Halothane Hepatitis Patients Generate an Antibody Response Toward a Covalently Bound Metabolite of Halothane


Halothane hepatitis (HH), a rare complication of halothane anesthesia, may in part result from an immune mediated injury. Investigators speculate that this immune response is due to the alteration of hepatocyte proteins by halothane or, more probably, one of its reactive intermediates formed during metabolism.1-2 Epidemiologic data in humans support an association between this disease and an immune response.3-5 Multiple administrations of halothane increase the incidence and intensity of hepatitis, the interval between anesthetic and jaundice is shorter after two or more exposures than after a single exposure,6 and patients are reported to evidence rash, arthralgia, and peripheral and/or hepatic eosinophilia.7 Moreover, early studies noted that, in patients with HH, an increased frequency of anti-mitochondrial and anti-smooth muscle antibodies,8 anti-thyroid antibodies,9 and anti-nucleic acid antibodies10 were present.

Previous investigations have suggested that the potential antigen in a halothane-induced immune response is not the parent drug, but, instead, a biotransformation intermediate of halothane which alters the antigenicity of liver cell constituents by serving as a hapten.11 Thus, the antigenicity of the hepatocyte membrane protein may be altered by biotransformation intermediates of halothane generated through either an oxidative [trifluoroacetyl halide (TFA)] or a reductive (free radicals or carbene intermediates) pathway.12

Work in other laboratories describe a humoral immune response in patients with liver complications following halothane anesthesia that is directed toward a hepatocyte protein altered by a reactive intermediate of halothane. These patients with fulminant halothane hepatitis evidenced a circulating antibody that appeared to react with the surface of rabbit hepatocytes altered by halothane exposure.10,11 In both an antibody-dependent cellular cytotoxicity assay and an immunofluorescent test using hepatocytes from rabbits exposed to halothane, this antibody could be detected only in sera from halothane hepatitis patients, and not from individuals with other liver complications. In addition, sera from two surgeons with hepatic damage following occupational exposure were also positive for this antibody.12 More recent studies using the sensitive and highly quantitative enzyme-linked immunosorbent assay (ELISA) have noted that sera from HH patients will

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Received from the Departments of Microbiology and Anesthesiology, University of Arizona, Tucson, Arizona; and the Division of Anesthesia, Clinical Research Centre, Harrow, Middlesex, England. Accepted for publication December 7, 1987. Supported in part by NIH grant GM34788. Presented in part at the ASPET-SOT meeting, August, 1986, Baltimore, Maryland, and at the ASA Annual Meeting, October, 1986, Las Vegas, Nevada.

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Key words: Anesthetic, volatile: halothane. Immune response: antibodies; halothane hepatitis. Liver: hepatitis.
react with subcellular fractions of hepatocytes from halothane-exposed rabbits, as well as with the halothane-reactive metabolite, TFA, conjugated to a carrier protein.

Detection of circulating antibody directed toward a reactive intermediate of halothane, such as the trifluoroacetyl moiety, provides two major advantages: 1) early diagnosis of this entity, thereby enabling potential therapeutic intervention, and 2) defining the mechanism of immune mediated hepatic damage by assessing the specificity of human antibodies. Preliminary studies in this laboratory reported that sera from a patient with HH had a high titer of antibody that reacted with trifluoroacetylated rabbit serum albumin (TFA-RSA) in an ELISA. Antibody binding to the carrier protein, RSA, was negligible, whereas binding to the antigen, TFA-RSA, could be substantially eliminated by pre-incubation with the hapten TFA-lysine.

The current work presents evidence that five of six patients with HH have a high titer of circulating antibody that recognizes the reactive intermediate of halothane (TFA), whereas ten patients exposed to halothane and not developing HH have minimal or no levels of this antibody. This paper, then, explores the association between titer/chronology of the anti-TFA antibody response and indicators/predisposing factors of halothane hepatitis in these patients with HH to determine if a cause (immune response)-and-effect (hepatotoxicity) relationship exists. Although the data presented here do not yet support a role for this humoral immune response in mediating liver damage, it is clear that, in patients developing unexplained hepatitis following halothane exposure, a persistent anti-TFA antibody response is elicited.

**Materials and Methods**

**Patient Information and Sera Collection.** Six of the patients examined in this study were referred to our laboratory based on the diagnosis of unexplained hepatitis following halothane anesthesia. Clinical records supplied for each patient provided the information for age, gender, obesity, previous medications, days post-exposure, duration of anesthesia, type of surgery, fever, jaundice, and levels of liver function enzymes. These records also reported that three of six patients were tested for serologic markers of type A and type B viral hepatitis and found negative. The presence of non-A, non-B viral hepatitis cannot be excluded in any of the patients. Sera from these patients were collected from clinics and hospitals throughout North America. Samples from an additional ten patients serving as controls were referred to us from the Clinical Research Centre, Northwick Park Hospital, Harrow, England. These individuals were also exposed to halothane, but did not develop clinical signs of unexplained hepatitis. Serum from all patients would arrive frozen and, upon thawing, the antibody titer was measured. Remaining serum would be portioned into 0.5-ml aliquots and frozen at −70°C until further use. An additional control group consisted of laboratory personnel and anesthetists and staff from the operating room.

**Enzyme Linked Immunosorbent Assay (ELISA).** The antigen, trifluoroacetylated-rabbit serum albumin (TFA-RSA), was synthesized as described by Callis et al., using a modification of the method by Goldberger and Anfinsen. This assay was performed as previously described. Any potential binding to the carrier, RSA, was also evaluated to ensure that reactivity was directed toward the TFA moiety of the antigen. To detect binding of the patient serum to this antigen, HRP-conjugated goat anti-human (IgG, IgM, IgA) (Cooper Biomedical Laboratories, Malvern, PA) was used. The degree of antibody binding was determined using a Titertek ELISA reader (Flow Laboratories, McLean, VA) with monitoring at 410 nm and a 490 nm reference wavelength. For quantitation of antibody in the ELISA, the reciprocal of the antibody dilution which gave an optical density of 0.500 was used.

**Results**

Table 1 is a composite of information on each of the six patients with unexplained hepatitis examined. Comparing the titer of anti-TFA antibody with days post-exposure provides a chronology for the halothane-induced immune response between patients, as well as within the same patient. The highest titer (12,850) was seen in patient A at 11 days post-exposure. Antibody was still present in patient B as long as 3 months following halothane. Patient D maintained a substantial antibody titer until 1 day prior to her death. The sudden fall in titer on the day of her death might be due to body fluid replacement by intravenous fluid therapy, or by an overall impairment of the immune system. Interestingly, patient C demonstrated a minimal antibody response on day 4, followed by a dramatic increase by day 6. This increase between days 4 and 6 probably reflects the induction of an immune response by an antigen generated through the metabolism of halothane. Patient F was the only patient who demonstrated all the signs of halothane-induced hepatitis, but did not evidence a positive antibody response. This may be due to the patient's chronic use of prednisone for allergy. None of the patients showed binding to the carrier protein, rabbit serum albumin. Personnel exposed to halothane in the operating theater or in the research laboratory did not evidence any anti-TFA antibody.
### TABLE 1. Medical History and Anti-TFA Antibody Titer of Patients Developing Hepatitis after Halothane Anesthesia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Obesity</th>
<th>Previous Medications</th>
<th>Days Post-exposure</th>
<th>Duration Anesthesia/Surgery Type</th>
<th>Fever</th>
<th>Jaundice</th>
<th>Type A, Type B Hepatitis</th>
<th>Liver Enzymes</th>
<th>Anti-TFA-RSA Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>49</td>
<td>F†</td>
<td>No</td>
<td>Thyroid</td>
<td>11 , 30, 304</td>
<td>Unknown/aroscopic surgery</td>
<td>Y</td>
<td>+/−</td>
<td>Negative</td>
<td>ALT 3520</td>
<td>12,850</td>
</tr>
<tr>
<td>B</td>
<td>51</td>
<td>F</td>
<td>Yes</td>
<td>Lithium, thyroid</td>
<td>25, 67</td>
<td>70 min/simple mastectomy</td>
<td>N</td>
<td>Y</td>
<td>Not tested</td>
<td>ALT 981</td>
<td>191</td>
</tr>
<tr>
<td>C</td>
<td>49</td>
<td>F</td>
<td>No</td>
<td>Antibiotics, estrogen</td>
<td>4, 6, 18, 21</td>
<td>30 min/consillectomy</td>
<td>Y</td>
<td>N</td>
<td>Negative</td>
<td>ALT 3960</td>
<td>180</td>
</tr>
<tr>
<td>D‡</td>
<td>70</td>
<td>F</td>
<td>Mildly</td>
<td>Unknown</td>
<td>14, 18, 23, 24</td>
<td>2.5 h/coronary bypass</td>
<td>Y</td>
<td>Y</td>
<td>Not tested</td>
<td>AST 1917</td>
<td>584</td>
</tr>
<tr>
<td>E</td>
<td>29</td>
<td>F</td>
<td>No</td>
<td>Birth control pills</td>
<td>19, 33</td>
<td>20 min/dental surgery</td>
<td>Y</td>
<td>Y</td>
<td>Not tested</td>
<td>AST 2850</td>
<td>364</td>
</tr>
<tr>
<td>F</td>
<td>52</td>
<td>F</td>
<td>Yes</td>
<td>Theophylline, thyroid, prednisone</td>
<td>15, 18</td>
<td>6 h/cholecystectomy</td>
<td>Y</td>
<td>Y</td>
<td>Negative</td>
<td>AST 3560</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

Lab personnel 25–50 M/F

* Titer is the reciprocal of the antibody dilution which yields an optical density of 0.5 at 410/490 nm.

† F = female; M = male.
‡ Patient died on day 25.

The patients’ medical history was then examined to determine if a relationship existed between any of the parameters measured and antibody presence or titer. All patients were female and, except for one (patient E), were above 45 yr of age. The majority of the patients demonstrated fever and jaundice, and all evidenced substantially elevated levels of liver function enzymes (ALT, AST, and/or LDH). Interestingly, three of six patients diagnosed with halothane hepatitis were taking chronic thyroid medication. Two of the women were obese, and three of the six patients were tested for type A and type B hepatitis virus and found to be negative. No real association, however, could be made between any of these parameters and antibody titer.

Table 2 is a composite of information on each of the ten patients examined who did not demonstrate hepatitis following halothane exposure. These control patients were comparable to the six patients in table 1 in age and duration of exposure. There was a mixture of men and women. None of these ten individuals had fever or jaundice and, of the four with available information on aspartate aminotransferase levels, all were within normal values. The majority of these control patients had anti-TFA antibody titers of less than 50 at times when halothane hepatitis patients demonstrated titers of 12,850, 756 and 584 (table 1, patients A, C, and D, respectively). Patients S, U, and X did, however, demonstrate a moderate level of serum reactivity with TFA-RSA. The binding by patients S and X can be accounted for as binding to the RSA carrier protein, since there was comparable binding to TFA-RSA and RSA by the sera from these two patients (data not shown). All other individuals in table 2 had no demonstrable reactivity with RSA (data not shown). Although the reactivity of patient U’s serum toward TFA-RSA is about twice that seen in other control samples, it is still at least sevenfold less than the titer seen in a patient with HH on the same day post-exposure (table 1, patient C, day 6).

### DISCUSSION

This study reports the detection of antibodies cross-reactive with trifluorooxacyl halide, a reactive intermediate formed during halothane metabolism, in patients diagnosed with unexplained hepatitis following halothane anesthesia. Five of six patients examined were positive for this antibody. Although demonstrating all the symptoms of halothane hepatitis, the sixth patient (patient F) was negative for anti-TFA antibody. One possible explanation for the lack of this antibody response was the fact that she was receiving chronic immunosuppressive therapy, prednisone, for treatment of allergy. Since patient F developed hepatitis without the presence of antibody, the argument could be made against a role for this antibody in mediating liver damage. Indeed, in all patients, it could be said that the presence of antibody appears to be more of an effect of
Table 2. Medical History and Anti-TFA Antibody Titer of Patients with No Post-Halothane Anesthesia Hepatic Complications

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Obesity</th>
<th>Previous Medications</th>
<th>Days Post-exposure</th>
<th>Duration Anesthesia/Surgery Type</th>
<th>Fever</th>
<th>Jaundice</th>
<th>Liver Enzymes</th>
<th>Anti-TFA-RSA Titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>67</td>
<td>M†</td>
<td>No</td>
<td>No</td>
<td>d0, d3, d6</td>
<td>65 min/unknown</td>
<td>N</td>
<td>N</td>
<td>NA‡</td>
<td>&lt;50</td>
</tr>
<tr>
<td>R</td>
<td>62</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>d0, d3, d6</td>
<td>45 min/unknown</td>
<td>N</td>
<td>N</td>
<td>NA</td>
<td>&lt;50</td>
</tr>
<tr>
<td>S</td>
<td>69</td>
<td>F</td>
<td>No</td>
<td>Atenolol</td>
<td>d0, d3, d9</td>
<td>215 min/unknown</td>
<td>N</td>
<td>N</td>
<td>AST 17</td>
<td>&lt;50</td>
</tr>
<tr>
<td>T</td>
<td>43</td>
<td>F</td>
<td>Mildly</td>
<td>No</td>
<td>d0, d3, d13</td>
<td>85 min/unknown</td>
<td>N</td>
<td>N</td>
<td>NA</td>
<td>60</td>
</tr>
<tr>
<td>U</td>
<td>46</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>d0, d3, d6</td>
<td>65 min/unknown</td>
<td>N</td>
<td>N</td>
<td>NA</td>
<td>&lt;50</td>
</tr>
<tr>
<td>V</td>
<td>58</td>
<td>F</td>
<td>No</td>
<td>Phyllocontin</td>
<td>d0, d3, d6</td>
<td>150 min/cholecystectomy</td>
<td>N</td>
<td>N</td>
<td>NA</td>
<td>&lt;50</td>
</tr>
<tr>
<td>W</td>
<td>65</td>
<td>M</td>
<td>No</td>
<td>Thyroxine</td>
<td>d0, d3, d6</td>
<td>90 min/unknown</td>
<td>N</td>
<td>N</td>
<td>NA</td>
<td>&lt;50</td>
</tr>
<tr>
<td>X</td>
<td>68</td>
<td>F</td>
<td>No</td>
<td>Disopyramide</td>
<td>d0, d3, d6</td>
<td>135 min/cholecystectomy</td>
<td>N</td>
<td>N</td>
<td>AST 15</td>
<td>130</td>
</tr>
<tr>
<td>Y</td>
<td>89</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>d4, d8</td>
<td>25 min/biopsy</td>
<td>N</td>
<td>N</td>
<td>AST 19</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Z</td>
<td>80</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>d4, d8</td>
<td>30 min/hernia</td>
<td>N</td>
<td>N</td>
<td>AST 15</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

* Titer is the reciprocal of the antibody dilution which yields an optical density of 0.5 at 410/490 nm.
† F = female; M = male.
‡ Not available.

hepatocyte damage than a cause. However, despite the lack of antibody in patient F, a role for the immune system in perpetuating halothane-induced liver damage cannot be ruled out. If a reactive intermediate of halothane conjugated to a liver protein has the potential for evoking a humoral immune response, it may also form the type of immunogen needed to elicit an cell-mediated immune response. The eliciting of a cell-mediated immune response and a role for this response in disease pathogenesis has yet to be examined. Alternately, patient F may be positive for halothane-induced antibodies with a specificity directed toward other halothane-reactive species or metabolites.

This is the first report to chronicle the rise and fall of anti-TFA antibodies over time in terms of days following halothane exposure. The titer of antibody decreased with time in all positive patients. Antibody was present as early as 4 days post-exposure in one patient, and remained elevated for 3 months following halothane in another patient. The induction of this anti-TFA antibody response was clearly demonstrated in patient C, who evidenced a moderate titer on day 4 post-exposure, followed by dramatic rise in titer 2 days later.

Previous reports have also screened patients using the ELISA for the presence of antibody reactive with halothane-associated antigens. Kenna et al. found that 16 of 24 patients with hepatitis following halothane exposure were positive for antibody reactive with halothane-induced antigen from rabbit hepatocytes. Their titers varied from 100 to 25,600. This antibody could not be detected in individuals who did not develop liver complications following multiple halothane exposures, anesthetists, patients with other liver diseases, or normal blood donors. Satoh et al. also reported that two of six patients with halothane-associated massive liver cell necrosis contained anti-TFA antibodies, as determined in a TFA-RSA ELISA. The level of antibody was significantly higher than levels measured in two paracetamol hepatitis patients. Although these investigators used TFA-RSA as antigen, as did the studies reported here, their titers appeared much lower. This result is apparently due to the fact that our laboratory used approximately threefold the amount of antigen in the ELISA analysis.

Three of the six patients reported as having unexplained hepatitis following halothane were also receiv-
ing thyroid therapy. This treatment may be classified as a pre-disposing factor based on previous animal studies. Rats, pretreated with triiodothyronine, developed hepatic necrosis following halothane (1%) anesthesia for 2 h at normal 21% oxygen concentration. Exposure conditions suggest that the oxidative pathway of halothane metabolism was operative in this model.

No true association could be made between the level of anti-TFA antibodies and any of the parameters cited. This is not surprising, given the number of patients examined and the diversity in age and genetic background. However, the detection of this antibody might aid physicians in the early diagnosis of halothane hepatitis, thereby preventing any subsequent exposure to these susceptible individuals. Indeed, the specificity of this antibody response for patients developing halothane-induced hepatitis is confirmed by the absence of antibody in individuals exposed to halothane and not developing liver disease. In addition, the finding that three of the six patients were negative for type A and type B viral hepatitis partially confirms the diagnosis in these three patients of unexplained hepatitis following halothane anesthesia. The presence of non-A, non-B viral hepatitis cannot be excluded in any of the patients.

Detection and characterization of these halothane-induced antibodies in humans has provided more evidence into defining the mechanism of halothane-induced liver damage and the role the immune system may play. Previous work in this laboratory has detected anti-TFA antibodies in halothane-exposed rabbits and guinea pigs. Studying the similarities and differences in antibody levels and specificities in humans and the two animal models suggests the following as a possible mechanism for the generation of a halothane-induced immune response. Intracellular halothane is rapidly metabolized within hepatocytes generating reactive metabolic species that react with intracellular or plasma membrane proteins to alter their antigenicity. Given the quantity and degree of foreignness of these proteins, an immune response (humoral and/or cell-mediated) may ensue. Several populations of antibodies may be formed with specificities directed toward the hapten (TFA), the carrier (altered hepatocyte proteins), or both. Antibodies may play a role in clearance of free antigen by the reticuloendothelial system with the formation of immune complexes, or may lyse any hepatocytes which have altered membrane proteins. Thus, liver damage initiated by metabolite mediated toxicity may be exacerbated during repeated exposure to halothane by immunologically mediated injury.

Despite the complexity of the disease pathogenesis of halothane-induced hepatitis, future studies of more patients, the presence and specificity of their antibodies, and other indicators of an immune response (e.g., immune complexes, cell mediated reactivity) will provide the means for more sensitive diagnostic capabilities for patients developing this disease.

The authors wish to thank John Levy for his synthesis and purification of the trifluoroacetateylated rabbit serum albumin, Susan Schuman for her technical expertise, and Patricia Kime for typing the manuscript. They also appreciate the cooperation of concerned anesthesiologists for referring the serum samples to our laboratory.

REFERENCES


The Use of Caffeine in the Control of Post-anesthetic Apnea in Former Premature Infants

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Premature infants undergoing general anesthesia within the first few months of life are prone to develop apnea and/or bradycardia in the postoperative period.1–5 The incidence of perioperative apneic episodes is inversely correlated with gestational age and weight.1–4 Methylxanthines have been widely used by neonatologists for the management of apnea of prematurity. Although therapeutic blood concentrations and pharmacokinetic profiles in premature infants have been established for both theophylline7 and caffeine,8,10 caffeine has the distinct advantage of being a more potent central nervous system and respiratory stimulant, and possesses fewer cardiac side effects than does theophylline.11

We designed a prospective, double blind, randomized study to examine the possible effectiveness of caffeine in the prevention of apnea following anesthesia and surgery in premature infants.

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Received from the Departments of Anesthesiology, Pulmonary Medicine, Laboratory Medicine, and Child Health and Development, Children's Hospital National Medical Center and George Washington University, Washington, DC. Accepted for publication December 8, 1987. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Atlanta, Georgia, October, 1987.

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Key words: Anesthesia; pediatric; Complications: apnea; periodic breathing; Pharmacology: caffeine.

MATERIALS AND METHODS

Informed consent and institutional approval for the study were obtained. Twenty otherwise healthy (ASA PS 1 or 2) premature infants born at ≤37 weeks gestational age undergoing general anesthesia for inguinal hernia repair were studied. All were ≤44 weeks conceptual age at the time of operation (range 35–44 weeks). Infants with pre-existing cardiac, neurologic, or metabolic diseases, as well as those already receiving methylxanthines, were not included.

No preoperative medication was used. General endotracheal inhaled anesthesia supplemented with neuromuscular blockade was used in all cases. Heart rate and sounds, arterial blood pressure, electrocardiogram, temperature, oxygen saturation, and end-tidal CO2 were monitored. No barbiturates or narcotics were administered in the perioperative period.

Infants were randomly divided into two groups. Group 1 patients received iv caffeine 5 mg/kg injected over a 2-min period. The drug was administered immediately following induction of anesthesia, so that its peak effect would be evident at the end of surgery. Patients in group 2 received iv saline and served as controls. The solutions were supplied by the hospital pharmacy in a double-blinded fashion. At the completion of surgery, the trachea was extubated in the operating room when the patient was fully awake, and a venous blood sample was drawn to measure caffeine level using the Emti® caffeine assay.12 The pattern of respiration and heart rate were continuously monitored and recorded13 using an impedance pneumograph (Healthdyne 16000®) with an Oxford® recorder for at least 12 h postoperatively. The recorded data were analyzed by a pulmonologist for evidence of apnea, periodic breath-