Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 461–465, 1983.

Potentiation of Halothane Hepatotoxicity by Chronic Ethanol Administration in Rat: An Animal Model of Halothane Hepatitis

TOSHIKAZU TAKAGI, HIROMASA ISHII, HISAO TAKAHASHI, SHINZO KATO, FUMIO OKUNO, YOKO EBIHARA, HIROSHI YAMAUCHI, SHIGEYUKI NAGATA, MASAO TASHIRO* AND MASAHARU TSUCHIYA[1]

Departments of Internal Medicine and Pathology, School of Medicine, Keio University Tokyo 160, Japan*

TAKAGI, T., H. ISHII, H. TAKAHASHI, S. KATO, F. OKUNO, Y. EBIHARA, H. YAMAUCHI, S. NAGATA, M. TASHIRO AND M. TSUCHIYA. *Potentiation of halothane hepatotoxicity by chronic ethanol administration in rat: An animal model of halothane hepatitis.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 461–465, 1983.—To determine if chronic ethanol administration modifies the effect of halothane on the liver, fourteen male Wistar rats were pair-fed nutritionally adequate liquid diets containing either ethanol (36% of calories) or isocaloric carbohydrate (controls) for 6 weeks. After halothane anesthesia of these animals under different oxygen concentration, the livers were examined light microscopically as well as biochemically. The livers from rats fed ethanol which received halothane at low oxygen concentration showed multifocal or patchy necrosis primarily in the centrilobular regions with parenchymal lipid accumulation, whereas no such lesions were not observed in pair-fed controls. Hepatic necrosis was also seen after halothane anesthesia even at ambient oxygen concentrations, although the degree of necrosis was much milder. Hepatic microsomal cytochrome P450 content was increased by 30% after ethanol but was decreased following halothane anesthesia. These data suggest that halothane is hepatotoxic to liver of rats chronically pretreated with ethanol, especially under hypoxic condition.

Chronic ethanol administration Halothane hepatitis Animal model Hypoxia

THE mechanism by which halothane causes hepatic injury in man still remains speculative. Recently, administration of enzyme inducing agents, such as phenobarbital, to rats in combination with hypoxia has been reported to enhance the hepatotoxicity of halothane resulting in centrilobular necrosis [10,15].

Since ethanol is known to produce hepatic microsomal induction both in man [16] and rat [4,7], we wondered if chronic ethanol administration modifies the effect of halothane on the liver.

In this communication we report that halothane causes centrilobular hepatic necrosis in rats fed ethanol chronically, especially under hypoxic conditions.

METHOD

Male Wistar rats weighing about 200 g were obtained from Nippon Clea Co. and maintained on a 12 hour dark and light cycle. Then the animals were housed in individual wire-bottom cages. The rats were fed a nutritionally adequate liquid diet [2] for 6 weeks. The diet was purchased from Bio-Serv. Inc., Frenchtown, NJ (Diet No. 711-A, C). As for control rats, carbohydrate, protein and fat provided 47%, 18% and 35%, respectively, of the total calories. Pair-fed rats consumed the same diet except that carbohydrate was isocalorically replaced by ethanol accounting for 36% of the total calories.

After 6 weeks of ethanol feeding, the rats were placed into Plexiglas chambers in groups of four per cage. These animals were anesthetized for 2 hours with 1% halothane at two different concentrations of oxygen, low (10%) and ambient (35%). The halothane and oxygen were delivered to chambers by Flotec III at a flow rate of 4 liters per minute. Halothane concentration was determined by the Flotec III and the chamber oxygen concentration was monitored with a Teledyne oxygen analyzer (Type 331). Another group of pair-fed animals was treated with 10% oxygen for 2 hours without exposure to halothane. Twenty-four hours after halothane anesthesia, the animals were killed by decapitation and the blood was collected from the neck vessels. A small portion of the liver was immediately removed and fixed in 10% formalin solution and further processed for light microscopy. The liver tissues stained with hematoxylin and eosin was evaluated for hepatotoxicity. The major portion of the liver was quickly perfused in situ via the portal vein with ice-cold physiological saline, quickly excised, weighed and

[1]Requests for reprints should be addressed to Masaharu Tsuchiya, M.D., Department of Internal Medicine, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.

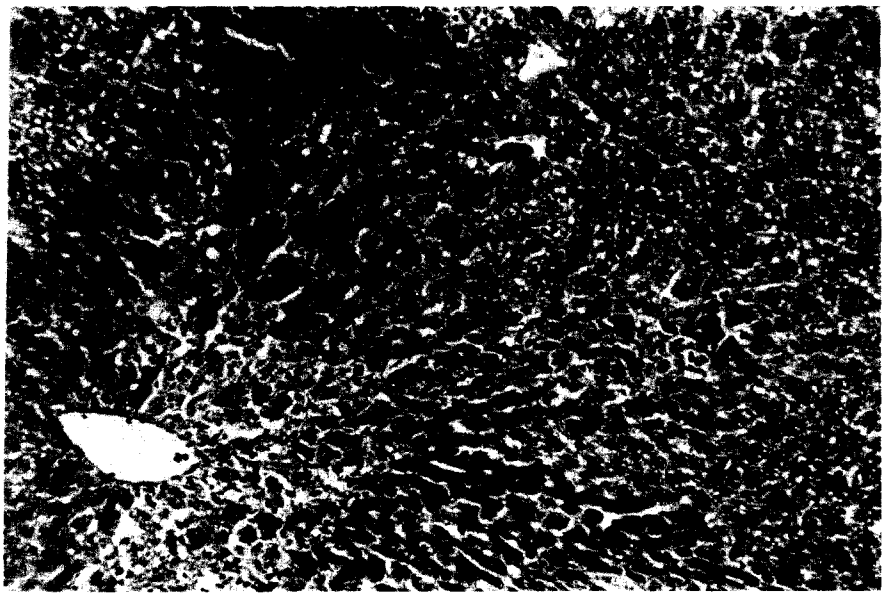

FIG. 1. Hepatic morphology 24 hours after 1% halothane at low oxygen concentrations (10%) in an ethanol treated rat. Centrilobular necrosis with leukocyte infiltration and coagulation necrosis in the midzonal area. Hematoxylin and eosin stain.

used for biochemical analyses. All subsequent steps were performed at 2° to 4°C. The liver was homogenized in three volumes of 1.15% KCl with a Potter-type glass homogenizer with teflon pestle. The homogenates were centrifuged at 10,000 ×g for 20 minutes followed by ultra-centrifugation of the 10,000 ×g supernatants at 105,000 ×g for 60 minutes using a Beckman L5-50 ultracentrifuge. The pellets harvested by this procedure were used as microsomal fractions. Hepatic microsomal cytochrome P450 and b5 contents were measured by the method of Omura and Sato [11] using a Shimazu MP5000 recording spectrophotometer. Protein concentration was determined according to Lowry *et al.* [8]. Hepatic injury was also assessed by estimating the activity of serum glutamic oxaloacetic transaminase (sGOT).

Histological grading of the hepatic lesions was performed by the pathologist (M.T.) who was not informed of the treatment received by the rats.

The Student's *t*-test was used to determine statistical significance of the difference among the various groups.

RESULTS

Under conditions of hypoxia with chronic ethanol feeding, halothane anesthesia produced extensive hepatic necrosis (Figs. 1, 2). The livers of animals that had been fed ethanol for 6 weeks and subsequently received halothane with low oxygen (10%) showed numerous areas of hepatic necrosis, primarily in pericentral area of the lobule. These necrotic areas were frequently infiltrated with clusters of lymphocytes and neutrophils. Acidophilic bodies were often seen (Fig. 2). A number of hepatic cells in the centrilobular areas were swollen, vacuolated with fat droplets and more eosinophilic than normal. It should be noted that halothane anesthesia with ambient oxygen (35%) also resulted in hepatic damage when the rats had been fed ethanol chronically. Damage was apparent in most of these animals, but hepatic necrosis was milder in degree and extent compared to the animals treated with low oxygen concentrations. Histologic study of the livers revealed only minimal changes in pair-fed animals receiving 10% oxygen without halothane, although accumulation of fat droplets was seen in the livers fed ethanol. Moreover, halothane anesthesia with 10% oxygen in pair-fed control rats showed no significant changes in hepatic morphology.

Hepatic microsomal cytochrome P450 content was increased by 30% after ethanol administration (Fig. 3). However the cytochrome P450 content of ethanol-fed rat was significantly decreased after halothane anesthesia with 10% oxygen. The hepatic cytochrome P450 content was not significantly changed after halothane anesthesia with 35% oxygen. Microsomal cytochrome b5 was found to be unaltered by halothane anesthesia in ethanol-fed rat whether they received adequate oxygen or not.

The levels of s-GOT were increased significantly in ethanol-fed animals receiving halothane irrespective of the oxygen concentration, but s-GOT values were higher with hypoxic conditions (Fig. 4).

DISCUSSION

Despite much controversy over the existence of halothane-induced hepatitis [1], a consensus has been reached that it is a distinct although rare syndrome [3]. Since the earlier studies on its pathogenesis, investigators [6,12] have attempted to demonstrate cell-mediated immune responses to halothane in patients with halothane hepatitis and suggested that halothane could produce hepatic injury by a hypersensitivity mechanism through halothane or its metabolites as a hapten. Although several studies [9, 13, 14] have shown skin hypersensitivity or in vitro antibody forma-

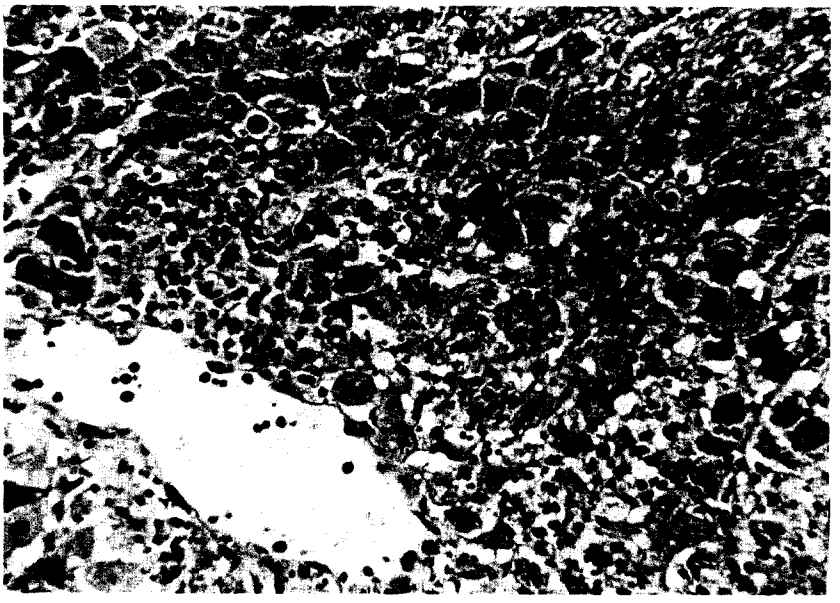

FIG. 2. Hepatic morphology 24 hours after 1% halothane at low oxygen concentrations (10%) in an ethanol treated rat. Centrilobular necrosis with polymorphonuclear and lymphocytic cell infiltration with scattered eosinophilic bodies in the pericentral area.

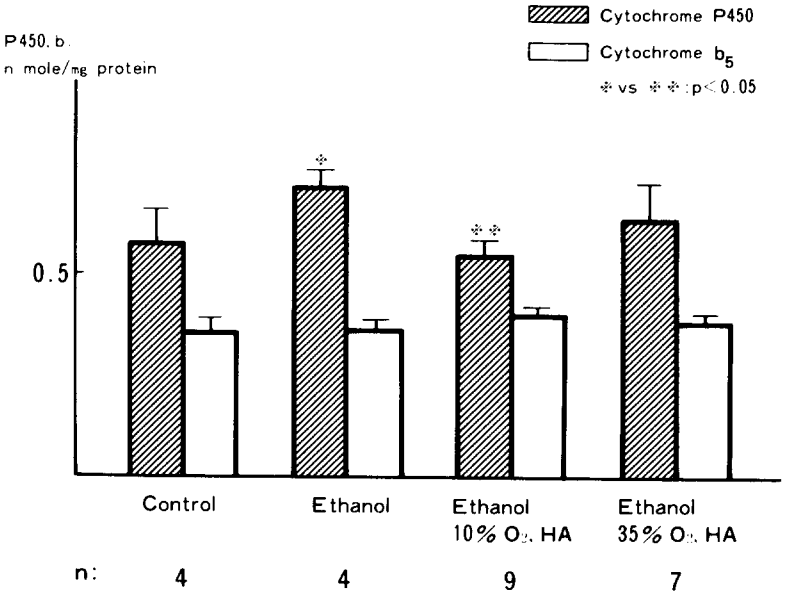

FIG. 3. Hepatic microsomal cytochrome P450 and b5 contents in rats treated with ethanol chronically and halothane at different oxygen concentrations. Data are expressed as mean±SEM; n: number of animals used for the experiment.

tion to halothane or its metabolites, they failed to demonstrate hepatic damage.

More recently, evidence has evolved that halothane may be a direct hepatotoxin, and animal models of halothane hepatotoxicity have been described [10,17]. McLain *et al.* [10] have demonstrated that halothane hepatotoxicity can be produced by pretreatment of rats with microsomal enzyme inducers, such as phenobarbital, in combination with hypoxic conditions. The hepatic lesions in these animals mainly consist of centrilobular necrosis often associated with infiltration of clusters of lymphocytes and neutrophils. Furthermore, it has been suggested [10,17] that halothane might

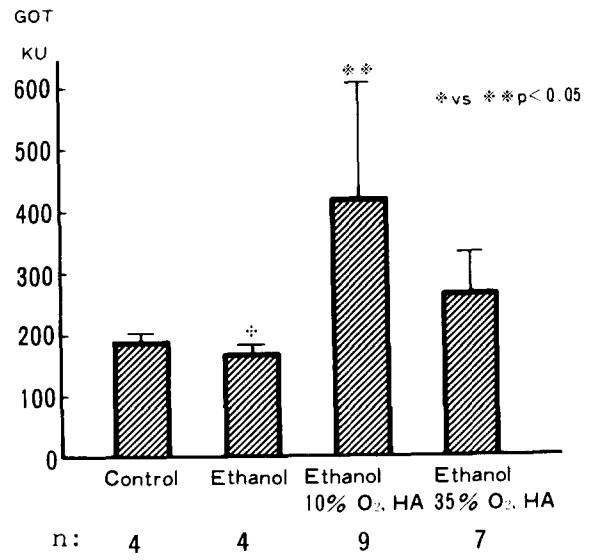

FIG. 4. Serum glutamic oxaloacetic transaminase activity in rats treated with ethanol chronically and halothane at different oxygen concentration. Data are expressed as mean±SEM; n: number of animals used for the experiment.

be metabolized to hepatotoxic intermediates by a reductive, cytochrome P450-dependent pathway in hepatic microsomes.

Since chronic ethanol administration has been shown to produce a proliferation of hepatic smooth endoplasmic reticulum associated with an enhancement of a variety of microsomal drug metabolizing enzymes in man [16] and rat [4,7], we wondered if halothane anesthesia affects the livers of rats fed ethanol chronically. It was found that halothane anesthesia produced extensive hepatic necrosis primarily in pericentral area of the lobules under conditions of hypoxia and chronic ethanol feeding. The histological finding was similar to those reported [10,15] previously with phenobarbital and hypoxia. Moreover, in the present study, hepatic necrosis was also demonstrated in rats fed ethanol chronically and subsequently anesthetized with halothane and 35% oxygen. Since it has been suggested [5] that the liver of ethanol treated animals consumes oxygen at higher rate and is more sensitive to reduction in the availability of oxygen, it is not unexpected that hepatic necrosis may occur in the rat fed ethanol and anesthetized with halothane even at ambient oxygen concentration. This finding is of interest in view of the report [20] that hepatic necrosis developed in rats pretreated with triiodothyronie and then anesthetized with halothane at an ambient oxygen concentration. They suggested that excess triiodothyronine induced an intracellular hypoxia causing halothane to undergo reductive biotransformation to reactive metabolites resulting in a production of hepatic damage.

Although precise mechanism by which halothane produces hepatic necrosis in these animals is now known, Uehleke *et al.* [18] have previously shown in vitro that halothane produces far more covalent binding to hepatic constitutents when oxygen is reduced. Moreover, Widger *et al.* [19] substantiated increased covalent binding, enhanced inorganic fluoride release and histologic damage with hypoxia during halothane anesthesia. Our preliminary observation on ethanol-fed rats also demonstrated that serum fluoride levels of rats were markedly increased after halothane anesthesia with low oxygen concentrations. These data suggest that altered halothane metabolism via a reductive pathway could lead to reactive metabolites interacting with hepatic cells or its organelles resulting in hepatic necrosis. Therefore, it is concluded that our animal model may be predicated on decreased oxygen availability to ethanol-induced microsomal enzymes so that reductive metabolism of halothane is activated.

ACKNOWLEDGEMENTS

This research was supported in part by the grant from the Ministry of Education of Japan.

REFERENCES

1. Brown, B. R., Jr. Halothane hepatitis is a reasonably well proved clinical entity. In: *Controversy in Anesthesiology*, edited by J. E. Eckenhoff. Philadelphia: Saunders, 1979, pp. 31–37.
2. DeCarli, L. M. and C. S. Lieber. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new diet. *J Nutr* **91:** 331–336, 1967.
3. Dienstag, J. L. Halothane hepatitis, allergy or idiosyncrasy? *N Engl J Med* **303:** 102–104, 1980.
4. Ishii, H., J. G. Joly and C. S. Lieber. Effect of ethanol on the amount of enzyme activities of hepatic rough and smooth microsomal mechanisms. *Biochim Biophys Acta* **291:** 411–420, 1973.
5. Israel, Y., H. Orrego, J. M. Khanna, D. J. Stewart, M. J. Phillips and H. Kalant. Alcohol induced susceptibility to hypoxic liver damage: Possible role in the pathogenesis of alcoholic liver disease? In: *Alcohol and the Liver*, edited by M. M. Fisher and J. G. Rankin. New York: Plenum Press, 1977, pp. 323–346.
6. Klatskin, G. and D. V. Kimberg. Recurrent hepatitis attributable to halothane sensitization in anesthesiologist. *N Engl J Med* **280:** 515–522, 1969.
7. Lieber, C. S. Liver adaptation and injury in alcoholism. *N Engl J Med* **288:** 356–362, 1973.
8. Lowry, O. H., N. J. Rosebrough, A. R. Farr and R. J. Randell. Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
9. Mathieu, A., D. D. Padna, B. D. Kahan and J. Mills. Humoral immunity to a metabolite of halothane, fluroxene and enflurane. *Anesthesiology* **42:** 612–616, 1975.
10. McLain, G. E., I. G. Sipes and B. R. Brown, Jr. An animal model of halothane hepatotoxicity: Roles of enzyme induction and hypoxia. *Anesthesiology* **51:** 321–326, 1979.
11. Omura, T. and R. Sato. The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239:** 2370–2378, 1964.
12. Paronetto, F. and H. Popper. Lymphocyte stimulation induced by halothane in patients with hepatitis following exposure to halothane. *N Engl J Med* **283:** 277–280, 1970.
13. Reeves, J. G. and L. E. McCracken, Jr. Failure to induce hepatic pathology in animals sensitized to a halothane metabolite and subsequently challenged with halothane. *Anest Analg* **55:** 235–242, 1976.

14. Rosenberg, P. H. and T. Wahlstorm. Trifluoroacetic acid and some possible intermediates of halothane as hapten. *Anesthesiology* **38:** 224–280, 1970.
15. Ross, W. T., Jr., B. P. Daggy and R. R. Cardell, Jr. Hepatic necrosis caused by halothane and hypoxia in phenobarbital-treated rats. *Anesthesiology* **51:** 327–333, 1979.
16. Rubin, E. and C. S. Lieber. Hepatic microsomal enzyme in man and rat: Induction and inhibition by ethanol. *Science* **162:** 690–691, 1968.
17. Sipes, I. G. and B. R. Brown, Jr. An animal model of hepatotoxicity associated with halothane anesthesia. *Anesthesiology* **45:** 622–628, 1976.
18. Uehleke, H., K. H. Hellmer and S. Tabarelli-Poplawski. Metabolic activation of halothane and its covalent binding to liver endoplasmic proteins in vitro. *Arch Pharmacol* **279:** 39–52, 1973.
19. Widger, L. A., A. J. Gandolfi and R. A. Van Dyke. Hypoxia and halothane metabolism in vivo: Release of inorganic fluoride and halothane metabolite binding to cellular constitutents. *Anesthesiology* **44:** 197–201, 1976.
20. Wood, M., M. L. Berman, R. D. Harbison, P. Holye, J. M. Phythyon and A. J. J. Wood. Halothane-induced hepatic necrosis in triiodothyronine pretreated rats. *Anesthesiology* **52:** 470–476, 1980.