Volatile Metabolites of Halothane in the Rabbit

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To date, carbon dioxide is the only volatile metabolite that has been identified to result from the biotransformation of halothane. This study was undertaken to determine whether other volatile metabolites might be formed. Expiratory gas from four rabbits given halothane by inhalation and from three rabbits into which the halothane was injected intraperitoneally was analyzed by gas chromatography. Qualitative analysis of the metabolites was made by injecting 50–70 µl of the expired halothane condensed in an ultralow-temperature device (–80°C) attached to the mass spectrometer. Gas chromatography revealed two volatile metabolites between the air peak and the halothane peak. They were identified by mass spectra to be CF₂CHCl and CF₂CH₂Cl. These volatile metabolites appeared immediately after the beginning of anesthesia. The present investigation suggests the possible existence of a previously unknown metabolic pathway of defluorination and debromination occurring in the early stage of halothane biotransformation. These volatile metabolites may be toxic, highly reactive intermediates that undergo further biotransformation. (Key words: Biotransformation, halothane; Anesthetics, volatile, halothane.)

According to present reports concerned with the biotransformation of halothane, no volatile metabolite except carbon dioxide has been demonstrated in the expired gas. The present investigation was performed to identify and quantify additional volatile metabolites found in the expired gas of rabbits given halothane by inhalation or intraperitoneal injection.

Methods

Studies were made in seven male rabbits weighing 2.5 to 3.0 kg, all of which had undergone tracheotomy under regional anesthesia. Four rabbits (Group I) were given halothane, 1.5 per cent inspired concentration with oxygen (5 l/min), for three hours using a nonrebreathing system (fig. 1). Controlled ventilation was used to maintain a respiratory minute volume of 1.0 l/min. After the termination of anesthesia, the lungs were ventilated with pure oxygen. Three rabbits (Group II) were each given a single injection of halothane into the intraperitoneal cavity, in doses of 0.5, 1.0 and 2.0 mg/kg, respectively. Following the administration of halothane, they breathed room air and the end-expired gas during spontaneous respiration was analyzed. The rectal temperatures of all rabbits were maintained at 36 to 38°C by heating pads.

Expired gas was collected at intervals of 5 to 15 minutes and analyzed by a flame ionization gas chromatograph equipped with a sampling tube to introduce a constant volume of gas. Prior to anesthesia, expired gas from the rabbits and gas samples from the anesthetic circuit were demonstrated to be uncontaminated by halothane. Concentrations of the volatile metabolites were measured by calibrating the peak areas with known concentrations of enfurane premixed in halothane.

The expired gas was condensed in a column passing through a refrigeration device filled with n-heptane and dry ice to maintain the temperature at –80°C. Qualitative analysis of the metabolites was made by injecting 50–70 µl into a mass spectrometer (table 1).

The chemical formulas of the metabolites were determined from the Mass Spectral Data.

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Results

Two gas chromatographic peaks representing unknown volatile metabolites were found between the air and halothane peaks in both experiments (fig. 2). Peak I had a retention time of 1.05 minutes and Peak II, 1.45 minutes. The retention times of air and halothane were 0.68 and 5.05 minutes, respectively. Mass spectral analysis of Peaks I and II showed them to have molecular weights (M/e) of 98 and 118, respectively (fig. 3). By comparing these with data from other fragment peaks, they were identified as CF₂-CHCl and CF₃CH₂Cl, respectively.

In Group I rabbits, CF₂-CHCl increased in the first hour and reached a plateau concentration of about 2 ppm v/v during the next two hours (fig. 4). There was no significant change in concentration during the first hour after the end of anesthesia. However, CF₂-CHCl decreased slowly in the next two hours. In contrast, CF₃CH₂Cl showed a concentration approximately tenfold higher than that of CF₂-CHCl throughout the procedure. CF₃CH₂Cl increased significantly in the initial two hours and reached a plateau. At the end of anesthesia, the plateau was followed by a slight increase for an hour, followed by a decrease for two hours. The highest concentrations of these metabolites observed in this group were 3.9 and 46.0 ppm v/v, respectively.

Group II rabbits showed dose-related increases of both metabolites (fig. 5). The two rabbits given 1.0 and 2.0 ml/kg of halothane died of respiratory depression five and three hours after injection, respectively. The remaining animal showed 0.1 and 0.5 ppm of the metabolites 39 hours after injection.

![Fig. 2. Left. gas chromatogram of expired gas of a rabbit collected for three hours after halothane anesthesia. Retention times of air, Peak I, Peak II and halothane are 0.68, 1.05, 1.45 and 5.05 minutes, respectively. Right. gas chromatogram of expired gas of a rabbit collected 45 minutes after intraperitoneal injection of 1.0 ml/kg halothane. Note Peak I/Peak II ratio is higher than that observed in Group I.](image)

![Fig. 3. Mass spectra of Peak I, Peak II, and halothane. Each fragment peak is expressed in relative percentage against the most abundant peak. Comparing this information with the Mass Spectral Data, Peak I and Peak II were identified to be CF₂-CHCl and CF₃CH₂Cl, respectively.](image)
of CF₂:CH₂Cl as a defluorinated intermediate in halothane biotransformation. Sakai et al.⁵ have also reported a slight increase of inorganic fluoride excreted in the urine after inhalation of halothane.

On the contrary, bromide appears to be easily removed from the halothane molecule both in vivo and in vitro, because of the relative unstability of C-Br bond. According to Stier et al.,⁶ maximum urinary excretion of bromide has been observed for a few days after halothane anesthesia. Tinker et al.⁷ reported a significant increase of plasma bromide levels following halothane anesthesia in 25 patients, of whom 16 showed peak bromide levels during the 48–72-hour period after anesthesia. Plasma bromide levels in some patients remained elevated for at least 22 days after anesthesia. The findings suggest that there may be a time gap for dehalogeneration of halothane in the body, increase in circulating halide levels, and excretion of halide in the urine.

In some rabbits of Group I, the highest concentrations of the metabolites were observed during the 30- or 60-minute period after recovery from anesthesia. This indicates that dehalogeneration continues even after termination of anesthesia. It is interesting that similar

**Discussion**

In 1964, Van Dyke et al.⁸ reported that in their animal experiments 0.84 per cent of the injected dose of halothane appeared as carbon dioxide. No other volatile metabolite was identified.

It has not been accepted that defluorination readily occurs because of the high bond energy of the trifluoride radical of the halothane molecule. However, the presence of CF₂:CHCl and CF₂:CH₂Cl in the expired gas implies that defluorination as well as debrimation definitely occurs during halothane metabolism. Dobkin et al.⁹ have suggested that the slight increase in serum fluoride level observed after halothane anesthesia is probably due to mobilization of fluoride ion in the body or accumulation in the air and the anesthetic device. However, Cohen and associates¹⁰ have suggested the possibility of formation

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**Fig. 4.** Time concentration courses of CF₂:CHCl and CF₂:CH₂Cl in four rabbits (Group I) anesthetized with halothane for three hours with a nonrebreathing technique. CF₂:CH₂Cl shows a concentration approximately tenfold higher than that of CF₂:CHCl.

**Fig. 5.** Time concentration courses of CF₂:CHCl and CF₂:CH₂Cl in three rabbits (Group II) following intraperitoneal injections of halothane. Both metabolites increased in a dose-related manner.

○○○○ = 0.5 ml/kg; ●●●● = 1.0 ml/kg; ○○○○ = 2.0 ml/kg.
findings were reported by Atallar et al. who suggested that de bromination occurred during the early phase of anesthesia and again following recovery from anesthesia. They attributed this to inhibition of dehalogenation of halothane when administered at anesthetic concentrations and to active de bromination when the blood concentration of halothane is low. The present investigation is also relevant to the findings of Cascorbi et al. who suggested that microsomal enzyme saturation or complete suppression of metabolism is associated with halothane at anesthetic concentrations.

Whether CF₂:CHCl is produced directly from halothane or secondarily from CF₃CH₂Cl is unknown. However, the metabolic pathway for producing CF₂:CHCl by the loss of HF from CF₃CH₂Cl is likely, because the concentration of CF₃CH₂Cl was always higher than that of CF₂:CHCl. Our data suggest the possible existence of a previously unknown metabolic pathway of halothane.

The concentration ratios of CF₂:CHCl/CF₃CH₂Cl of Group II ranged from 0.21 to 0.37 (mean 0.30), which differed significantly (P < 0.05) from the 0.07 to 0.16 (mean 0.11) observed in Group I. This may be due to the difference between the expired oxygen concentrations in these two groups. In contrast to ventilation with pure oxygen for Group I, the three rabbits of Group II may have been hypoxic because they were allowed to breathe room air spontaneously after the intraperitoneal injection of halothane. The present data suggest that hypoxic conditions facilitate the formation of CF₂:CHCl. Widger et al. reported a significant increase of plasma inorganic fluoride levels after halothane anesthesia with hypoxia. They also observed very high levels of inorganic fluoride in some animals for which hypoxia and depression from halothane were sufficient to cause death.

Group II showed dose-related increases of both metabolites. This suggests that microsomal enzyme activity is facilitated, depending upon the administered dose of halothane.

The pharmacologic effect of CF₂:CHCl is not known. However, Rudo et al. have reported that CF₂:CHF, a similar substance, possesses flammability and a very slight anesthetic effect at 70 per cent v/v and that CF₂:CHI is unstable and produces anesthesia and tremors at 6.0 per cent. Likewise, Widger et al. have suggested that CF₂:CHBrCl is highly reactive and can bind to cell components. Raventos et al. reported that CF₃CH₂Cl produces convulsions in anesthetized mice. Hence, it is likely that the volatile metabolites identified in this study may be toxic, highly reactive intermediates that may undergo further biotransformation.

References