Halothane hepatotoxicity and hepatic free radical metabolism in guinea pigs; the effects of vitamin E

I. Durak PhD,* T. Güven PhD,† M. Birey PhD,†
H.S. Öztürk MD PhD,* Ö. Kurtipek MD,‡ M. Yel PhD,§
B. Dikmen MD,‡ O. Canbolat MD PhD,*
M. Kavutcu PhD,* M.Kaçmaz MD*

Purpose: *The aim of this study was to investigate the relation between halothane hepatotoxicity and hepatic free radical metabolism and to establish a possible protective role of vitamin E against halothane hepatotoxicity.*

Methods: *Twenty-eight guinea pigs were used in the experiments. Halothane (1.5% v/v) in oxygen (100%) was given to the animals for 90 min over three days. Livers from animals were then taken and prepared for the assays. In the enzymatic study, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities were measured. As a peroxidation index, the malondialdehyde (MDA) concentration was determined. Also, electron spin resonance (ESR) analysis and electron microscopy (EM) were performed.*

Results: *Superoxide dismutase (1168.3 ± 78.2 $U \cdot mg^{-1}$) and glutathione peroxidase (14.9 ± 6.2 $mIU \cdot mg^{-1}$) activities were decreased, but catalase activity (1260.0 ± 250.6 $IU \cdot mg^{-1}$) and malondialdehyde concentration (11.5 ± 1.8 ppb) were increased in liver tissues exposed to halothane compared with control values (1382.2 ± 91.8 $U \cdot mg^{-1}$ for SOD, 27.8 ± 5.2 $mIU \cdot mg^{-1}$ for GSH-Px, 840.2 ± 252.4 $IU \cdot mg^{-1}$ for CAT and 10.0 ± 1.0 ppb for MDA). Electron spin resonance analysis revealed a peak of $CF_3CHCl^{\cdot}$ radical in the exposed tissue. Electron microscopy indicated ultrastructural changes in the hepatic cells of both halothane groups with and without vitamin E treatment.*

Conclusion: *Halothane causes impairment in the hepatic antioxidant defense system and accelerates peroxidation reactions. As a result, some ultrastructural changes in hepatic tissues occur due to halothane treatment. Although vitamin E prevents peroxidative damage, it does not ameliorate ultrastructural changes caused by halothane treatment. This shows that halothane toxicity results not only from impaired hepatic antioxidant defense system but also from other, unknown causes.*

Objectif: *Cette étude visait à examiner la relation possible entre l'hépatotoxicité à l'halothane et le métabolisme des radicaux libres et à vérifier si la vitamine E protège contre l'hépatotoxicité à l'halothane.*

Méthodes: *Vingt-huit cobayes ont été utilisés. De l'halothane (1,5% v/v) en oxygène (100%) a été administré aux animaux pendant 90 min sur une période de trois jours. Les foies ont alors été prélevés et préparés pour fin d'analyse. Pour l'étude enzymatique, l'activité de la superoxyde dismutase (SOD), de la glutathione peroxydase (GSH-Px) et de la catalase (CAT) a été mesurée. En tant qu'indice de la peroxydation, la concentration de la malondialdéhyde (MDA) a été déterminée. En outre, on a procédé à des examens à la résonance paramagnétique électronique (Electronic spin resonance: ESR) et à la microscopie électronique (EM).*

Résultats: *L'activité de la superoxyde dismutase (1168,3 ± 78,2 $U \cdot mg^{-1}$) et de la glutathione peroxydase (14,9 ± 6,2 $UI \cdot mg^{-1}$) a diminué, mais celle de la catalase (1260,0 ± 250,6 $UI \cdot mg^{-1}$) ainsi que la concentration de la malondialdéhyde (11,5 ± 1,8 ppb) ont augmenté dans le tissus hépatique exposé à l'halothane comparativement aux valeurs de contrôle (1382,2 ± 91,8 $U \cdot mg^{-1}$ pour SOD, 27,8 ± 5.2 $mUI \cdot mg^{-1}$ pour SGH-px, 840 ± 252,4 $UI \cdot mg^{-1}$ pour CAT et 10,0 ± 1,0 ppb pour MDA). La résonance paramagnétique a révélé un pic de radical $CF_3CHCl^{\cdot}$ dans les tissus exposés. La microscopie électronique a montré des changements ultrastructuraux dans les cellules hépatiques chez les deux groupes halothane traités ou non à la vitamine E.*

Conclusion: *L'halothane provoque une altération du système*

Key words
ANAESTHETICS, VOLATILE: halothane toxicity;
COMPLICATIONS: hepatic;
TOXICITY: hepatic.

*From the Department of Biochemistry, Ankara University, Medical Faculty and †Departments of Biology and Physics, Ankara University, Science Faculty and ‡Anaesthesiology Clinics of İbn-i Sina and Numune Hospitals and §Department of Biology, Gazi University, Education Faculty, Ankara-Turkey.

Address correspondence to: Prof. Dr. İlker Durak, Ankara Universitesi Tip Fakültesi, Biyokimya A.B.D (Dekanlik Binasý), 06100 Sihhiye - Ankara / Türkíye.

Phone: (90) 312 3092219. Fax: (90) 312 3106370.

Accepted for publication 2nd March, 1996.

de défense hépatique antioxydant et accélère les réactions de peroxydation. Il en résulte des changements ultrastructuraux des tissus hépatiques produits par l'exposition à l'halothane. Bien qu'elle prévienne le dommage peroxydatif, la vitamine E n'atténue pas les changements ultrastucturaux produits par l'exposition à l'halothane. Ceci montre que la toxicité à l'halothane résulte non seulement de l'altération du système de défense antioxydant mais aussi d'autres causes non déterminées.

Halothane is associated with occasional hepatotoxicity[1] and causes hepatic necrosis in some people,[2] especially after multiple exposure over short periods.[3,4,5] In 1985, Lunam *et al.* demonstrated that extensive acute centrilobular necrosis consistently developed in naive guinea pigs after four hour anaesthesia with halothane 1% (v/v).[6] Pathological characteristics of the liver injury in guinea pigs exposed to halothane have been found to resemble the spectrum of injury observed in nonfatal hepatitis in humans exposed to halothane.[7] More recently, oxidative biotransformation of halothane by the hepatic cytochrome P-450 system has been implicated as a leading mechanism in the hepatotoxicity.[8]

It has been suggested that some reactive intermediates which are created during oxidative biotransformation reactions are involved in cell death.[9] Although the majority (>98%) of halothane metabolism *in vivo* is along the oxidative route,[8] lack of oxygen will also lead to the insertion of electrons into the molecule by the Cyt-P-450 system,[10] known as the reductive pathway, and generate free radical intermediates. Thus, both hypoxia and the cyt-P-450 system enhance the production of potentially toxic free radicals. Several researchers have postulated that free radicals generated by the reductive pathway during halothane metabolism might be the primary factor in the halothane hepatotoxicity.[11-13]

Although several investigators have tried to establish the relationship between tissue glutathione content and halothane use, conflicting results have been obtained. Some have found unchanged,[14] others decreased[15,16] glutathione levels in the hepatic tissues affected. There are some suggestions that halothane hepatitis is mediated by free radicals, but no work has been conducted to elucidate the possible relationship between halothane and free radical metabolism from enzymatic points of view.

To this aim, we attempted to establish the status of the enzymatic free radical defence system in hepatic tissues exposed to halothane and wished to elucidate possible relationships between free radical metabolism and halothane hepatitis.

Activities of enzymes participating in free radical metabolism (superoxide dismutase, glutathione peroxidase and catalase) and the malondialdehyde concentration, an important indicator of lipid peroxidation, were measured in hepatic tissues from guinea pigs treated with halothane, halothane plus vitamin E, vitamin E alone and control animals. Furthermore, electron spin resonance examinations were made in an attempt to determine free radicals derived from halothane in the hepatic tissues by using electron spin resonance spectrometry (ESR).

Methods

Chemicals

Halothane was obtained from Hoechst and Vitamin E from Roche Corporations. All the chemicals used in the experiments were of analytical grade.

Animals

Animal studies were performed in accordance with approval of Hifzissihha Institute of Health Ministry. Twenty-eight guinea pigs (two-months-old and approx 450 g weight) were used in the experiments. The animals were fed on a laboratory diet. At the end of the experiments, the animals were killed by cervical dislocation and their livers were placed in an ice bath for about one hour until homogenisation. Part of each liver was immediately put in liquid nitrogen to be used in Electron spin resonance assays and the other part was put in glutaraldehyde 3% solution to be used in the electron microscope examinations. From the animals' inferior vena cava, blood was taken and used for ALP (alkaline phosphatase), LDH (lactate dehydrogenase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), BUN (blood urine nitrogen) and creatinine assays.

Intramuscular vitamin E injection (300 $mg \cdot kg^{-1} \cdot day^{-1}$) was started three days before the first halothane treatment and continued for three days. Relatively high doses of vitamin E were injected in the animals to provide enough vitamin stores in their livers during the short period of three days.[17-19] As a multiple exposure model, halothane (1.5% v/v) in oxygen (100%) was given to the animals at the rate of 2 $L \cdot min^{-1}$ for 30 min each day for three days. Gas mixtures were inspired using face masks. Animals were killed two days after the last halothane treatment.

Preparation and analysis

Livers were first washed with deionized water to discard blood and then homogenized in an homogenisator

(B.Braun Melsungen model) at 1000 U for about three minutes. After centrifugation at 10,000 × g for about 60 min, the upper layer was taken. In this fraction, the protein concentration and glutathione peroxidase activity were measured.[20,21] Part of the homogenate was extracted in an ethanol/chloroform mixture (5/3 v/v) to discard the lipid fraction, which caused interference in the activity measurements of superoxide dismutase and catalase enzymes. After centrifugation at 10 000 × g for 60 min, the upper clear layer was taken and used in the measurements of superoxide dismutase and catalase activities. All the experiments were carried out at 4°C. Superoxide dismutase and catalase activities were measured.[22–24] One unit of superoxide dismutase activity was expressed as the protein amount causing 50% inhibition in nitroblue tetrazolium salt (NBT) reduction rate. The catalase and glutathione peroxidase activities were given in IU · mg^{-1} protein. All activities are expressed as specific activity (IU · mg^{-1} protein). The Malondialdehyde concentrations were determined by using thiobarbutiric acid reaction.[25] The lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase,urea and creatinine analyses in the serum samples were made in the routine biochemistry laboratory.

Precision analysis of the methods

(A) Within, (same day, ten times), (B) Between (once a day for consecutive five days) batch analysis results of the methods:

	Superoxide dismutase	*Glutathione peroxidase*	*Catalase*	*Malondialdehyde*
A ($n = 10$) CV %	6.2	5.2	3.8	5.3
B ($n = 5$) CV %	5.4	3.6	4.2	6.2

Electron microscopy

Electron microscope examinations were made in order to determine ultrastructural changes due to halothane use by using JEOL-100 CX-II TEM model electron microscope.In the first fixation of tissue samples, glutaraldehyde 3% solution prepared in phosphate buffer (200 mM, pH 7.2) and in the second fixation, osmium tetroxide 1 % solution prepared in the same buffer were used.Samples were stained by Sato's lead citrate and uranyl acetate 1% solutions.

Electron spin resonance (ESR) analysis

The spectra of the samples were recorded with a Varian X-Band E 109 C Model ESR spectrometer and investigated between 100–300 K. The g and a values were obtained by comparison of the centers of gravity of the spectra with a DPPH sample g = 2.0036. The conditions used were:Scan range 2000 G, power 6 dB, gain 2.5 × 10^3, microwave frequency 9.105 Ghz, field setting 3250 G.[26,27]

Statistics

In the statistical analysis, Tukey's HSD (honestly significant difference, ANOVA) procedure was used.

Results

Oxidant/antioxidant parameters

The GSH-Px and SOD activities were lower and CAT activity higher in the halothane group than in controls. Hepatic MDA concentrations were also higher in this group. However, GSH-Px activity was higher and, SOD and CAT activities were lower in the liver tissues of the halothane plus vitamin group. The MDA concentrations were higher in the hepatic tissues of the halothane group than in controls. In the vitamin groups, MDA concentrations were lower than in the halothane group (Table I).

Serum parameters

Serum ALT and AST concentrations were higher in the halothane group than in controls. The ALT concentrations were also high in the halothane plus vitamin group. There were no differences among groups with respect to other serum parameters (Table II).

Electron microscopy

Electron micrographs show considerable cellular changes and derangements such as vacuolisation in some mitochondria and cytoplasm, wrinkling in nucleus and, accumulation of lipid droplets in cell cytoplasm in the hepatocytes of halothane-treated animals. (Figure 2). In the electron micrographs of the hepatocytes from guinea pigs treated with halothane plus vitamin E, there was vacuolisation in some mitochondria and cytoplasm. (Figure 3). Moreover, enlargement and fusion were observed in some mitochondria in the electron micrographs of the hepatocytes from guinea pigs treated with vitamin E alone (Figure 4). Electron micrograph of a normal hepatocyte was also included for comparison (Figure 1).

ESR analysis

There was a considerable amount of $CF_3CHCl^{\bullet}$ radical in hepatic tissues exposed to halothane (relative radical concentration 10.3 ± 0.3) but the amount was not increased in hepatic tissues exposed to halothane *plus* vitamin (relative radical concentration 3.1 ± 0.3, which was similar to the control group) (Table III).

TABLE I Glutathione peroxidase (mIU · mg^{-1}), superoxide dismutase (U · mg^{-1}), catalase (IU · mg^{-1}) activities and malondialdehyde (ppb) concentrations in liver tissues from guinea pigs.

Groups	*GSH-Px*	*SOD*	*CAT*	*MDA*
Control	27.8 ± 5.2	1382.2 ± 91.8	840.2 ± 252.4	10.0 ± 1.0
Halothane	14.9 ± 6.2	1168.3 ± 78.2	1260.0 ± 250.6	11.5 ± 1.8
Halot.+Vit.E	32.3 ± 8.6	1197.4 ± 204.0	450.0 ± 90.1	9.1 ± 1.1
Vitamin E	34.0 ± 8.0	1445.2 ± 105.0	1440.8 ± 390.2	8.9 ± 1.0
Statistical analysis (Tukey's HSD procedure)				
Control-Halothane	***	***	***	*
Control-Halothane+Vit. E	***	**	***	NS
Halothane-Halot.+Vit. E	*	*	****	*
Control-Vit. E	***	NS	****	*

*$P < 0.05$; **$P < 0.025$; ***$P < 0.005$; ****$P < 0.0005$ and NS: non significant ($P > 0.05$).
(mean ± SD, $n = 7$ for all groups).

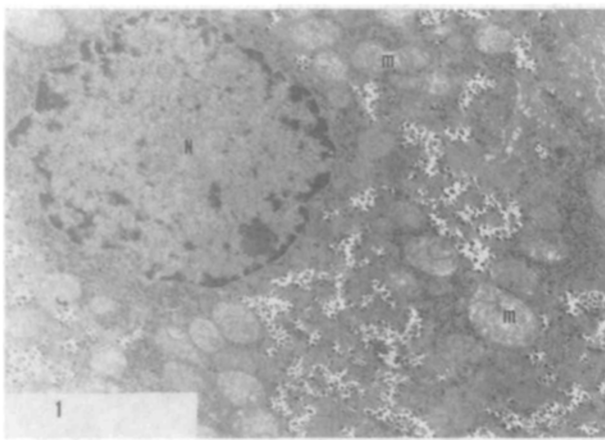

FIGURE 1 Electron micrograph of normal hepatocyte from guinea pig. Nucleus (N), mitochondria (m) × 10300.

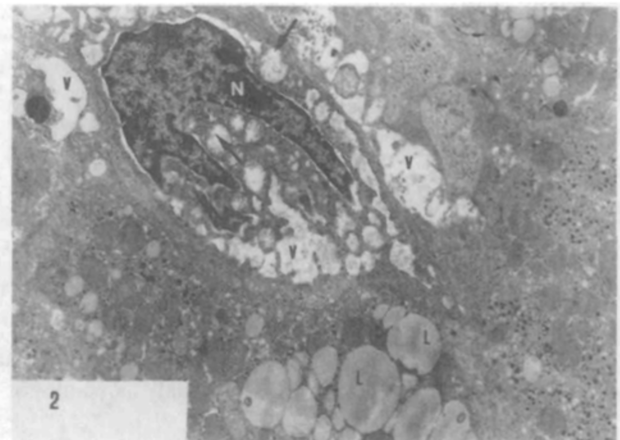

FIGURE 2 Electron micrograph of hepatocyte from guinea pig treated with halothane. Note vacuoles in some mitochondria (arrows) and cytoplasm (v), wrinkled nucleus (N) and lipid droplets in cytoplasm (L) × 7800.

Discussion

Halothane is associated with occasional hepatotoxicity and hepatic necrosis.[1,2] There is accumulating evidence on the hepatotoxicity of halothane.[6,7,28] However, the mechanism leading to halothane hepatotoxicity has not been completely described. Some have suggested that oxidative biotransformation of halothane by cyt P-450 microsomal system was the primary event.[8] Halothane undergoes oxidative and reductive pathway reactions in the microsomal fraction of the liver. Oxidative metabolism is greater in the presence of high oxygen tensions while reductive metabolism is favoured by hypoxic conditions. Both pathways exist in man and predominantly reductive metabolism occurs in patients undergoing halothane anaesthesia.[29,30] Trifluoroacetic acid and bromide ions are the main products of oxidative metabolism whereas 2-chloro-1,1,1-trifluoroethane, 2-chloro-1,1-difluoroethylene and fluoride ion are the predominant products of reductive metabolism.[29,30] Some reactive intermediates that are created during biotransformation are involved in the development of cell death, perhaps through their ability to bind covalently to free amino groups on subcellular proteins.[9] In addition, these intermediates can react with water within the hepatocyte to produce more toxic metabolites.[31] Others have suggested that, although the majority of halothane metabolism is by the oxidative route,[8] hypoxia is also an important factor leading to cellular degeneration.[10] This second route of halothane biotransformation generates free radical intermediates, which cause peroxidative damages on the lipid, protein and carbonhydrate components in some cell structures.[10] Indeed, toxic free radical intermediates produced by the reductive pathway are held to be responsible for halothane hepatotoxicity.[11-13,31,32]

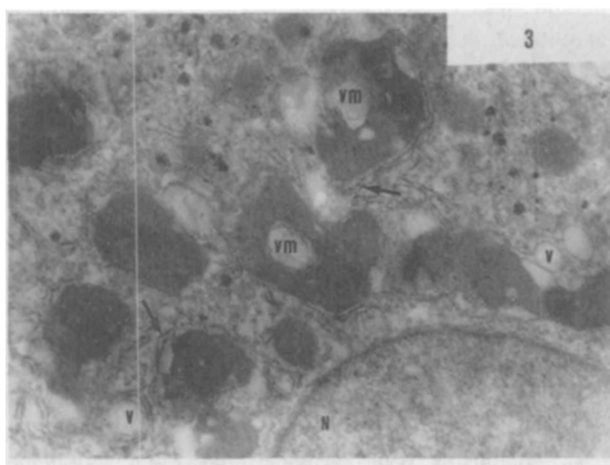

FIGURE 3 Electron micrograph of hepatocyte from guinea pig treated with halothane plus vitamin E. Note vacuoles in some mitochondria (vm) and cytoplasm (v). Nucleus (N), rough endoplasmic reticulum (arrows) × 21000.

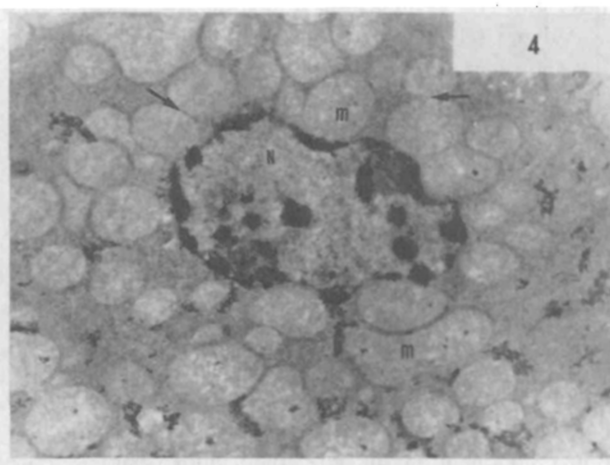

Figure 4 Electron micrograph of hepatocyte from guinea pig treated with vitamin E alone. Note enlargement (m) and fusion (arrows) of some mitochondria × 8700.

TABLE II Alkaline phosphatase ($U \cdot L^{-1}$), lactate dehydrogenase ($U \cdot L^{-1}$), aspartate aminotransferase ($U \cdot L^{-1}$), alanine aminotransferase ($U \cdot L^{-1}$), blood urine nitrogen ($mg \cdot dl^{-1}$) and creatinine ($mg \cdot dl^{-1}$) in sera from guinea-pigs

Groups	*ALP*	*LDH*	*AST*	*ALT*	*BUN*	*Creatinine*
Control	72.3 ± 24.2	542.4 ± 115.2	22.5 ± 6.5	21.5 ± 6.2	26.2 ± 7.3	0.9 ± 0.2
Halothane	78.4 ± 31.3	606.4 ± 280.2	30.2 ± 11.2*	38.6 ± 10.1†	28.4 ± 9.3	1.1 ± 0.4
Halot.+Vit E	76.3 ± 22.6	605.2 ± 230.4	26.6 ± 9.3	30.4 ± 18.7*	27.2 ± 10.1	1.0 ± 0.4
Vitamin E	73.3 ± 25.6	570.6 ± 240.4	24.5 ± 12.3	27.6 ± 13.2	24.7 ± 9.6	0.9 ± 0.3

*$P < 0.05$ and †$P < 0.005$, relative to control group with Tukey's test.
Mean ± SD, $n = 7$ for all groups.

TABLE III Some parameters established in liver tissues at 140 K in ESR analysis

	Control G	*Halothane G*	*Halot.+Vit. G*	*Vit.G*
Radical type	NTS	$CF_3CHCl^{\bullet}$	NTS	NTS
a (Gauss)	0	145	0	0
g	2.0032	2.0035	2.0033	2.0032
RRC	2.4 ± 0.3	10.8 ± 0.3	3.1 ± 0.3	2.6 ± 0.3

NTS: Normal tissue spectrum. *a*: Hyperfine splitting constant.
g: Spectroscopic splitting factor. RRC: Relative radical concentration / g tissue.

Some investigators have suggested that halothane itself may not cause severe toxicity and that toxicity mainly arises from tissue hypoxia.[33,34] Halothane hepatotoxicity in hypoxic whole animals suggests that factors other than a direct interaction between halothane and the hepatocyte are important in producing toxicity.[13,34–36]

Although free radicals have been implicated in the production of halothane hepatitis, there is no information on the status of the enzymatic defence system in hepatic tissues after multiple exposure to halothane. In this study, we investigated this area using a guinea pig animal model. Although there are limitations in applying the model to humans, the information may assist understanding the mechanism of halothane hepatotoxicity because it is impossible to carry out such experiments in humans. Also, the dose of vitamin E was very high although high doses are preferred in animal studies to obtain definitive results.[17–19] The other reason for the use of high vitamin doses was to provide animals with a sufficient vitamin store for the three days.

The activities of glutathione peroxidase and superoxide dismutase, cytoplasmic components of enzymatic free radical defense system, decreased but catalase activity, mainly functioning in cell peroxisomes, increased in hepatic tissues from guinea pigs treated with halothane (Table I). Hepatic malondialdehyde level of the halothane group was found to be moderately higher than that of the control group, which was an indication of increased peroxidative process in the tissues

affected. The cytoplasmic antioxidant defence system was depressed due to halothane and this was probably the main reason of the increased peroxidation process in the liver tissues exposed to halothane.

What factors might be responsible of these changes? The decreases in glutathione peroxidase and superoxide dismutase activities might arise from two reasons. First, halothane or its radical intermediates, such as the $CF_3CHCl^•$ radical that may be determined by electron spin resonance analysis, may inhibit these enzymes as do they some subcellular proteins[9] or second, inhibition may arise from tissue hypoxia, which is known to cause suppression in the activity of free radical metabolising enzyme systems.[37–41] Either suppression of the cytoplasmic component of the enzymatic antioxidant defence system, or radical metabolites of halothane may cause oxidative stress in hepatic tissue, the consequences of which are peroxidation of some cellular components including unsaturated lipids, and increased tissue malondialdehyde level. The increase in catalase activity may occur as a compensatory mechanism against decreased glutathione peroxidase activity since both enzymes use H_2O_2 as their main substrates. Alternatively, factors relating to oxidative degredation processes in peroxisomes might be responsible. Whatever the reasons, it seems that the enzymatic antioxidant defence system is impaired and peroxidation process is accelerated due to halothane exposure. This can be seen from the moderately increased malondialdehyde concentration.

Mild degeneration in hepatic cells and moderate increases in serum ALT and AST activities were observed in the halothane group (Table II). However, there were no differences between other serum parameters in the control and working groups. Electron microscopy showed mitochondrial degeneration in hepatic cells of both halothane treated groups, with and without vitamin E pretreatment. No pathology was seen in the hepatic cells of the animals treated with vitamin E alone. This suggests that halothane causes hepatotoxicity not only through free radical metabolism but also through e other unknown mechanisms such as immune-mediated cellular reactions.

In the halothane plus vitamin E group, glutathione peroxidase activity was increased and catalase activity was decreased compared with that in the control group. No effect of vitamin E was observed on the superoxide dismutase activity in this group. Lowered malondialdehyde concentrations in the vitamin group demonstrated that vitamin E prevented peroxidative reactions caused by free radical intermediates derived from halothane or other sources. This was also established by Sato *et al.*[42] In addition to a direct scavenging effect, vitamin E might also eliminate the factor(s) which suppress glutathione peroxidase and, induce and/or activate catalase activities in hepatic tissues of the halothane group. This was indicated by the increased glutathione peroxidase and decreased catalase activities in the hepatic tissues of halothane plus vitamin E group. The results of electron spin resonance analysis showed that the amounts of $CF_3CHCl^•$ radical as well as malondialdehyde concentrations were considerably reduced in the hepatic tissues of vitamin-treated groups. In fact, all of the free radical metabolising enzyme activities were higher in the hepatic tissues of the animals treated with vitamin E alone than in controls. Electron microscopy also demonstrated that vitamin E did not produce severe effects on cellular organelles.

In conclusion, our results suggest that halothane reduces the enzymatic potential of the free radical defence system, particularly in cell cytoplasm, and causes acceleration of cellular peroxidative reactions due either to this impairment or to the direct effects of free radical intermediates derived from halothane. The important indication of reduced free radical defence potential is the increased malondialdehyde level in the liver tissues from animals exposed to halothane. Although vitamin E pretreatment prevents peroxidative reactions, it does not prevent cellular, in particular mitochondrial, deformations observed in the electron microscope. This indicates that halothane hepatotoxicity results not only from changed free radical metabolism or free radical species produced during metabolism, but also from other undetermined factors. We suggest that vitamin E pretreatment may be useful in the prevention of some peroxidative damages caused by halothane but it does not prevent the development of cellular changes, which may occur via mechanisms other than free radical metabolism.

References

1 *Stock JGL, Strunin L.* Unexplained hepatitis following halothane. Anesthesiology 1985; 63: 424–39.

2 *Hughes HC, Lang CM.* Hepatic necrosis produced by repeated administration of halothane to guinea pigs. Anesthesiology 1972; 36: 466–72.

3 *Inman WHW, Mushin WW.* Jaundice after repeated exposure to halothane: an analysis of reports to the Committee on Safety of Medicines. BMJ 1974; 1: 5–10.

4 *Inman WHW, Mushin WW.* Jaundice after repeated exposure to halothane: a further analysis of reports to the Committee on Safety of Medicines. BMJ 1978; 2: 1455–6.

5 *Moult PJA, Sherlock S.* Halothane-related hepatitis. QJM 1975; 44:99–114.

6 *Lunam CA, Cousins MJ, Hall P de La M.* Guinea-pig model of halothane-associated hepatotoxicity in the

absence of enzyme induction and hypoxia. J Pharmacol Exp Ther 1985; 232: 802–9.

7 *Lunam CA, Hall P de La M, Cousins MJ.* The pathology of halothane hepatotoxicity in a guinea-pig model: a comparison with human halothane hepatitis. British Journal of Experimental Pathology 1989; 70: 533–41.

8 *Lind RC, Gandolfi AJ, Hall P de la M.* The role of oxidative biotransformation of halothane in the guinea pig model of halothane-associated hepatotoxicity. Anesthesiology 1989; 70: 649–53.

9 *Kenna JG, Satoh H, Christ DD, Pohl LR.* Metabolic basis for a drug hypersensitivity: antibodies in sera from patients with halothane hepatitis recognize liver neoantigens that contain the trifluoroacetyl group derived from halothane. J Pharmacol Exp Ther 1988; 245: 1103–9.

10 *De Groot H, Noll T.* Halothane hepatotoxicity: relation between metabolic activation, hypoxia, covalent binding, lipid peroxidation and liver cell damage. Hepatology 1983; 3: 601–6.

11 *Gandolfi AJ, Sipes IG, Brown BR Jr.* Detection of covalently bound halothane metabolites in the hypoxic rat model for halothane hepatotoxicity. Fundam Appl Toxicol 1981; 1: 255–9.

12 *Plummer JL, Beckwith ALJ, Bastin FN, Adams JF, Cousins MJ, Hall P.* Free radical formation *in vivo* and hepatotoxicity due to anesthesia with halothane. Anesthesiology 1982; 57: 160–6.

13 *Lind RC, Gandolfi AJ, Sipes IG, Brown BR Jr, Waters SJ.* Oxygen concentrations required for reductive defluorination of halothane by rat hepatic microsomes. Anesth Analg 1986; 65: 835–9.

14 *McLain GE, Sipes IG, Brown BR Jr.* An animal model of halothane hepatotoxicity: roles of enzyme induction and hypoxia. Anesthesiology 1979; 51: 321–6.

15 *Van Dyke RA.* Hepatic centrilobular necrosis in rats after exposure to halothane, enflurane or isoflurane. Anesth Analg 1982; 61: 812–9.

16 *Siegers CP, Frühling A, Younes M.* Halothane hepatotoxicity in hyperthyroid rats as compared to the phenobarbital-hypoxia model. Toxicol Appl Pharmacol 1983; 69: 257–64.

17 *Ramsammy LS, Josepovitz C, Ling K-Y, Lane BP, Kaloyanides GJ.* Failure of inhibition of lipid peroxidation by vitamin E to protect against gentamicin nephrotoxicity in the rat. Biochemical Pharmacology 1987; 36: 2125–32.

18 *Wang Y-M, Madanat FF, Kimball JC et al.* Effect of vitamin E against adriamycin-induced toxicity in rabbits. Cancer Res 1980; 40: 1022–7.

19 *Kappus H, Diplock AT.* Tolerance and safety of vitamin E. A toxicological position report. VERIS, the Vitamin E Research & Information Service. LaGrange, Illinois: 1991.

20 *Lowry O, Rosenbrough N, Farr L, Rondall R.* Protein measurement with the the folin phenol reagent. J Biol Chem 1951; 183: 265–75.

21 *Paglia DE, Valentine WN.* Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158–69.

22 *Sun Y, Oberley LW, Li Y.* A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 479–500.

23 *Durak I, Canbolat O, Kavutçu M, Öztürk HS, Yurtarslaný Z.* Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. Journal of Clinical Laboratory Analysis 1996; 10: 17–20.

24 *Aebi H.* Catalase. *In:* Bergmeyer HU (Ed.). Methods of Enzymatic Analysis. New York and London: Academic Press Inc. 1974: 673.

25 *Dahle LK, Hill EG, Holman RT.* The thiobarbituric acid reaction and the autooxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys 1962 98: 253–61.

26 *Lontz RJ.* Electron spin resonance analysis of a γ-irradiated single crystal of pentafluoropropionamide. Journal of Chemical Physics 1966; 45: 1339.

27 *Rogers MT, Kispert LD.* Trifluoromethyl, and other radicals, in irradiated single crystals of trifluoroacetamide. Journal of Chemical Physics 1967; 46: 3193–9.

28 *Gut J, Christen U, Huwyler J.* Mechanisms of halothane toxicity: novel insights. Pharmacol Ther 1993; 58: 133–55.

29 *Cousins MJ, Gourlay GK, Knights KM, Hall P de la M, Lunam CA, O'Brien P.* A randomised prospective controlled study of the metabolism and hepatotoxicity of halothane in humans. Anesth Analg 1987; 66: 299–308.

30 *Sharp JH, Trudell JR, Cohen EN.* Volatile metabolites and decomposition products of halothane in man. Anesthesiology 1979; 50: 2–8.

31 *Royston D.* Free radicals. Formation, function and potential relevance in anaesthesia. Anaesthesia 1988; 43: 315–20.

32 *Sipes IG, Gandolfi AJ, Pohl LR, Krishna G, Brown BR Jr.* Comparison of the biotransformation and hepatotoxicity of halothane and deuterated halothane. J Pharmacol Exp Ther 1980; 214: 716–20.

33 *Lind RC, Gandolfi AJ, Hall P de la M.* Covalent binding of oxidative biotransformation intermediates is associated with halothane hepatotoxicity in guinea pigs. Anesthesiology 1990; 73: 1208–13.

34 *Shingu K, Eger EI II, Johnson BH, et al.* Hepatic injury induced by anesthetic agents in rats. Anesth Analg 1983; 62: 140–5.

35 *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. Anesth Intensive Care 1979; 7: 9–24.

36 *Jee RC, Sipes IG, Gandolfi AJ, Brown BR Jr.* Factors influencing halothane hepatotoxicity in the rat hypoxic model. Toxicol Appl Pharmacol 1980; 52: 267–77.
37 *Lind RC, Gandolfi AJ, Sipes IG, Brown BR Jr.* Comparison of the requirements for hepatic injury with halothane and enflurane in rats. Anesth Analg 1985; 64: 955–63.
38 *Ross WT Jr, Daggy BP, Cardell RR Jr.* Hepatic necrosis caused by halothane and hypoxia in phenobarbital treated rats. Anesthesiology 1979; 51: 327–33.
39 *Copin J-C, Ledig M, Tholey G.* Almitrine prevents some hypoxia-induced metabolic injury in rat astrocytes. Mol Chem Neuropathol 1993; 20: 97–109.
40 *Kirshenbaum LA, Singal PK.* Antioxidant changes in heart hypertrophy: significance during hypoxia-reoxygenation injury. Can J Physiol Pharmacol 1992; 70: 1330–5.
41 *Kirshenbaum LA, Singal PK.* Changes in antioxidant enzymes in isolated cardiac myocytes subjected to hypoxia-reoxygenation. Lab Invest 1992, 67: 796–803.
42 *Sato N, Fujii K, Yuge O, Tanaka A, Morio M.* Suppressive effect of vitamin E on lipid peroxidation in halothane-administrated guinea pig liver. In Vivo 1992; 6: 503–5.