/kg, and tracheostomies were performed. Each animal received pancuronium, 1 mg/kg, ip, and ventilation was controlled using a standard Air Shields® ventimeter. One per cent halothane in 99 per cent oxygen was administered for 2 h. To analyze halothane and carbon dioxide, we obtained end-tidal samples via a stopcock connected to the tracheostomy tube using a previously described sampling method.[4] Ventilation was adjusted to maintain an end-tidal CO_2 concentration of 35–45 mmHg. End-tidal samples were obtained every 5 min for the first 15 min of anesthesia and every 15 min thereafter. The same sampling sequence was repeated for 1 h after halothane had been discontinued. Differences in liver damage scores between groups were tested for significance using the Mann-Whitney U-test for ranking.

Results

Under conditions of hypoxia ($F_{I_{O_2}}$ = 0.12) and induction of microsomal enzymes, halothane (Group 1) but not enflurane (Group 2) anesthesia produced extensive hepatic injury ($P < 0.01$) (table 1). Histologic damage that occurred when hypoxia ($F_{I_{O_2}}$ = 0.08) immediately followed halothane anesthesia (Group 3) was significantly greater ($P < 0.05$) than that found when animals

TABLE 2. Effect of Enzyme Induction and Postanesthesia Hypoxia on Hepatic Injury with Halothane, Enflurane, and Isoflurane Anesthesia

Group	n	Agent	Histological Score*	
			$F_{I_{O_2}}$	(Mean ± SE)
3	19	1 per cent halothane	0.08	3.8 ± 0.3
4	19	2 per cent enflurane	0.08	1.3 ± 0.4
5	15	1.4 per cent isoflurane	0.08	1.2 ± 0.5
C1	20	None (with phenobarbital)	0.08	1.3 ± 0.3
C2	10	None (without phenobarbital)	0.08	0
C3	10	None (with phenobarbital)	0.21	0
C4	10	Cage controls	0.21	0.1 ± 0.1

* Scores are based on a scale of 0 (no injury) to 5 (confluent centrilobular necrosis).

were treated similarly with enflurane (Group 4) or isoflurane (Group 5) (fig. 1, table 2). When a 15-min interval of oxygen was imposed between halothane anesthesia and hypoxia, a difference of $P < 0.06$ in histologic score existed between control animals (Group C1) and those anesthetized with halothane (Group 3). Combined results from the 15- and 30-min delay groups (Groups 6–7) also differed from those of control animals, Group C1 ($P < 0.05$) (fig. 1). There was no difference between halothane (Groups 8 and 9) and control (Group C1) when the oxygen interval was 60 or 120 min. The injury scores of the isoflurane (Group 4) and enflurane (Group 5) animals were comparable to the score for Control Group 1 (phenobarbital and hypoxia) (table 2). No evidence of hepatic injury occurred in the remaining control groups.

Elimination of halothane was rapid but not complete one hour after anesthesia. End-tidal concentrations were appreciable at 15 and 30 min and were comparable to end-tidal concentrations found at 30 and 60 min in man (fig. 2).[5]

Discussion

Our study confirms the finding of McLain *et al.*[1] that halothane is hepatotoxic when enzyme induction and hypoxia coexist. Imposition of hypoxia at the end of anesthesia resulted in hepatic damage approaching that found with concomitant hypoxia and anesthesia when the agent was halothane. Neither enflurane nor isoflurane anesthesia produced hepatic damage under the same conditions. As recovery from anesthesia proceeded, the amount of damage from hypoxia decreased, rats being vulnerable for 15 to 30 min after anesthesia.

Several reports present data that suggest a similarity between the hepatic damage found in the hypoxic model and that occurring in humans. The morphologic findings are strikingly similar and characteristic. The appearance of swollen hepatocytes and vacuoles with confinement of damage to the centrilobular region occurs in both the model and humans.[6] Identical volatile reductive metabolites have been measured in the model and humans during normoxic anesthesia. Reductive metabolite production is markedly increased in the model following enzyme induction and halothane anesthesia with hypoxia.[7] We speculate that the mechanism has relevance to humans since reductive metabolism has been demonstrated in both species. It is likely that large amounts of metabolic products are necessary to produce injury and that conditions which simulate enzyme induction and hypoxia must coexist before clinically significant hepatic damage occurs.

The absence of hepatic damage following enflurane and isoflurane may be the result of two factors. First, the metabolism of enflurane and isoflurane is minimal relative to that of halothane. In humans, 15 to 20 per cent of the halothane taken up can be recovered as metabolites.[8,9] With enflurane, only 2.4 per cent can be recovered;[10] and with isoflurane, only 0.2 per cent.[11] Second, metabolism may result in the development of reactive intermediates, probably free radicals. The formation of such radicals during the course of reductive metabolism of halothane has been suggested by Cohen *et al.*[12] and Sharp *et al.*[13] As of this time, no reductive pathways have been demonstrated for enflurane or isoflurane.

From our measurements of halothane elimination, we found concentrations of approximately 0.1 per cent at 15 and 30 min after anesthesia. A period of 30 to 60 min is necessary for alveolar concentrations to reach com-

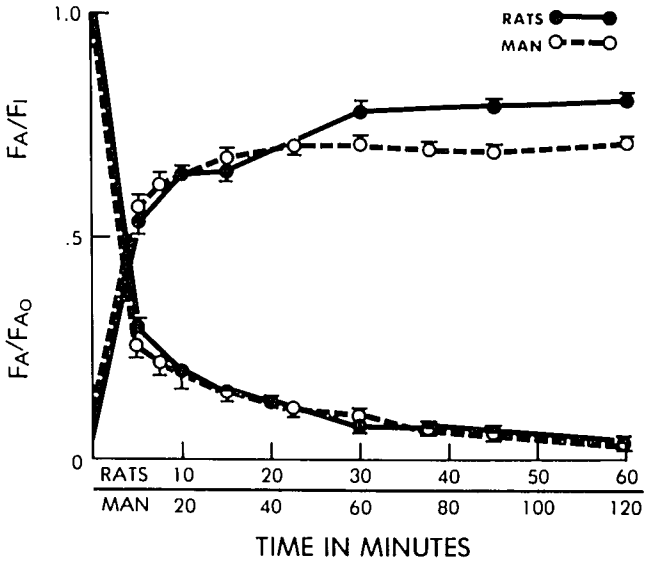

FIG. 2. The uptake and elimination of halothane in rats and man. FA/FI is the ratio of end-tidal to inspired concentration and defines the ratio of uptake. FA/FA_0 is the ratio of alveolar concentration during the course of recovery to the concentration immediately preceding recovery.[5]

parable levels in humans.[5] We suggest that a potential for hypoxia-induced liver injury exists during this time.

In summary, we have shown that the rat hypoxic model is valid during recovery from anesthesia with halothane. Hepatic damage did not occur with enflurane or isoflurane. Insofar as the rat model is applicable to the clinical situation, a potential for hepatic injury exists in the early postanesthesia period as well as intraoperatively.

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