Excretion of Trifluoroacetic Acid as a Metabolite of Halothane in Digestive Juices

Miroslav I. MIRKOV, Michio MORIO, Michio KAWAHARA, Osafumi YUGE, Hiroyuki KINOSHITA and Kohyu FUJII

The excretion of trifluoroacetic acid (TFAA) in bile, saliva and gastric juice of two groups of guinea pigs with bile fistulae was measured by ion-chromatography during inhalation of halothane (0.25% and 1.0%) for two hours and after inhalation of halothane. In another two groups without bile fistulae, excretion of TFAA was measured in saliva and gastric juice during and after inhalation of same concentrations of halothane.

The excretion of TFAA increased with time and showed the highest concentrations in the saliva. The highest excretion rate and cumulative amounts of excreted TFAA were observed in bile. The cumulative amounts of TFAA excreted into the bile, saliva and gastric juice was 4.85 ± 1.87 μmol, 0.89 ± 0.62 μmol, 0.11 ± 0.06 μmol, respectively, after inhalation of 0.25% halothane and 5.36 ± 2.29 μmol, 1.50 ± 0.59 μmol, 0.25 ± 0.19 μmol, respectively, after inhalation of 1.0% halothane. The excretion of TFAA in bile and saliva was saturated after inhalation of the higher concentration of halothane. The excretion of TFAA into the gastric juice was higher with 1.0% concentration of halothane and in animals without bile fistulae.

We concluded that TFAA a metabolite of halothane is excreted not only in bile but also in saliva and gastric juice. Biotransformation of halothane in salivary glands seems very likely. A small amount of TFAA excreted in bile enters the enterohepatic circulation. The excretion of TFAA in digestive juice seems to be controlled by a rate-limiting mechanism. (Key words: Anesthetics, halothane, trifluoroacetic acid, salivary glands)

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The metabolic pathway of halothane has been reported[1–3], and it is known that TFAA is the major urinary metabolite of halothane[4,5]. TFAA accumulates in body tissues of rats in almost equal concentrations after exposure to subanesthetic concentrations of halothane[9]. A small fraction of the halothane taken up during anesthesia is degraded into TFAA but most of this metabolite is formed following anesthesia[7,8]. The TFAA excreted in urine after halothane anesthesia in humans shows a late peak – 48 hours after anesthesia[8]. Enterohepatic circulation of TFAA has proposed as an explanation for its delayed renal excretion[9]. Halothane metabolites had been detected in the feces and sweat of human following intravenous injection or inhalation[7].

The purpose of this study was to establish the excretion of TFAA as a metabolite of

Department of Anesthesiology, Hiroshima University School of Medicine, Hiroshima, Japan

Address reprint requests to Dr. Morio: Department of Anesthesiology, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima, 734 Japan

halothane in saliva and gastric juice and to investigate the enterohepatic circulation of this metabolite.

Methods

Reagents

Halothane was obtained from Hoechst Japan Ltd. The other reagents were commercial products of analytical grade.

Animals

Twenty eight male guinea pigs (body weight 332 ± 37, mean ± sd) were used in this study. They were housed in metal mesh cages, five in a cage. All of them were allowed free access to food and water except overnight fasting before the experiment. The animals were divided in to four groups, with seven guinea pigs in each group.

Halothane in oxygen was administered to the animales from a Fluotec Mark 2 vaporizer. Those of groups 1 and 2 inhaled 0.25% halothane for two hours and those of groups 3 and 4 inhaled 1.0% halothane for two hours. Bile fistulae and ligation of the pyloric part of the stomach were performed in groups 1 and 3.

Preparation of the animals

Under anesthesia with pentobarbital (Nembutal) 30 mg/kg body weight intravenously, a gastric tube was inserted through the mouth and tracheostomy was performed. The esophagus was ligated in the area adjacent to the trachea over the gastric tube. Body temperature was maintained at 37°C by a warming mattress (Gorman-Rupp, Industries Division, Bellvile, Ohio, USA, Model K-1C-3). The left jugular vein was cannulated and Ringer's Lactate solution was infused throughout the experiment at a mean rate of 10 ml/kg body weight/hr.

Mechanical ventilation of the lungs was maintained, using a Harvard Apparatus Rodent Respirator (Model H-681), with an oxygen supply of 5 l/min., tidal volume of 6–8 ml/kg. body weight, and respiratory frequency of 100/min., through a non-rebreathing anesthetic system.

In the animals from groups 1 and 3, ligation of the pyloric part of the stomach was performed, and bile fistulae were constructed by means of cystic bile duct ligation and common bile duct cannulation.

Collection of the samples

All animals were ventilated for 1 hour with 100% oxygen until the collection of the control samples was performed. Thereafter, inhalation of halothane was started.

Control samples of saliva, gastric juice and bile were collected as follows: saliva – before insertion of the gastric tube; gastric juice after insertion of the tube into the stomach; bile – from bile cyst and from the common bile duct during the time of bile fistula construction.

Samples of saliva, gastric juice and bile were collected every 60 min during the inhalation of halothane and for 6 hours after the discontinuation of the anesthetic.

Bile and gastric juice were drained in plastic tube containers. Saliva was collected and measured as the weight gain of pieces of absorbent paper placed into the mouth at the end of the every 60 min interval throughout the observation period.

All samples were stored in a refrigerator at 4°C until the measurements were performed.

Preparations of the samples

i) Bile was diluted 100 times and 100 μl were injected into the Ion Chromatographic Analyser through a cation exchange filter.

ii) Gastric juice was centrifuged for 10 min. at 500 × g for deproteinization, using an Amicon^{R}(Amicon, USA) ultrafiltration membrane cone. The ultrafiltrate was diluted 100 times and 100 μl were injected into the Ion Chromatographic Analyser through the same type of cation exchange filter as was used for bile.

iii) The pieces of absorbent paper soaked with saliva were centrifuged by means of the same equipment used for gastric juice. The ultrafiltrate was diluted 100 to 300 times and 100 μl were injected into the Ion Chromatographic Analyzer.

Measurement of (TFAA)

The concentration of TFAA in the samples was measured by an Ion Chromatographic Analyzer IC-100 (Yokogava Electric Co., Japan), equipped with a suppressor

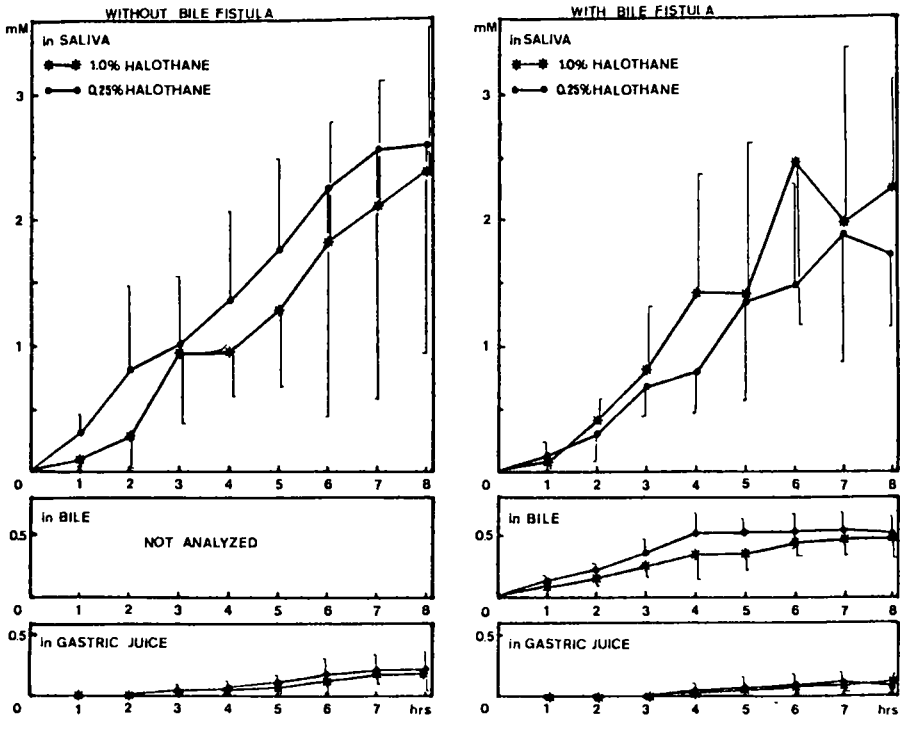

Fig. 1. Concentrations for TFAA excreted in saliva, bile and gastric juice for animals with and without bile fistula, who inhaled two different concentrations of halothane. The concentrations in saliva are highest, increased with time and do not reach plateau. The concentrations of TFAA excreted under different halothane concentrations are similar for all three juices.

and an electro-conductive detector. An anion exchange resin SAX-1, packed in 25 cm(L) × 4.6 mm(D) stainless column was used as a separator. As an eluent and scavenger carboxilic buffer (2mM Na_2CO_3, 4mM $NaHCO_3$) and 50mM of dodecylbenzensulfonic acid were used, respectively, at a flow rate of 2 ml/min.

From the data on the concentrations of TFAA excreted in bile gastric juice, and saliva and from the volumes of bile, and gastric juice and the weight of saliva the amounts of TFAA excreted into these fluids were calculated.

Statistics

The means and standard deviations of the different groups of data were calculated. The two-sample t-test and paired-sample, t-test were used to assess the significance of difference between values.

Results

Bile

i) Biliary volume and secretion rate.

The cumulative volume of bile secreted did not differ significantly between groups 1 and 3. The values were 12,607 ± 6,159 μl (mean ± sd) for group 1 and 15,047 ± 3,008 μl for group 3. The secretion rate of bile was 28 μl/min in group 1 and 31 μl/min in group 3.

ii) Concentration of TFAA excreted in the bile

TFAA was detected in the first sample during inhalation of halothane and in all following samples. Its concentration gradually increased with time and there was no difference in the values for groups 1 and 3 at the end of the experiment (fig. 1).

iii) Amount of TFAA excreted in bile

Table 1. Cumulative amount of TFAA excreted in bile, saliva and gastric juice during 8 hours

Halothane	Bile Fisturae		Bile	Saliva	Gastric Juice
0.25%	+	Volume (during 8hr)	12,607 ± 6.159μl	1.49 ± 1.41g	5,535 ± 2,081μl
		Secretion Rate	28μl/min	3.6mg/min	11.5μl/min
		TFAA (μmol/8hrs)	4.85 ± 1.87	0.89 ± 0.62	0.11 ± 0.06
0.25%	−	Volume (during 8hr)	−	1.24 ± 0.62g	6,014 ± 1.241μl
		Secretion Rate	−	2.5mg/min	12.5μl/min
		TFAA (μmol/8hrs)	−	1.65 ± 0.75	0.56 ± 0.40
1.0%	+	Volume (during 8hr)	15,047 ± 3,008μl	0.89 ± 0.37g	8,400 ± 3,867μl
		Secretion Rate	31μl/min	1.7mg/min	17.5μl/min
		TFAA (μmol/8hrs)	5.36 ± 2.29	1.50 ± 0.59	0.25 ± 0.19
1.0%	−	Volume (during 8hr)	−	1.28 ± 0.77g	7,371 ± 3,727μl
		Secretion Rate	−	2.5mg/min	15.3μl/min
		TFAA (μmol/8hrs)	−	1.69 ± 1.04	1.02 ± 0.71

The cumulative amounts are significantly higher in the gastric juice of animals without bile fisturae ($P<0.05$ by the two-sample t-test). The cumulative amounts excreted in gastric juice are alose higher with the higer concentration of halothane. The relative proportions of the cumulative amount of TFAA excreted in bile, saliva and gastric juice are 44:8:1 for animals receiving 0.25% halothane and 26:6:1 for receiving 1.0% halothane.

The excretion of TFAA gradually increased with time in both groups.

Saliva

i) The secretion of saliva was significantly suppressed during the inhalation of Halothane in all groups. Thereafter it gradually and significantly increased. The mean secretion rate of saliva was 2.5 mg/min in the groups without bile fistulae – i.e. groupes 2 and 4. The rate was 3.6 mg/min in group 1 and 1.7 mg/min in group 3. The cumulative weights of secreted saliva for the groups without bile fistulae were 1.24 ± 0.62 g in group 2 and 1.28 ± 0.77 g in group 4. The weight were 1.49 ± 1.41 g in group 1 and 0.89 ± 0.37 g in group 3.

ii) TFAA was detected in the first sample of saliva collected during the inhalation of halothane and in all samples thereafter. The concentrations of TFAA excreted in saliva increased with time and was similar in animals who inhaled different concentration of halothane (Fig.1).

In the group without bile fistulae, the concentration of TFAA excreted in saliva was higher, but not significantly, than in the group with bile fistulae when the concentration of inhaled halothane was 0.25%. The concentrations of TFAA excreted in saliva were similar in the animals with and without fisturae when the inspiratory concentration of halothane was 1.0% (fig.1).

iii) The amounts of TFAA excreted in saliva increased with time. There was no significant difference between animals who inhaled different concentrations of halothane. The cumulative amount of excreted TFAA was higher in the group without bile fistulae when the inspiratory concentration of halothane was 0.25%. The cumulative amounts of TFAA excreted in saliva of animals with or without bile fistulae were similar when inspiratory concentration of halothane was 1.0% (table 1).

Gastric juice

i) The secretion of gastric juice decreased during halothane inhalation. It showed a comparatively constant rate thereafter. The mean secretion rate was 12.5 μl/min in group 2 and 15.3 μl/min in group 4. The cumulative volumes of the secreted gastric juice in those groups were 6,014 ± 1,241 μl

and 7,371 ± 3,727 μl, respectively.

The rate of secretion of gastric juice in the groups with bile fistulae and ligated pyloric regions was showed comparatively constant at 11.5 μl/min in group 1 and 17.5 μl/min in group 3. The cumulative volumes of gastric juice secreted in these two groups were 5,535 ± 2,081 μl and 8,400 ± 3,867 μl respectively.

ii) The concentration of TFAA excreted in gastric juice of animals who inhaled different concentrations of halothane were similar (fig. 1).

In gastric juice, TFAA was detected after anesthesia. It appeared in the first sample during this period in animals without bile fistula and was detected and measured in all of them. In the animal who had undergone bile fistulae TFAA was detected one hour later and we failed to detect it in two animals. The concentrations of TFAA excreted in gastric juice were higher in the animals without bile fistulae (fig. 1)

iii) The excretion of TFAA in gastric juice gradually and only slightly increased with time. The cumulative amounts of TFAA excreted in gastric juice were higher in the groups who inhaled the higher concentration of halothane (table 1). The cumulative amounts of TFAA in gastric juice of the animals without bile fistulae were higher than with bile fisturae.

Discussion

Cohen and Hood[10] reported a high rate of metabolism for halothane in mice and alse found that nonvolatile metabolites were produced and accumulated in liver and intestine because of their slow elimination. Fiserova – Bergerova[6] et al. postulated that the metabolism of halothane in rats by all metabolic pathways was flow-limited at low concentrations and capacity-limited at high concentrations and they also reported that the major metabolite of halothane was likely to be TFAA and that its metabolic processes were saturated at a very low concentration of halothane. Their study has shown that the volatile and nonvolatile metabolites mainly TFAA were produced from halothane, and that these products were distributed quite differently in body tissues. The volatile metabolites were easily eliminated through lungs, whereas TFAA was accumulated in the body tissues because of its slow renal clearance.

Since TFAA is mainly produced from halothane in liver[1,2,11], it is considered that the guinea pigs exposed higher concentrations of halothane have to excrete more TFAA in bile. In this study, the concentration of TFAA in bile obtained from bile fistulae in the guinea pigs which inhaled 0.25% halothane attained to a plateau 2 hours after cessation of inhalation. In the guinea pigs exposed to 1.0% halothane for 2 hours the concentrations of TFAA in bile were increased continuously throughout the experiment. The concentrations of TFAA in bile were higher in the guinea pigs exposed to 0.25% halothane than those exposed to 1.0% halothane. The total amounts of TFAA excreted in bile throughout the experiment were 4.85 μmol/8 hours in the guinea pigs exposed to 0.25% halothane and 5.36 μmol/8 hr in the guinea pigs exposed to 1.0% halothane. It was also shown that the concentration and amount of TFAA excreted in bile per hour were higher after cessation of inhalation than during inhalation in both groups. These findings probably resulted from following mechanisms: 1) The elimination of TFAA was so slow that a considerable amount of TFAA was accumulated in blood or tissues, 2) The higher tissue concentrations of halothane induced by exposure to 1.0% halothane exceeded the metabolic capacity for halothane so that TFAA production could not increase in proportion to the inhalational concentrations, and 3) The higher tissue concentrations of halothane depressed the matabolic rate of halothane[12] and/or the excretion process of TFAA. In this study it was not possible to clarify the exact mechanism.

Kikuchi[9] has reported the possibility of enterohepatic circulation of TFAA. If it takes place, the excretion of TFAA in saliva and gastric juice might be diminished by external drainage of bile. In this study it was found that the total amount of TFAA excreted

in gastric juice was significantly less in the guinea pigs with bile fistulae than those in the guinea pigs without bile fistulae. The total amount of TFAA excreted in saliva changed insignificantly in the guinea pigs with bile fistulae and those without fistulae. The concentration of TFAA in saliva were several times higher than those found in gastric juice and bile in any group. According to the report of Fiserova-Bergerova[6], TFAA accumulated in liver, brain, muscle and kidney of rats after exposure to halothane in almost equal concentrations. Thus TFAA seems to be carried to these organs through blood stream and to be excreted in gastric juice and saliva in similar concentrations. The higher concentrations of TFAA found in saliva in this study probably resulted from the considerable large amounts of TFAA production and/or from the active elimination mechanisms for TFAA in salivary glands. Since Murakami et al.[13] reported on the cytochrome P-450 activity in salivary glands of rats, biodegradation of halothane in salivary glands should not be ignored in guinea pigs.

In 1970 Cascorbi et al.[7] reported on the elimination of halothane metabolites in human feces and sweat. It may be considered that a greater amount of TFAA than that excreted in bile is uptaken into circulating blood due to the high protein binding of TFAA and low biliary clearance of TFAA. A part of TFAA excreted in bile can be reabsorbed through the intestinal mucosa, so that enterohepatic circuration of TFAA can be resulted. The rest excreted in bile may be eliminated in feces.

In conclusion, it is suggested that TFAA produced from biodegradation of halothane in liver is removed from hepatocytes in bile and circulating blood. In this study we were able to confirm two previously unkown elimination process of TFAA via saliva and gastric juice. The possibility of active metabolism of halothane in salivary glands was also shown.

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