Anesthesiology
60:141–143, 1984

Effects of Hypersensitivity to a Halothane Metabolite on Halothane-induced Liver Damage

Douglas J. Ford, Ph.D., Dennis E. Coyle, Ph.D.,* Jean F. Harrington, M.D.†*

The effect of immunologic hypersensitivity to a metabolite of halothane (trifluoroacetate) on the halothane–hypoxia–induction model was tested in mice and rats. Male Fisher 344 rats (200 g) were immunized with ovalbumin–trifluoroacetate (OVA-TFA) and the time course of the delayed hypersensitivity response determined. The animals had a peak response between 4 and 6 weeks after immunization. Rats were immunized with OVA–TFA, OVA, or saline 5 weeks before being anesthetized. Ten days before anesthesia, the animals were started on 0.1% phenobarbital in the drinking water. The animals were anesthetized with 1% halothane and 14% oxygen for 2 h. Hypersensitivity to TFA had no effect on the liver damage in either the mouse or the rat. These results do not rule out an immunologic vector in halothane hepatitis but make the involvement of TFA unlikely. Key words: Anesthetics, volatile: halothane. Immune response. Liver: hepatotoxicity. Metabolism: metabolites. Toxicity: hepatic; metabolites.

IT GENERALLY IS ACCEPTED that under certain circumstances, halothane can be hepatotoxic. Two general theories have emerged to explain halothane's idiosyncratic hepatotoxicity. In the first theory, damage is the result of a toxic metabolite of halothane.[1,2] The animal models supporting this theory require that the liver enzymes be induced and that the halothane be administered under hypoxic conditions. This model is best established in the rat.

In the second theory, liver damage results from an immunologic hypersensitivity reaction.[3,4] This mechanism is supported by the observation that the incidence of hepatic damage increases with multiple exposures to halothane[5] and that patients develop antibodies to halothane-altered hepatocytes following halothane and massive hepatic necrosis.[6]

In this study, we examined the possible interaction of the two mechanisms. We asked the question: If an animal is immunologically hypersensitive to a metabolite of halothane, will the hepatic damage seen when halothane is administered to induced, hypoxic animals be exacerbated?

* Assistant Professor of Anesthesia Research.

† Assistant Professor of Anesthesia.

Received from the Department of Anesthesia, University of Cincinnati, Cincinnati, Ohio. Accepted for publication July 18, 1983. Supported in part by a grant from the American Society of Anesthesiologists and the Parker B. Francis Foundation and NIH Grant GM28155.

Address for reprint requests to Dr. Ford: Department of Anesthesia, Mail Location #531, University of Cincinnati, College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267.

Methods

Male Fisher 344 rats (200 g) were purchased from Harlen Laboratories, Indianapolis, Indiana, and housed in the University animal quarters until used. Ovalbumin (OVA) and human serum albumin (HSA) were trifluoroacetylated (OVA–TFA, HSA–TFA) according to Goldberger and Anfinsen.[7] Control OVA and HSA were treated the same, but no trifluoracetylating agent was added. All preparations were dialyzed exhaustively against distilled water, lyophilized, and stored at −20° C.

The rats were immunized with OVA, OVA–TFA, or saline. OVA and OVA–TFA were made up to 2 mg/ml in saline and then mixed with an equal volume of complete Freund adjuvant (CFA). The saline control also was mixed with CFA. A total of 500 μl of the appropriate emulsion was injected subcutaneously and divided equally among each hind footpad and the nape of the neck.

Delayed hypersensitivity to the proteins or hapten (TFA) was assessed by skin testing. The back of each rat was shaved and 20 μl of OVA, HSA, and HSA–TFA (100 μg) in saline were injected intradermally. Since CFA contains killed mycobacteria, purified protein derivative (PPD) also was injected intradermally as a positive control of an immunologic reaction. HSA was used as a negative control of an immunologic response, since it fails to induce a response in animals made hypersensitive to OVA (non-cross-reactive). OVA was used as a measure of the immune reaction to the native protein. HSA–TFA tested the immune response to the hapten. Twenty-four hours after the intradermal injections, the diameters of the induration and erythema were measured with a caliper.

Liver P-450 enzymes were induced by giving the rats free access to 0.1% phenobarbital in their drinking water for 10 days. The animals were fasted overnight before anesthesia. The rats were anesthetized in a 16 l plexiglass chamber, using a flow of 10–12 l/min of 14% O_2, balance N_2. For the first 10 min of anesthesia, 2% halothane was delivered to the chamber for induction, followed by 1% halothane for the remaining 110 min.

The time course of the primary response to OVA–TFA was determined by immunizing 18 animals with OVA–TFA as described above. At 1, 2, 4, 6, 8, and 10 weeks, three animals were selected randomly and skin tested as described above (table 1).

TABLE 1. Primary Response to OVA–TFA in Fisher 344 Rats

Weeks post-immunization	OVA	HSA–TFA	HSA	PPD
1	0	0	0	0
2	0.95(H)	0	0	0.80
4	0.95(H)	0.53/0.75	0	0.93
6	1.0/1.2(H)	0.88/1.01	0	0.98
8	0.79/1.3(H)	0.77*	0	0.85
10	0.84	0.31†	0	0.78

Erythema (cm)/induration (cm).
H = hemorrhage.
* = two of three responded.
† = one of three responded.

To determine the effect of hypersensitivity to TFA on the halothane–hypoxia–induction model, 64 animals were divided into seven groups (table 2). Group A animals (N = 8) were immunized with OVA–TFA, induced with phenobarbital, and anesthetized with halothane and 14% O_2. Group B animals were immunized with OVA (N = 10), induced with phenobarbital and anesthetized with halothane. This group was used to assess the effect of hypersensitivity to OVA on liver damage. Group C animals were immunized with saline (N = 8), induced, and anesthetized with halothane. Group C animals were used to determine the effect of immunization with CFA. Group D animals (N = 10) were immunized with OVA–TFA, induced with phenobarbital, and exposed to 14% O_2 for 2 h. Group E and F animals were immunized, respectively, with OVA (N = 8) or saline (N = 8), induced with phenobarbital, and exposed to 14% O_2 for 2 h. Group G animals (N = 12) were not treated and served as healthy controls. Five weeks prior to anesthesia, the rats were immunized with OVA–TFA, OVA, or saline. Ten days before anesthesia, the rats were induced with phenobarbital. One day or 3 days after anesthesia, the animals were exsanguinated via the inferior vena cava (under ether anesthesia). A section of the median lobe and a section of the left lobe were removed, fixed in glutaraldehyde–formalin buffer, and mounted in paraffin. Two-micron sections of the fixed tissue were stained with hematoxylin and eosin. The liver sections were evaluated for necrosis and cellular infiltration by a pathologist who had no knowledge of the groups.

SGOT and SGPT levels were determined on an Abbott 100 Autoanalyzer. The data were analyzed using a nonlinear parametric analysis. $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the primary immunologic response of Fisher 344 rats to TFA. Since the animals were immunized with OVA–TFA in CFA, the delayed hypersensitivity response to OVA and PPD was expected. The response to HSA–TFA could be either a response to TFA or a cross-reactivity between OVA and HSA. However, since the rats did not respond to HSA, the hypersensitivity must have been to TFA. The TFA response peaked between 4 and 6 weeks. For this reason, we anesthetized the rats with halothane 5 weeks after being immunized.

Table 2 shows the effect of hypersensitivity on halothane-hypoxia-induction in the rat. Since the animals immunized with OVA and saline (both were control immunization) responded equally to the other experimental variables, the results were combined. Although Group A exhibits higher SGPT levels than Groups B and C, these differences are not significant. In both groups, resolution of the liver necrosis is underway at 3 days. The microscopic examination showed no difference between Groups A, B, and C in the degree of necrosis and the number of infiltrating cells at 24 and 72 h.

The experiments also were conducted in mice with the result that hypersensitivity to TFA had no effect on the halothane–hypoxia–induction model (data not shown).

Discussion

One feature of human halothane hepatitis not present in rodent models is the development of life-threatening, fulminant hepatic necrosis. Therefore, there is apparently more to some cases of human halothane hepatitis than

TABLE 2. Effect of Hypersensitivity on the Halothane–Hypoxia–Induction Model in Rats

Group	Induced Hypoxic*	Halothane†	Immunized‡	SGPT (IU/l) 24 h ± SEM	SGPT (IU/l) 72 h ± SEM	N
A	+	+	+	3848 ± 2844	930 ± 312	4
B & C	+	+	−	2588 ± 1002	778 ± 185	9
D	+	0	+	47 ± 4	42 ± 2	5
E & F	+	0	−	53 ± 3	42 ± 1	8
G	0	0	0	34 ± 3	34 ± 3	6

* + = 0.1% phenobarbital for 10 days and 14% O_2; 0 = No phenobarbital, 20% O_2.
† + = 1% halothane; 0 = no halothane.
‡ + = OVA–TFA; − = saline or OVA; 0 = no injection.

what is reflected in rodent models. A possible feature missing from the strictly metabolic model is an immunologic vector. An inflammatory reaction in the liver triggered by delayed hypersensitivity to a halothane metabolite may lead to massive necrosis.

We wished to know if hypersensitivity would exacerbate the damage caused by halothane, hypoxia, and enzyme induction in rats. A similar experiment was done by Mathieu *et al.*[8] with guinea pigs. However, after immunizing the guinea pigs with HSA–TFA, he anesthetized his animals with halothane in 100% O_2. Mathieu's protocol was designed to determine if hypersensitivity to TFA could initiate liver damage. However, since halothane is metabolized intracellularly, reactive metabolites might react and remain intracellular. In this case, the presumed reactive metabolite is trifluoracetyl chloride, which is either hydrolyzed to trifluoracetic acid or reacts with a nucleophile such as an amine.

The interior of a cell is an immunologically privileged area. Consequently, an intracellular hapten will not elicit an immunologic response even if the animal is hypersensitive to that hapten. However, once the cell is damaged, the hapten is accessible to the immune system, and an inflammatory response can follow. This is the rationale behind our causing some damage to the liver when the animals were anesthetized. Twenty-four hours after anesthesia, the hypersensitive Group A had a higher SGPT level than the nonhypersensitive Group B. If this difference were due to an immunologic reaction, the damage should have progressed over the following 2 days before resolving. However, at 72 h, both groups were resolving at the same rate. The conclusion is that immunologic hypersensitivity to trifluoroacetate does not exacerbate the metabolic damage produced by halothane, hypoxia, and enzyme induction.

The results of this experiment do not rule out immunologic involvement in human halothane hepatitis. But they do argue against TFA involvement in any such reaction.

References

1. Cousins MJ, Sharp JH, Gourlay GK, Adams JF, Haynes WD, Whitehead R: Hepatotoxicity and halothane metabolism in an animal model with application for human toxicity. Anaesth Intensive Care 7:9–24, 1979
2. Jee RC, Sipes IG, Gandolfi AJ, Brown BR: Factors influencing halothane hepatotoxicity in the rat hypoxic model. Toxicol Appl Pharmacol 52:267–277, 1980
3. Walton B, Simpson BR, Strunin L: Unexplained hepatitis following halothane. Br Med J 1:1171–1176, 1976
4. Paronetto F, Popper H: Lymphocyte stimulation induced by halothane in patients with hepatitis following exposure to halothane. N Engl J Med 283:277–280, 1970
5. Carney FMT, Van Dyke RA: Halothane hepatitis: A critical review. Anesth Analg 51:135–160, 1972
6. Vergani D, Miili-Vergani G, Albert A: Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. N Engl J Med 303:66–71, 1980
7. Goldberger RG, Anfinsen CB: The reversible masking of amino groups in ribonuclease and its possible usefulness in the synthesis of protein. Biochemistry 1:401–405, 1962
8. Mathieu A, Depadua D, Kahan BD, Galdabini JJ, Mills J: Correlation between specific immunity to a metabolite of halothane and hepatic lesions after multiple exposures. Anesth Analg 54:332–339, 1975