

Halothane macrophage migration inhibition factor test in halothane-associated hepatitis

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SUMMARY As an index of delayed hypersensitivity *in vitro* halothane macrophage migration inhibition factor tests (halothane-MIF tests) were performed on peripheral blood lymphocytes from five patients with halothane hepatitis. Twenty-two subjects exposed to halothane, but with no evidence of jaundice, five 'healthy' hospital anaesthetists, nine jaundiced subjects without halothane exposure, and 10 healthy subjects with no history of exposure to halothane were also tested.

The halothane-MIF test was positive in four of the five patients with halothane-induced hepatitis; the negative result was in a patient on steroid treatment. The test was negative in all other subjects. Our findings suggest that the halothane-MIF test may be of value in the diagnosis of halothane-induced hepatitis and as a screening procedure for the identification of susceptible subjects.

Halothane has been used as an anaesthetic agent in clinical medicine for 20 years and is considered to be both safe and reliable. However, in a very small number of patients, it has been implicated in the causation of postoperative jaundice (Burnap *et al.*, 1958; Virtue and Payne, 1958; Slater *et al.*, 1964; Peters *et al.*, 1969; Sharpstone *et al.*, 1971). Mushin *et al.* (1971) estimated that the risk of jaundice after halothane exposure, repeated within a four-week period, was between 1 in 6000 and 1 in 20 000.

The occurrence of jaundice after halothane exposure is unpredictable, but there are several known predisposing factors, *viz.* multiple exposures, particularly within a short time interval (Klion *et al.*, 1969; Inman and Mushin, 1974), obesity (Carney and Van Dyke, 1972), and middle age, but not the severity of operation or duration of exposure (Moult and Sherlock, 1975).

The diagnosis of halothane-induced hepatitis depends on a history of exposure, appropriate clinical features, and the absence of other known causes of hepatitis. Suggestive features include the occurrence of postoperative fever, without any obvious cause (Klion *et al.*, 1969; Hughes and Powell, 1970), jaundice, usually of hepatocellular type, occurring within two weeks of halothane exposure (Inman and Mushin, 1974), the presence of eosinophilia (Klion *et al.*, 1969), and auto-antibodies (Walton *et al.*, 1976).

The above criteria, though not diagnostic, suggest

a delayed hypersensitivity type mechanism. Preliminary investigations on two patients with established halothane-induced hepatitis showed the production of macrophage migration inhibition factor (MIF) after incubation of their lymphocytes with halothane (halothane-MIF test) (Jones Williams *et al.*, 1972a). We were thus encouraged to further our studies of '*in vitro*' testing for delayed hypersensitivity.

Material and methods

SUBJECTS (Table 1)

We investigated six groups of subjects:

Group 1

Five patients with established halothane-induced hepatitis (Table 2) included two previously reported (Jones Williams *et al.*, 1972a). All were tested between seven and 21 days after the development of jaundice. One of the cases (WC) had had a previous episode of postoperative jaundice after halothane exposure at another hospital, four years previously, which was ascribed to halothane. After recovery from the last episode of halothane-induced jaundice the patient received several more non-halothane anaesthetics without any further complications.

All were Australia antigen negative and showed the biochemical profile of hepatocellular jaundice, and no other cause for the jaundice was found. Liver biopsy on one patient (ER) showed areas of focal cellular necrosis with some centrilobular cholestasis.

Table 1 *Subjects examined*

Group	Halothane exposure	Jaundice	Number of cases
1	Multiple	With	5
2	Multiple	Without	3
3	Single	Without	19
4	Potential	Without	5
5	None	Without	10
6	None	With	
		Infective	4 9
		Obstructive	5

Table 2 *Group 1 halothane jaundice patients (5)*

Cases ¹	Age	Dates of halothane exposure	Onset jaundice (days)	Postop pyrexia	Disease
ME	53 F	3 Mar 72	—	—	Carcinoma cervix
		23 Mar 72	11	Yes	
WC ²	60 M	20 June 72	—	Yes	Carcinoma bladder
		27 June 72	2	Yes	
ER ³	51 F	7 Aug 72	—	—	Sarcoma uterus
		10 Aug 72	—	—	
		24 Aug 72	4	Yes	
EH	30 F	15 Sept 75	—	Yes	Fractured tibia
		23 Oct 75	3	Yes	
MR ³	75 F	29 July 75	—	Yes	Fractured femur
		31 July 75	—	Yes	
		28 Aug 75	6	Yes	

¹LFT—all showed hepatocellular change and negative Australian antigen.

²Eosinophilia present.

³On steroids.

Clinically, all recovered from the illness within two months.

Group 2

Three patients exposed more than once to halothane but without development of jaundice were tested at 3, 11, and 14 days after exposure.

Group 3

Nineteen patients with a single halothane exposure, without development of jaundice, were tested between six and 23 days, mostly six to 10 days, after exposure.

Group 4

Five healthy anaesthetists had been potentially exposed to halothane over a long period.

Group 5

Ten healthy subjects had no history of jaundice or halothane exposure.

Group 6

Nine jaundiced patients had had no halothane exposure. These included four with viral hepatitis type A, two with carcinoma of the head of the

pancreas, two with gallstones, and one with cirrhosis of the liver.

METHODS

White blood cells were separated from 20 ml of heparinised blood by sedimenting for $\frac{1}{2}$ -1 hour with 2.5 ml Dextran 110 injection BP in 0.9% sodium chloride. Cell concentrations were adjusted with Eagle's Minimum Medium to give final concentrations of 2.5 and 5.0×10^6 cells per ml. The cells were then cultured with three concentrations of halothane (Fluothane ICI), 5, 15, and 25 μ l. Halothane was omitted from the control cultures.

Peritoneal macrophages were obtained from Hartley guinea-pigs, seven days after oiling with paraffin oil, and macrophage migration chambers (Sterilin) were set up as described by Bloom and Bennett (1971). Briefly, each culture supernatant was used to fill three wells of a migration plate, each containing two 20 lambda micropipette tips (Camlab, Cambridge). The areas of migration were read at 18 and 24 hours. The migration index was expressed as:

$$\frac{\text{area of migration with antigen}}{\text{area of migration without antigen}}$$

It was found that, by an analysis of variance, there was no optimum concentration(s) for the experiment; consequently, the 12 readings for each subject were averaged to give a mean migration index, which is used throughout this report.

Results

MIF READINGS

The values for the halothane-MIF test are shown in the Figure.

Group 1

The halothane hepatitis patients not on steroid treatment (four) had MIF readings in the 0.64 to 0.69 range, whereas the fifth subject (ER), who was on steroid treatment, showed a MIF index of 1.08.

Group 2

The three patients with multiple exposure to halothane, but without jaundice, had MIF readings in the 0.94 to 1.08 range.

Group 3

Of 19 patients with a single exposure and without jaundice, 12 had MIF indices within the range 0.85 to 1.15; two had MIF indices below 0.85 but above 0.80, and five subjects had MIF indices above 1.15.

Group 4

The five healthy anaesthetists had MIF indices within the range 0.95 to 1.06.

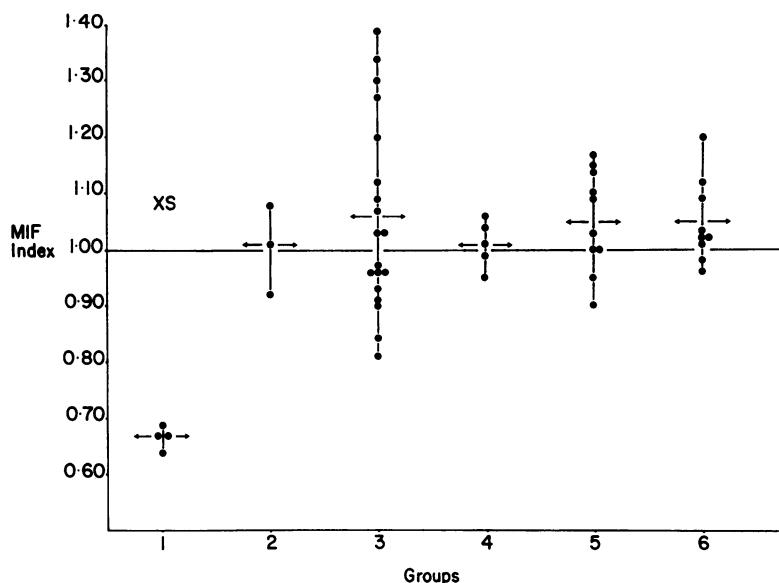


Figure *Halothane-MIF results; group 1—halothane hepatitis patients and comparison groups 2-6 (see text). XS—patient on steroids.*

Group 5

The 10 healthy subjects had MIF indices within the range 0.90 to 1.17.

Group 6

Of the nine jaundiced patients unexposed to halothane, eight had MIF indices in the range 0.96-1.12, and one patient had a much higher MIF index of 1.20.

No correlation was found in any jaundiced subject between the age and sex of the subject, duration of jaundice, liver function tests, the presence or absence of serum auto-antibodies and eosinophilia, and the halothane-MIF indices.

No statistically significant difference could be shown between the single halothane exposed group (group 3), the healthy anaesthetists (group 4), and the 10 healthy subjects (group 5). For purposes of comparison, therefore, they are combined to form a control group.

Comparison of patients exposed more than once to halothane who developed jaundice (group 1) with patients having multiple exposures but without developing jaundice (group 2) (Table 3) shows a very significant difference of 0.3425 in the mean MIF result. From these results, therefore, there is evidence that patients who are exposed more than once to halothane and develop jaundice have significantly lower MIF test readings than those similarly exposed who do not develop jaundice.

Comparison between both groups exposed to halothane more than once (1 and 2), the control groups (3, 4, and 5), and the jaundice group (6)

shows that patients who did not develop jaundice after multiple halothane exposure (group 2) could not be differentiated from either the control or jaundice groups. Patients who developed jaundice (group 1) after multiple halothane exposure, however, were very significantly different, the MIF test readings being on average 0.38 lower than for the other groups.

Thus, neither halothane exposure on one occasion only, potential halothane exposure, nor jaundice due to causes other than halothane appear to affect the MIF test to a significant degree.

Discussion

There was strong circumstantial evidence that halothane was the agent responsible for the onset of jaundice in the five patients described above. All had had multiple exposures to the anaesthetic, developed jaundice of hepatocellular type with no other obvious cause within two weeks of the last exposure, and developed pyrexia after the last exposure to the anaesthetic. Unexplained pyrexia after the penultimate halothane exposure was seen in three out of the five patients. Two showed eosinophilia in the peripheral blood. As in other series reported, the majority (80%) of our patients were female and middle-aged.

It is very difficult, on the basis of light microscopy, to differentiate between halothane and viral hepatitis. Features which suggest halothane hepatitis, such as the presence of significant numbers of eosinophils,

Table 3 Analysis of halothane-MIF test

Group	No.	Mean	SD	Standard error
1 Exposed more than once and jaundiced	4	0.67 ¹	±0.0206	0.0103
2 Exposed more than once and not jaundiced	3	1.01 ²	±0.0700	0.0404
3 Exposed once and not jaundiced	19	1.06	±0.1714	0.0393
4 Potentially exposed	5	1.01	±0.0430	0.0192
5 Healthy controls	10	1.05	±0.0909	0.0288
(3, 4, and 5 combined)	34	1.05	±0.1370	0.0235
6 Obstructive and infective jaundice	9	1.05	±0.0756	0.0252

¹P < 0.001 significant $t = 8.213$ (compared to group 2)

P < 0.001 significant $t = 14.84$ (compared with groups 3, 4, and 5 combined)

P < 0.001 significant $t = 13.98$ (compared with group 6).

²Not significant $t = 0.8$ (compared to groups 3, 4, and 5)
 $t = 0.8$ (compared to group 6).

granulomas, and fatty change (Klion *et al.*, 1969), were absent in our one patient who had a biopsy (ER).

In our investigation, four of the five patients who had developed hepatitis after halothane anaesthesia were positive on halothane-MIF testing. The one negative result may be explained by the fact that this patient (ER) was receiving prednisone at the time of testing. It is well known that steroids can reduce MIF production (Jones Williams *et al.*, 1972b; Price *et al.*, 1977).

Paronetto and Popper (1970) have supported an immunological mechanism in halothane-induced hepatitis. They performed the lymphocyte transformation test, using halothane as antigen on 15 patients with halothane hepatitis, and obtained a positive result in 10. Our earlier report (Jones Williams *et al.*, 1972a), using the MIF test, confirmed their conclusion in two cases. However, two further lymphocyte transformation studies on 10 patients (Walton *et al.*, 1973) and on 17 patients with halothane jaundice (Moult and Sherlock, 1975) failed to confirm these findings.

Klatskin and Kimberg (1969) described an anaesthetist with a history of asthma and hay fever in whom recurrent hepatitis led to the development of cirrhosis. Each of the relapses coincided with the patient's return to work and re-exposure to halothane. When he was challenged with a non-anaesthetic dose of halothane (0.1 to 0.2% oxygen for 5 min) he responded with an identical relapse, characterised by fever, and acute hepatitis. This was documented both biochemically and histologically. Once he abandoned the use of halothane and used other anaesthetic agents no relapse occurred. The five anaesthetists we tested, who had all worked with halothane over long periods, showed negative responses to halothane-MIF testing, indicating that they had not become sensitised to halothane.

Most of the reported cases of halothane-induced hepatitis have occurred in patients who have received

multiple exposures in a short time interval (about four weeks). It has been shown, using serial transaminase estimations, that minor hepatic cell damage in the absence of jaundice may occur with repeated halothane administration (Trowell *et al.*, 1975; Wright *et al.*, 1975). It may be that repeated minor hepatic cellular damage may set off a severe immunological reaction in certain individuals resulting in halothane hepatitis.

Other evidence for an immunological mechanism is the presence of various autoantibodies in the serum of patients with halothane-induced hepatitis. These include smooth muscle antibody, and antinuclear factor (Moult and Sherlock, 1975), and the liver/kidney microsomal antibody (Walton *et al.*, 1976). These are probably secondary phenomena, since they are not present in all patients and disappear after recovery from the disease.

The results of our investigations have been very encouraging as the halothane-MIF index has been found to be specific for patients with halothane-induced hepatitis. This suggests that the halothane-MIF test will be a valuable diagnostic tool in patients with halothane-induced hepatitis. Secondly, it gives additional support for an immunological pathogenesis. Finally, it may be of value in the detection of individuals who are susceptible to the development of jaundice after halothane anaesthesia.

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