Drug Metabolism and Genetic Polymorphism in Subjects with Previous Halothane Hepatitis

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To test the hypothesis that halothane hepatitis is caused by a combination of altered drug metabolism and an immunoallergic disposition, the metabolism of antipyrine, metronidazole, sparteine, phenytoin, and racemic R- and *S*-mephenytoin was investigated in seven subjects with previous halothane hepatitis. The HLA tissue types and the complement C3 phenotypes were also determined. The metabolism of antipyrine and metronidazole was within normal range in all subjects, and they were all fast or extensive metabolizers of sparteine, mephenytoin, and phenytoin. HLA tissue types were unremarkable. Five of the seven subjects had complement C3 phenotypes F or FS. In the general population phenotype S is the most common, but the difference in complement C3 phenotypes is not statistically significant ($p = 0.07$). We conclude, although in a limited number of patients, that subjects with previous halothane hepatitis do not appear to be different from controls with regard to drug metabolism and HLA tissue type. The possibility of a higher frequency of complement C3 phenotype F and FS needs further investigation.

Key words: Antipyrine; complement 3C; halothane; hepatitis; HLA antigens; mephenytoin; metronidazole; polymorphism (genetics); sparteine

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The pathogenetic mechanisms of halothane hepatitis have still not been solved. The formation of reactive metabolites during hypoxia (1), autoimmunity (2), and a genetically determined susceptibility factor (3) have been suggested as causative factors.

Absorbed halothane is mainly metabolized by the P450 system in the liver. In humans the number of drug-metabolizing P450 enzymes may be in the range of 30–100 (4), and each may have a different but overlapping substrate specificity.

Each P450 enzyme is encoded by a separate gene, and P450 genes may be highly polymorphic (5). In humans the best known examples are the sparteine/debrisoquine and the mephenytoin oxidation polymorphisms. In white Caucasian populations about 7–9% are poor metabolizers of sparteine/debrisoquine, but only about 3% are poor metabolizers of mephenytoin (6, 7).

The sparteine/debrisoquine oxidation polymorphism expresses the activity of a distinct P450, the P450IID6, whereas the mephenytoin oxidation polymorphism expresses P450 in the IIC subfamily (5). The sparteine/debrisoquine oxidation polymorphism is important for the elimination of about 30 clinically used drugs, whereas a broader clinical significance of the mephenytoin polymorphism remains to be established (8).

The hydroxylation of phenytoin is also under genetic control with an assumed frequency of 9% heterozygous slow metabolizers and 0.5% homozygous extremely slow metabolizers (9). The metabolism of two further model compounds, antipyrine and metronidazole, is mainly regulated by environmental factors (10), although the inducibility of the metabolism of the former may be under separate genetic control and cosegregate with arylhydrocarbonhydroxylase inducibility (11).

In the poor metabolizers an impairment of drug metabolism may result in accumulation of the parent compound or active metabolite, less formation of an active metabolite, or increased formation of a toxic metabolite (8).

It is at present not known which P450 isozyme catalyses the metabolism of halothane, and there is no published evidence of a relationship between halothane metabolism and the genetic polymorphisms of drug oxidation.

A genetically determined toxic effect of halothane metabolism is probably not the only explanation of the fulminant cases of halothane hepatitis, as autoimmune factors appear to be involved as well (12). It has therefore been proposed (13) that severe cases of halothane hepatitis are the result both of a genetically determined formation of toxic metabolites and an autoimmune disposition leading to an immunoallergic reaction to halothane-induced neoantigens.

Table I. Age, sex, number of halothane exposures, most abnormal values of serum alanine aminotransferases (ALAT) (normal range, 0.17–0.67 μkat/l), serum bilirubin (normal range, 4–17 μmol/l), and prothrombin (PP) index (normal range, 0.75–1.15) at the time of halothane hepatitis. Present determination of complement C3 phenotype and HLA tissue type

Subject no.	Age, years	Sex	H-exposure times	ALAT, μkat/l	Bilirubin, μmol/l	PP index	C3 type	HLA type
1	28	M	2	533.44	477	0.06	FS	A11, 28; B35, 40; Cw3, 4; DRw10
2	44	F	3	32.67	405	0.23	F	A2, 24; B7; DR2w6
3	65	F	4	46.71	335	0.39	FS	A1, 33; B8, 14; Cw8; DR3
4	67	F	3	30.01	170	0.29	S	A11, 30; B15, 35; Cw3, 4; DR1w6
5	61	F	2	18.84	34	0.60	S	A2, 24; B35, 37; Cw4; DR3
6	40	F	2	33.34	95	0.51	FS	A28, 29; B8, 27; Cw2; DR5w6
7	47	F	3	11.34	77	0.80	FS	A1, 30; B17, 21; Cw6; DR7

To test this hypothesis further, we have assessed the oxidation of several different model drugs in seven patients with previous halothane hepatitis. We also looked for genetic markers of autoimmune disposition by HLA and complement C3 phenotyping.

MATERIALS AND METHODS

Seven subjects, six women and one man, all Caucasians, with halothane hepatitis 1–9 years previous to the present investigation, volunteered to participate in this study.

They were all admitted to our department because of acute liver disease; the final diagnosis was halothane hepatitis.

As there is no specific test for halothane hepatitis, the diagnosis is based on halothane exposure followed by otherwise unexplained liver damage. Our subjects had multiple exposures to halothane. Clinically, they all developed unexplained fever within 1–4 days after the last halothane exposure. The highest recorded transaminase values were observed 4–8 days after exposure to halothane. All patients were negative for hepatitis A and B virus markers, and other drug-related damage could be excluded. None of the patients had alcohol or drug abuse, and none had preexisting liver disease.

Patients 1 and 2 developed hepatic coma grade II and I, respectively. On a clinical and biochemical basis four patients (Patients 1–4) had severe hepatitis, and three mild hepatitis. Patient 1 (Table I) had the most severe hepatitis, and Patient 7 the mildest. Eosinophilia was demonstrated in Patients 3 and 7. Liver biopsy was performed in Patients 1, 2, 3, and 7, all compatible with halothane hepatitis.

Investigational design

Blood was drawn from the fasting patient for routine chemical chemistry such as haemoglobin, serum creatinine, alanine aminotransferase, alkaline phosphatases, bilirubin, and prothrombin index and for HLA tissue typing and complement C3-phenotype determination (14). Then 1000 mg antipyrine, 100 mg sparteine (Depasan®, Giulini), and 500 mg metronidazole were given orally.

Urine was collected for the following 48 h. After 12 h the urine volume was recorded, and an aliquot kept frozen for determination of sparteine and its metabolites, dehydrosparteines (15, 16). Saliva was collected after 24 h for determination of antipyrine and metronidazole clearances (17). After 48 h blood and urine were investigated for antipyrine and metronidazole and their metabolites by the methods indicated (18, 19). On the 3rd day 100 mg phenytoin was given orally. Blood was drawn 8, 10, and 12 h later for determination of phenytoin and *p*-hydroxyphenytoin (20).

One month later 100 mg racemic R- and *S*-mephenytoin (Mesantoin®, Sandoz) was given orally, and urine collected for the following 12 h. A urine sample was used for determination of the ratio between chromatographic peak areas of *S*- and R-mephenytoin (21).

The frequency of poor metabolizers of sparteine, mephenytoin, and phenytoin was compared with the frequency known from the population (9, 16) by means of chi-square tests. The data regarding the metabolism of antipyrine and metronidazole were compared with the data from matched control groups consisting of nine and seven subjects, respectively, by means of *t* tests. The controls were selected from a database on antipyrine clearance in healthy subjects and a similar database on metronidazole clearance. The controls were matched for age, sex, body weight, height, and smoking habits.

HLA antigen frequency was not subjected to any statistical calculation.

The complement C3 phenotypes were compared with those of 154 healthy blood donors investigated at the same time, using Fisher's exact test.

RESULTS

The results with regard to the metabolism of sparteine, mephenytoin, and phenytoin are given in Table II. All persons were extensive or fast metabolizers of the test drugs used; that is, they had a metabolic ratio of sparteine-dehydrosparteines less than 20 (22), a ratio of peak areas of *S*- and R-mephenytoin less than 1.0 (21), and a ratio between plasma phenytoin and *p*-hydroxyphenytoin (conjugated and unconjugated) less than 3.8 (23).

Table II. Metabolism of sparteine, mephenytoin, phenytoin, antipyrine, and metronidazole in seven subjects with previous halothane hepatitis

Subject no.	Sparteine ratio*	Mephenytoin ratio†	DPH/pHPPH‡	Antipyrine clearance§				Metronidazole clearance‖			
				Plasma	To HMA	To NOR	To OHA	Plasma	To MAA	To HM	To gluc
1	0.56	0.30	2.40	41.3	6.6	8.3	12.8	70.8	9.8	39.6	4.1
2	0.13	0.34	0.85	30.5	3.2	3.1	7.6	76.4	6.4	47.0	5.1
3	4.05	0.54	1.35	22.7	2.6	3.2	6.8	44.9	1.4	20.0	2.6
4	1.48	0.23	1.00	42.6	6.4	8.6	13.7	78.6	5.2	50.4	5.0
5	0.85	0.25	2.60	36.1	4.9	3.3	10.6	78.7	10.8	31.0	1.9
6	1.02	<0.10	1.75	64.1	13.8	17.2	22.2	76.6	17.6	27.3	7.1
7	0.39	<0.10	2.15	46.2	8.3	9.7	14.4	51.2	8.9	22.9	4.3
Mean ± SD	None PM	None PM	None PM	40.5 ± 13.1	6.5 ± 3.7	7.6 ± 5.1	12.6 ± 5.1	68.1 ± 14.0	8.6 ± 5.1	34.0 ± 11.8	4.3 ± 1.7
Control values¶	9%	3%	9% (0.5%)	43 ± 12	4.7 ± 3.2	6.9 ± 4.5	10.2 ± 5.6	69 ± 11	9.5 ± 4.7	23.3 ± 10.4	2.2 ± 0.4

* Metabolic ratio between sparteine and dehydrosparteine in urine (16); poor metabolizer (PM) cutoff value, >20.
† Ratio of peak areas of *S*- and R-mephenytoin in urine (16); PM cutoff value, ≥1.0.
‡ Ratio between phenytoin (DPH) and *p*-hydrophenytoin (pHPPH) in plasma (9, 23); PM cutoff value, ≥3.8.
§ Antipyrine saliva clearance and fractional clearance to the hydroxymethyl (HMA), nor- (NOR), and 4-hydroxy (OHA) metabolites, in ml min^{-1}.
‖ Metronidazole plasma clearance and fractional clearance to the acetic acid (MAA), hydroxy- (HM), and glucuronide metabolite, in ml min^{-1}.
¶ Frequency in a control population (9, 16) or mean ± SD of control groups matched for sex, age, body weight, and smoking; $n = 9$ for antipyrine; $n = 7$ for metronidazole.

The mean clearance values of antipyrine and metronidazole and the formation of metabolites and their clearance were not different from controls (Table II).

None of the seven examined patients were poor metabolizers of sparteine, mephenytoin, or phenytoin (95% confidence limits, 0–3). This rules out the possibility of a considerable poor metabolizer frequency among patients with halothane hepatitis.

The 95% confidence intervals for the ratio of antipyrine and metronidazole clearance between subjects with previous halothane hepatitis and controls are 64–124% and 79–119%, respectively. The results of the HLA tissue typing and the C3 phenotyping are given in Table I. The HLA tissue types did not show any consistent pattern. The complement C3 phenotypes in the subjects with previous halothane hepatitis showed that five of the seven subjects with previous halothane hepatitis had phenotypes F and FS, compared with 56 of 154 healthy blood donors. This does not, however, reach statistical significance ($p = 0.073$).

DISCUSSION

Halothane hepatitis has, after much debate, been accepted as a clinical entity. Whereas the figures on the incidence vary from 1 per 300 anesthesias (24) to 1 per 9000 (25), there seems to be agreement on a mortality figure of around 1 per 40,000 halothane anesthesias (25, 26).

Farrel et al. (3) have described a familial, constitutional susceptibility factor that predisposes subjects to halothane hepatitis. They used an in vitro test that detects lymphocyte damage from toxic metabolites of phenytoin. The nature of this 'susceptibility factor' is unknown, but both altered drug metabolism and a genetic predisposition are possible explanations.

Our hypothesis was that halothane hepatitis is caused by genetically determined metabolism of halothane, resulting in liver cell damage. In disposed individuals the liver cell damage triggers an immunoallergic reaction. It may be the combination of the two factors that gives rise to the rare, fatal cases.

To assess the activities of different P450 isozymes, we administered different test drugs. In the present study subjects with previous halothane hepatitis did not differ from control populations with regard to any of the measured cytochrome P450 activities. The battery of five test compounds covered three mainly genetically regulated cytochrome P450s as well as several not yet identified P450 forms, which are mainly under environmental control. Each of the metabolites of antipyrine and metronidazole is thought to consist mainly of different P450 forms with partly overlapping substrate specificity (10). The formation of each metabolite is more or less selectively induced by different environmental factors. The inducibility of several of the involved P450 forms may well be under genetic control (11, 27).

Although the number of subjects, seven, may appear small, the study excludes with a high probability that poor metabolizers of sparteine, mephenytoin, and phenytoin are at increased risk with regard to halothane hepatitis. The similar rates of antipyrine and metronidazole metabolism in the subjects and matched controls suggest that the risk of halothane hepatitis is not related to high inducibility of cytochrome P450s. Thus, the present data cannot support the notion that the risk of halothane hepatitis is related to

an increased formation of reactive metabolites due to high cytochrome P450 activities or lack of alternative pathways. However, the present battery of test compounds did not cover several possibly important P450 forms, such as the ethanol-inducible P4502E1. This P450 form may be involved in the reductive formation of reactive metabolites from halothane (28), as it is with regard to other halogenated solvents, such as carbon tetrachloride (29).

As genetic markers we also investigated the HLA tissue types and the complement C3 phenotypes. We found no indication of a specific HLA tissue type associated with halothane hepatitis. Complement C3 shows genetic polymorphism and is grouped in phenotype F, FS, and S, the latter being by far the most common. Phenotypes F and FS have been associated with an autoimmune disease, rheumatoid arthritis (30). Indian childhood cirrhosis (31) and hepatitis (30) (not specified) are also associated with the F and FS phenotypes, and Farhud & Walter (30) speculated that a genetic factor could operate in the predisposition to hepatitis.

Phenotypes F and FS were found in five of the seven subjects in the present investigation. This did not reach statistical significance but merits further investigation.

In conclusion, we did not find alterations in the metabolism of the test drugs used indicative of mechanisms explaining halothane hepatitis, but an immunoallergic disposition, as reflected in the high frequency of complement C3 phenotypes F and FS, cannot be excluded.

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