HEPATIC PATHOLOGY AND SKIN TEST REACTIONS TO TRIFLUORO-ACETYLATED AUTOLOGOUS PROTEIN AFTER REPEATED HALOTHANE ANAESTHESIA IN THE GUINEAPEG*  

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SUMMARY  

The aetiology of the rare hepatic failure following halothane anaesthesia is not known. In an attempt to develop an experimental model, three groups of guinea pigs were exposed to air or oxygen, a single 1% halothane administration, or five exposures of 1% halothane at weekly intervals. In an attempt to identify a hypersensitivity reaction, all animals were skin-tested with the common final metabolite of halothane, fluroxene and isoflurane: trifluoroacetic acid, prepared as a complex with autologous serum protein. Hepatic necrosis was found in all groups and did not correlate with positive skin reactions. There was an increase in fatty changes in the liver in animals anaesthetized with halothane.

The clinical problem of unexplained liver dysfunction after halothane anaesthesia is rare (Bunker et al., 1966) and the aetiology is unknown, but, because its course may be fulminant and lead to death, it is important to elucidate its pathophysiology. Among the suggested mechanisms is hypersensitivity to halothane or a metabolite (Combes, 1969; Klion, Schaffner and Popper, 1969; Doniach, 1970). The primary reason for the acceptance of this hypothesis are two case reports of anaesthetists who were thought to have suffered from halothane-induced hepatitis, both of whom had an exacerbation of the symptoms of recurrent hepatitis after a brief challenge with the drug (Belfrage, Ahlgren and Axelson, 1966; Klatskin and Kimberg, 1969). Although these authors did not establish clearly a cause-and-effect relationship, by in vitro tests or other means, the temporal relationship of halothane exposure followed by symptoms of hepatitis has been regarded as persuasive evidence.

METHODS  

Twenty-nine Naval Medical Research Institute Hartley strain (NMRI[H]) guinea pigs, weight 350-550 g, fed on a commercially produced guinea pig diet, and given water containing ascorbic acid 2 mg/ml were studied.

Preparation of the antigen  

Trifluoroacetate (TFA) was conjugated with guinea pig albumen (GPA) by the method of Goldberg and Anfinsen (1962). The reagents involved were ethyl thiom trifluoroacetate (Pierce Chemical Company) and guinea pig serum albumen (Sigma Chemical Company). The conjugated TFA-GPA was lyophilized and analysed for fluorine content (Schwarzkopf Microanalytical Laboratories, Woodside, New York): the fluorine content was 4.27%.
Halothane exposures

The animals were divided randomly into three groups. Ten animals served as control; five were exposed to 100% oxygen, and five to air for 2 h. Ten animals received a single exposure to halothane 1% in oxygen for 2 h, and nine animals were exposed to halothane 1% in oxygen for 2 h each week for 5 consecutive weeks.

Skin tests

Skin tests were performed 3 days after the final exposure of all animals. The flanks were shaved, complete depilation was performed with calcium thioglycolate (Neet Lotion Hair Remover), and intradermal injections of 0.1 ml of physiological saline and 10 μg of TFA-GPA in physiological saline were performed with a 25-gauge needle. Skin reactions were observed at 2, 4, 6 and 24 h and scored as either “immediate” or “delayed”.

Immediate skin reactions were graded in severity according to the following criteria:
(1) slight oedema
(2) severe haemorrhage
(3) severe oedema with necrosis and haemorrhage
(4) marked haemorrhage and necrosis.

Delayed skin reactions were graded according to the diameter of erythema and induration at 24 h after skin-testing.

Liver study

The animals were sacrificed 5 days after the last exposure by stunning blow to the head and exsanguination. The liver was examined with the naked eye for any abnormalities, and representative 5-mm slices from each lobe were placed in 10% formalin buffer. The fixed tissue was then sectioned and stained with haematoxylin and eosin for microscopic examination, and the degree of fatty changes were scored on a scale of 0 to 4:
0—less than 20% of all cells’ cytoplasm contained less than 25% fat.
1—20–40% of all cells’ cytoplasm contained less than 25% fat.
2—40–60% of all cells’ cytoplasm contained 25–50% fat.
3—60–80% of all cells’ cytoplasm contained 50–75% fat.
4—over 80% of all cells’ cytoplasm contained over 75% fat.

The presence or absence of areas of hepatic necrosis was noted on microscopic examination, and recorded.

Statistical analysis

The means for immediate and delayed results of TFA-GPA and saline skin tests and the mean score results of fatty changes of the three groups were compared with an unpaired $t$ test. The incidence of

| Table I. Mean reaction scores (and SEM) of skin tests in guineapigs exposed to air or oxygen, halothane (once) and halothane (five times) at 2, 4 and 6 h after tests |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Time (h) | Control | Single halothane | Multiple halothane |
| TFA-GPA | Saline | TFA-GPA | Saline | TFA-GPA | Saline |
| 2 | 0.6 ± 0.21 (10) | 0.7 ± 0.16 (10) | 0.1 ± 0.10 (10) | 0.0 ± 0.0 (10) | 0.2 ± 0.15 (9) | 0.2 ± 0.15 (9) |
| 4 | 0.0 ± 0.0 (10) | 0.2 ± 0.13 (10) | 0.3 ± 0.15 (10) | 0.3 ± 0.15 (10) | 0.3 ± 0.17 (9) | 0.1 ± 0.11 (9) |
| 6 | 0.2 ± 0.13 (10) | 0.1 ± 0.90 (10) | 0.3 ± 0.15 (10) | 0.2 ± 0.13 (10) | 0.4 ± 0.18 (9) | 0.4 ± 0.13 (9) |

TFA-GPA = trifluoroacetylated guineapig albumen. Score 0–4 in increasing reactivity (see text). Parentheses indicate number of animals.

| Table II. Skin test reaction in guineapigs exposed to air or oxygen, halothane (once) and halothane (five times) at 24 h after testing |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Control | Single halothane | Multiple halothane |
| TFA-GPA | Saline | TFA-GPA | Saline | TFA-GPA | Saline |
| Mean | 3.4 ± 0.33 (10) | 3.0 ± 0.48 (10) | 2.5 ± 0.39 (10) | 1.5 ± 0.58 (10) | 3.8 ± 0.59 (9) | 3.1 ± 0.60 (9) |

TFA-GPA = trifluoroacetylated guineapig albumen. Skin reaction expressed as mean (± SEM) diameter (mm) of induration.
necrosis in the three groups was compared using Chi-square analysis with Yates' correction factor. A value for $P$ of less than 0.05 was accepted as significant.

**RESULTS**

**Skin tests**

The results of skin-testing expressed as a mean score during the first 6 h are given in table I. The highest mean score was 0.7 ± 0.21 for the saline-tested control group at the 2-h reading, compared with 0.6 ± 0.16 for the TFA-GPA-tested control group at the same reading. The responses were not significantly different between the TFA-GPA skin tests and the saline control skin tests during the first 6 h. The diameters of induration to the two test substances at 24 h are given in table II. There is no significant difference between the responses to TFA-GPA and saline, nor is there a difference (in TFA-GPA and saline skin tests) between the control, single and multiple exposure groups.

**Liver**

Six of the 10 animals in the control groups, five of 10 from single halothane exposure, and seven of nine from the multiple halothane exposure had livers which appeared abnormal to the eye with small subcapsular areas of white tissue. There was no macroscopic evidence of fatty changes in the liver, but microscopic examination revealed significant differences in the groups shown in table III. The fatty changes were general throughout the parenchyma, but were predominant in the centrilobular area. Commonly there were mild fatty changes in the cytoplasm with intact cell nuclei and borders. The degree of fatty change was greater in the single and multiple halothane groups (table III) ($P<0.05$), but there was no difference between the single and multiple halothane groups.

Areas of necrosis were found in animals from all the groups. The incidence was two of 10 (control), three of 10 (single halothane exposure) and four of nine (multiple halothane). These are not significantly different at a confidence level of 5%. A typical area of necrosis is shown in figure 1. The necrosis was usually focal and subcapsular in the various hepatic lobes. It was consistent with coagulation necrosis with a persistent cellular outline, but pyknotic nuclei in some areas and karyolysis in other areas were noted. Fatty metamorphosis was not a prominent feature. The necrosis encountered in the controls was identical with the necrosis seen in the two experimental groups.

**DISCUSSION**

Trifluoroacetic acid is the final metabolite of halothane and 12% to 20% of halothane is metabolized to...
induce both immediate and delayed hypersensitivity. When forming a complex with a carrier protein, may the final halothane metabolite, trifluoroacetic acid, tissue containing the compound. Mathieu and co-workers (1973, 1974, 1975) have shown clearly that sensitized thymic (T) lymphocytes directed against the compound, and immediate, mediated by antibody reactions among the 29 animals studied. In none of the animals with positive skin reactions was there hepatic necrosis. Thus, there appears to be no relationship between the hepatic necrosis and positive skin tests. Other in vitro methods, available for the study of delayed hypersensitivity, such as lymphocyte stimulation and leucocyte migration were not employed in this experiment.

It is concluded from these data that repeated halothane administration does not cause hepatic necrosis as an immune response which may be detected in the guineapig with a skin test. Whether or not the guineapig is a suitable animal, the sample was large enough, the antigen TFA-GPA was appropriate, or cutaneous testing is sufficiently sensitive, are valid questions. Other studies have indicated that the skin test employed is sensitive at the 24-h interval when animals are immunized with TFA-GPA (Reves and McCracken, 1976). It is possible that the skin test with the carrier protein, guineapig serum albumen (autologous serum protein), is not appropriate, but it appears from other studies that the skin response is hapten-specific using similar carrier proteins (Mathieu et al., 1975).

Since all experiments with trifluoroacetic acid as a complex with homologous carrier protein have been
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negative in demonstrating a cause-and-effect relationship between halothane and liver necrosis, other hapten-carrier complexes or antigens must be used. One such antigen is trifluoroacetaldehyde as a complex with autologous liver lipoprotein. We have used this antigen to induce hypersensitivity in the rat, but have been unable to demonstrate hepatic necrosis in animals immunized with this compound and exposed to halothane.

It would be useful to develop an experimental model of halothane hepatitis as has been done in other drug hypersensitivity states, such as penicillin. If the basis of this enigmatic disease is immunological, reproducible in vivo and in vitro studies will be developed to render the clinical diagnosis easier. We are continuing to attempt to develop such a model.

The opinions or assertions contained herein are those of the authors and should not be construed as official or reflecting the views of the Department of the Navy or the Department of Defense.

Experiments reported herein were conducted according to the principles enumerated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on Animal Facilities and Care of the National Academy of Sciences, National Research Council, Washington, D.C.

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PATHOLOGIE HEPATIQUE ET REACTIONS DES TESTS DE LA PEAU A LA PROTEINE AUTOLOGUE TRIFLUORO-ACETYLEE APRES ANESTHESIES REPETEES PAR L'HALOTHANE SUR DES COBAYES

RESUME

On ne connaît pas l'étiologie de la rare insuffisance hépatique qui suit l'anaesthésie par l'halothane. Dans un essai effectué pour mettre au point un modèle expérimental, on a exposé trois groupes de cobayes à l'air ou à l'oxygène, à une seule administration d'halothane à 1% ou à cinq expositions d'halothane à 1%, à intervalles d'une semaine. En vue d'identifier une réaction d'hypersensibilité, tous les animaux ont subi un test de la peau à l'aide du métabolite ordinaire final d'halothane, de fluoroène et d'isofluorane: l'acide trifluoro-acétique, préparée comme complexe avec protéine autologue du sérum. On a trouvé dans tous les groupes une nécrose hépatique et il n'y a eu aucune corrélation avec les réactions positives de la peau. Il y a eu une augmentation des changements des corps gras dans le foie des animaux anesthésiés à l'halothane.
LEBER- UND HAUTREAKTIONEN AUF DIE VERABREICHUNG VON TRIFLUORAZETYLIERTEM AUTOLOGEM PROTEIN NACH WIEDERHOLTER HALOTHAN-NARKOSE IM MEERSCHWEINCHEN

ZUSAMMENFASSUNG
Man weiß nichts über die Aetiologie des seltenen Leberversagens nach Halothan-Narkose. Bei einem Versuch, ein experimentelles Modell zu gestalten, wurden drei Gruppen von Meerschweinchen Luft oder Sauerstoff ausgesetzt, eine einzelne 1% Halothan-Narkose verabreicht, oder fünf, zu 1% Halothan im wöchentlichen Abständen exponiert. Um die Überempfindlichkeitsreaktion zu identifizieren, wurden bei Tieren mit dem allgemeinen Endprodukt von Halothan, Fluroxen und Isofluroxen, Haut-Teste durchgeführt, nämlich dem Metabolikum trifluorazetischer Säure, die als ein komplexes autologes Serum Protein hergestellt worden war. Lebernekrose wurde in allen drei Gruppen festgestellt, zeigte jedoch keinerlei positive Korrelation zur Hautreaktion. Es ergab sich eine Steigerung der Fettveränderungen in den Lebern jener Tiere, die mit Halothan narkotisiert worden waren.

PATOLOGIA HEPATICA Y DERMORREACCIONES A PROTEINA AUTOLOGA TRIFLUOROACETILADA TRAS REPETIDA ANESTESIA CON HALOTANO EN EL COBAYO

SUMARIO
Se desconoce la etiologia del fallo hepático infrecuente, tras la anestesia con halotano. Intentando desarrollar un modelo experimental, se expusieron tres grupos de cobayos a aire o oxígeno, una sola administración de halotano al 1%, o cinco exposiciones a halotano al 1% a intervalos semanales. A fin de identificar una reacción de hipersensibilidad, todos los animales fueron sometidos a pruebas cutáneas con el metabolito final común de halotano, fluroxeno e isoflurano: el ácido trifluoroacético, en forma de un complejo con proteína sérica autóloga. En todos los grupos se halló necrosis hepática que no se correlacionó con dermorreacciones positivas. En los animales anestesiados con halotano había un aumento de alteraciones adiposas hepáticas.