

Biotransformation of Isoflurane: Urinary and Serum Fluoride Ion and Organic Fluorine

Tatiana Davidkova, M.D.,* Hiroto Kikuchi, M.D., Ph.D.,† Kohyu Fujii, Ph.D.,‡ Keiko Mukaida, M.D.,§ Nobuyoshi Sato, M.D.,§ Shoji Kawachi, M.D.,§ Michio Morio, M.D., Ph.D.¶

The serum and urinary concentrations of fluorinated metabolites of isoflurane after inhalation of three different concentrations of isoflurane were studied in 18 ASA physical status 1 or 2 patients, scheduled for orthopedic or otolaryngeal surgery. Isoflurane was administered for 60 min during fentanyl-nitrous oxide-oxygen, and its end-tidal concentration was maintained at 0.3, 0.6, or 1.15% (groups I, II, and III). The organic fluorine was determined by combustion and fluoride ions were analyzed by ion chromatography. The amounts were expressed in terms of fluoride ion. The concentrations of serum fluoride ion and organic fluorine increased significantly 15 min after the onset of inhalation of isoflurane. The mean peak values of fluoride ions were 3.8 ± 1.1 , 3.9 ± 1.4 , and 4.2 ± 0.9 $\mu\text{mole/l}$ ($M \pm SD$) in patients in groups I, II, and III, respectively. The half-lives of fluoride ion and of organic fluorine as metabolites of isoflurane, calculated from the amounts excreted in urine, were 36 h and 41 h, respectively. The cumulative amounts of fluoride ion and organic fluorine excreted up to the 6th postoperative day were 548 ± 230 and 785 ± 452 μmoles in group I, 594 ± 138 and $1,378 \pm 807$ μmoles in group II, and $1,032 \pm 496$ and 728 ± 265 μmoles ($M \pm SD$) in group III, respectively. The urinary excreted fluoride ion increased in proportion to the dose of isoflurane and approximately 1.3 mmol was excreted per 1 MAC \times hour inhalation of isoflurane. The authors concluded that isoflurane might be biotransformed to a greater extent than reported previously, although the serum fluoride ion level was found to be low. (Key words: Anesthetics, volatile: isoflurane. Biotransformation: fluorometabolites. Ions, fluoride: excretion.)

ISOFLURANE, 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, is the least metabolized among the fluorinated inhaled anesthetics.^{1,2} The ethyl group is protected against degradation by the trifluorocarbon end, and the difluoromethyl group is considered stable.^{3,4} The limited metabolism of isoflurane is mostly the result of oxidation of the alpha carbon, which is likely to occur in the endoplasmic reticulum of the liver.

In 1974, Hitt *et al.* reported that the urine fluorine content increased after exposure to isoflurane.⁵ Fluorine was identified as fluoride ions, and the organic flu-

orine was strongly suspected to be trifluoroacetic acid (TFAA). From urine samples taken over 72 h after anesthesia, Holaday *et al.* calculated the overall biodegradation rate of isoflurane to be less than 0.2% (w/w) on the basis of organic fluorine and fluoride ion excreted in urine.¹ Recently, in animal experiments in which a very low concentration of anesthetics were inhaled for a long period, the extent of biotransformation of isoflurane was found to be slightly lower than that of enflurane.⁶

We examined the effects of the blood concentration of isoflurane on the blood concentration of organic fluorine and fluoride ion, and on the excreted amounts of organic fluorine and fluoride ion in urine. By obtaining the urinary half-lives of organic fluorine and fluoride ion, the predicted total amounts of excretion of these fluoride metabolites of isoflurane could be assessed.

Materials and Methods

Eighteen ASA physical status 1 or 2 patients who were scheduled for orthopedic or otolaryngeal surgery were investigated. This study was approved by the Ethical Committee on Human Studies of Hiroshima University Hospital and the Japanese Ministry of Health and Welfare. Written informed consent was obtained from all the patients.

The patients who had not been given barbiturates and any other enzyme inducing drugs were premedicated with atropine sulfate, 0.4–0.5 mg i.m., 45–60 min prior to anesthesia. Flunitrazepam, 1 mg iv, and fentanyl, 10 $\mu\text{g/kg}$ iv, were used for induction of anesthesia, and tracheal intubation was performed after muscular relaxation with 0.1 mg/kg pancuronium bromide. Anesthesia was maintained with 70% nitrous oxide in oxygen and, if necessary, with additional doses of fentanyl. The anesthetic machine with a new breathing circuit and attachments was flushed with oxygen for 72 h to avoid contamination by other anesthetics. This machine was used throughout the study. The lungs of all patients were ventilated using an Acoma Anespirator® with a tidal volume of 8 ml/kg and respiratory rate of 12/min. These parameters were altered, if necessary, according to blood gas analyses. Thirty minutes after initiation of surgery, isoflurane was administered for 60 min through a vaporizer calibrated by gas chromatography, and the end-tidal concentration was kept at 0.3,

* Research Associate.

† Associate Professor.

‡ Assistant Professor.

§ Instructor.

¶ Chairman and Professor.

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Address reprint requests to Dr. Morio.

0.6, or 1.15%, respectively, in six patients each (groups I, II, and III). In all cases, the end-tidal isoflurane concentration was monitored with a NORMAC® (Datex, Finland) equipped with an infra-red sensor, calibrated by gas chromatography and recorded simultaneously with arterial blood pressure.

Arterial blood samples were obtained for measurement of inorganic and organic fluorine and isoflurane concentration before inhalation; every 15 min during inhalation; 15, 30, 60, 120, and 240 min after discontinuation of isoflurane; and once a day for 6 postoperative days.

Urine excreted during the 12 h before surgery, during inhalation, and every 24 h for 6 postoperative days, was sampled for measurement of organic fluorine and fluoride ion.

MEASUREMENT OF BLOOD CONCENTRATION OF ISOFLURANE

The blood concentration of isoflurane was measured by the head space gas method with a Shimadzu GC-4A PTF gas chromatograph equipped with a flame ionization detector. A 3 m × 4 mm stainless steel column was packed with 20% dioctylphthalate and kept at 100° C. The injection port and flame ionization detector were maintained at 110° C. Helium (30 ml/min) was used as the carrier gas. Authentic isoflurane added to blood obtained from humans not exposed to anesthesia was used for obtaining the calibration curve (range of the concentration: 0.1 μmole/l to 10 mmole/l, with 0.9997 of correlation coefficient).

MEASUREMENT OF FLUORIDE ION

The fluoride ion was measured by an Ion Chromatographic Analyzer IC-100 (Yokogawa Electric Co., Japan) equipped with a suppressor and an electro-conductive detector. Anion exchange resin (SAX-1), packed in a 25 cm × 4.6 mm column, was used as a separator. As an eluent solution and a scavenger, 5 mM of sodium tetraborate and 50 mM of dodecylbenzenesulphonic acid were used, respectively, at a flow rate of 2 ml/min. Sodium fluoride as a standard solution was used for obtaining a calibration curve (range of concentration: 0.1–1 μmole/l, with a correlation coefficient of 0.9998).

Fluoride Ion in Serum and Urine. For measuring the concentration of serum fluoride ions, 50 μl of serum was diluted 10 times with deionized water and centrifuged for 10 min at 500 × G for deproteinization using an AMICON® ultrafiltration membrane cone (AMICON Corp., Danvers, MA). The ultrafiltrate (100 μl) was injected into the ion chromatographic analyzer through a cation exchange filter.

TABLE 1. Patient Characteristics (M ± SD)

Group	Age (Yr)	Weight (kg)	Height (cm)
I (n = 6)	33.3 ± 12.0	54.0 ± 7.0	161.1 ± 8.0
II (n = 6)	32.2 ± 13.2	55.7 ± 11.4	158.8 ± 8.6
III (n = 6)	35.5 ± 3.4	63.3 ± 11.9	163.5 ± 9.8

The urine samples were prepared in the same manner, but diluted 100 times without deproteinization.

Organic Fluorine in Serum and Urine. Total fluoride was measured by a modified oxygen-flask combustion method.⁷ The difference between the total nonvolatile fluoride and the fluoride ion was defined as fluoride ions from organic compounds.

Measurement of Trifluoroacetic Acid (TFAA). Measurement of TFAA was the same as for fluoride ions, except that the eluent solution was 4 mmoles/l of monosodium phosphate and 2 mmoles/l of disodium phosphate, and the amount of TFAA was determined using authentic TFAA. The minimum measurable concentration of TFAA was 1 μmole/l with the ion chromatography.

PHARMACOKINETICS⁸

From the urine volume and urine concentration of fluoride ions and organic fluorine, their excretion amounts per day were calculated. The amount of the substance excreted was plotted on semilogarithmic paper against the midpoint of the collection time. From the slope of the regression lines were calculated the elimination half-lives ($T_{1/2}$). Renal clearance was calculated as the ratio of the excretion rate at time *t* and serum concentration at time *t*.

For all the calculations, the control values of fluoride ion and organic fluorine were subtracted from the values of the measured samples.

REAGENTS

Isoflurane was provided by Dainabot Co., Japan. The other reagents were commercial products of analytical grade.

STATISTICS

The mean values and standard deviations for each group were calculated. Regression analysis was performed according to the least square method. Student's *t* test was used to assess the significance of differences between the data. Differences with a random probability of 5% or less were considered significant.

Results

There was no significant difference in age, height, or body weight among the three groups (table 1).

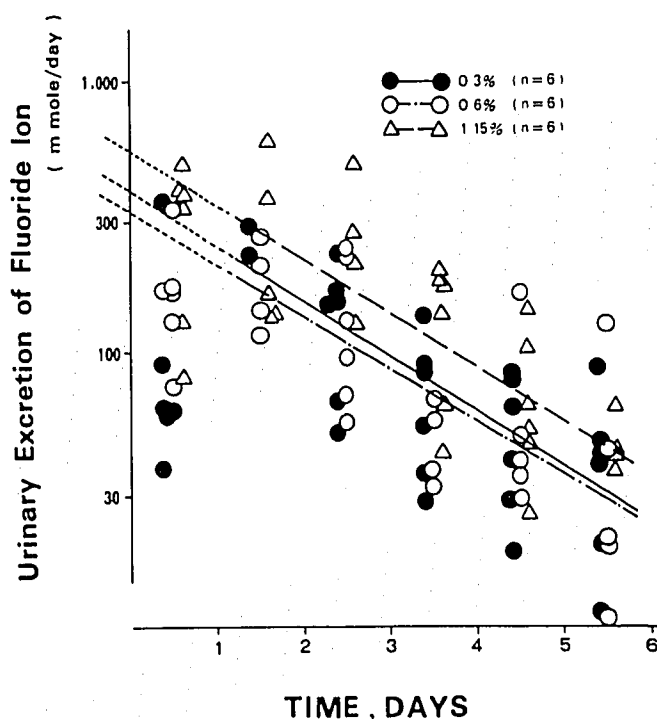


FIG. 1. Daily change in the amount of fluoride ion excreted in the urine after inhalation of isoflurane for 1 h at three different end-expiratory concentrations. The decrease was a single exponential function of time, showing the equations $\log Y = 2.584 - 0.202 X$ ($r = -0.718$), $\log Y = 2.513 - 0.191 X$ ($r = -0.706$), and $\log Y = 2.745 - 0.199 X$ ($r = -0.750$) in groups I, II, and III, respectively, where Y represents the amount of urinary excreted fluoride ion in m mole/day; X, the postoperative time in day; and r, the correlation coefficient.

THE END-TIDAL AND BLOOD CONCENTRATION OF ISOFLURANE

The expected end-tidal concentration of isoflurane was achieved in 3–5 min in all three groups. The vaporizer had to be adjusted frequently for a few minutes, but, thereafter, a stable concentration was maintained with only minor readjustment. The end-tidal isoflurane concentration decreased quickly after being discontinued.

The blood concentration of isoflurane was almost constant during the inhalation. Its mean value was $106 \pm 15 \mu\text{mole/l}$ in group I, $214 \pm 37 \mu\text{mole/l}$ in group II, and $418 \pm 51 \mu\text{mole/l}$ in group III. No other volatile anesthetic was detected in the blood.

SERUM CONCENTRATION OF FLUORIDE ION AND ORGANIC FLUORINE

The serum concentration of fluoride ion increased significantly 15 min after the onset of inhalation, continued to increase until the end of inhalation, and then remained at a plateau for about 4 h. The mean values at

the plateau were 3.0 ± 0.7 , 3.2 ± 1.2 , and $3.3 \pm 1.0 \mu\text{mole/l}$, in groups I, II, and III, respectively, and the mean control values were 1.6 ± 0.4 , 1.5 ± 0.5 , and $1.6 \pm 0.5 \mu\text{mole/l}$, respectively. Peak values for each individual, the means of which were 3.8 ± 1.1 , 3.9 ± 1.4 , and $4.2 \pm 0.9 \mu\text{mole/l}$, respectively, were observed at different times. The highest value was $6.6 \mu\text{mole/l}$ (control: $1.9 \mu\text{mole/l}$) obtained in a patient from group II 1 h after the end of inhalation.

In groups I and II patients, the serum concentration of fluoride ion returned to the preoperative level on the first postoperative day. In group III patients, the serum concentration of fluoride ion decreased significantly on the first postoperative day, and returned to the preoperative level on the second postoperative day.

The serum concentrations of organic fluorine were significantly increased 15 min after the start of inhalation from the control values of 34.8 ± 13.4 , 38.7 ± 10.7 , and $50.5 \pm 13.9 \mu\text{mole/l}$ to 51.0 ± 33.8 , 54.8 ± 16.7 , and $69.5 \pm 30.6 \mu\text{mole/l}$ in groups I, II, and III, respectively. Thereafter, the serum concentration remained relatively constant. The highest values were $79.1 \pm 53.4 \mu\text{mole/l}$ in group I, $88.7 \pm 60.2 \mu\text{mole/l}$ in group II, and $84.6 \pm 69.2 \mu\text{mole/l}$ in group III. The serum concentration of organic fluorine was slightly decreased on the sixth postoperative day, but did not return to the control value.

URINARY FLUORIDE ION AND ORGANIC FLUORINE

Fluoride Ion. Figure 1 shows the amount of fluoride ion excreted in every 24 h for 6 postoperative days. The highest mean value of fluoride ion excreted was observed on the first or second postoperative day. Thereafter, it decreased as a single exponential function of time. From the regression lines, the half-life of fluoride ion was calculated to be 35.8, 37.8, and 36.3 h in groups I, II, and III, respectively.

The renal clearance on the first and third postoperative day was calculated to be 45.0 and 61.0 ml/min in group I, 148.0 and 83.0 ml/min in group II, and 125.0 and 112.0 ml/min in group III.

The cumulative amount of fluoride ion excreted was $548 \pm 230 \mu\text{moles}$ in group I, $594 \pm 138 \mu\text{moles}$ in group II, and $1,032 \pm 496 \mu\text{moles}$ in group III. Figure 2 shows the relationship between the cumulative amount of fluoride ion excreted and the blood concentration of isoflurane during a 1-h inhalation, showing a regression line with an equation ($Y = 141 + 2.25 X$, $r = 0.83$; $P < 0.05$), where Y represents the cumulative amount of fluoride ion excreted; X, the blood concentration of isoflurane; and r, the correlation coefficient.

Organic Fluorine. The largest amount of organic fluorine was excreted later than the fluoride ion between 48–120 h. Thereafter, the excretion of organic fluorine

also decreased as a single exponential function of time. The regression lines were $\log Y = 3.276 - 0.189 X$ ($r = -0.746$), $\log Y = 3.328 - 0.153 X$ ($r = -0.758$), and $\log Y = 3.000 - 0.192 X$ ($r = -0.710$) in groups I, II, and III, respectively, where Y represents the excreted amount of organic fluorine; X, the time in day; and r, correlation coefficient. From the regression lines, the half-life of organic fluorine was calculated to be 38.2, 47.2, and 37.6 h in groups I, II, and III, respectively.

The cumulative amount of organic fluorine excreted was 785.1 ± 451.6 μ moles in group I, $1,378 \pm 807.0$ μ moles in group II, and 728.0 ± 264.9 μ moles in group III. The cumulative amount of organic fluorine in group III was smaller than that in group II, although there is no significant difference between groups III and II.

TFA was detected by the ion chromatographic analyzer in all the urine samples, but it could be measured quantitatively only in the samples that had a high concentration of organic fluorine. The amount of TFAA was similar to that measured by the oxygen flask combustion method.

Discussion

When biotransformation of an anesthetic is evaluated, the amount of other agents used concomitantly should be kept to a minimum to avoid their effects upon metabolism.^{9,10} One milligram of flunitrazepam, which contains a total of 3.1 μ moles of fluorine, was used as a hypnotic. When distributed to the body compartments, the resulting concentration will be negligible. Moreover, flunitrazepam metabolism does not release fluoride ions during metabolism.¹¹

The serum fluoride ion concentration increased, but remained at a very low level, as reported previously.^{12,13} The mean half-life of the fluoride ion for the three groups was calculated to be 36 h, which is shorter than the half-life of fluoride ions following methoxyflurane (48 h).¹⁴ This is related to the low lipid solubility of isoflurane, or fat/gas partition coefficient (69), when compared with methoxyflurane (885).¹⁴ Unlike a bolus injection of fluoride ions, the urinary half-life of fluoride ions produced from a fluorinated anesthetic could be influenced by the duration of metabolism.

Although traces of TFA were detected by the ion chromatographic analyzer in all the urine samples, it could be measured only in the samples that had a high concentration of organic fluorine. The amounts were almost equivalent to those measured by the oxygen flask combustion method. The urinary excretion of organic fluoride was decreased at high inhaled concentration of isoflurane, it might be that the metabolism of isoflurane to organic fluoride was inhibited by itself and/or its metabolites. Therefore, a large proportion of the or-

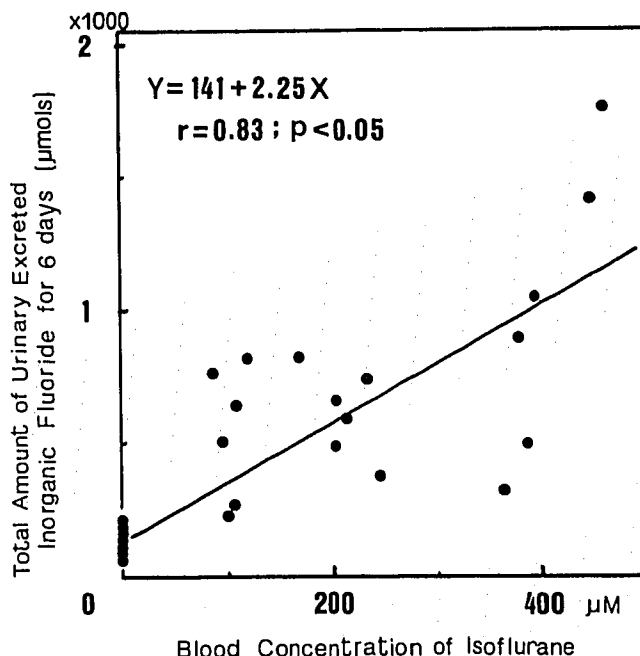


FIG. 2. Relationship between the measured cumulative amounts of fluoride ion excreted in the urine for 6 postoperative days and the blood concentration of isoflurane. The increase was dose-dependent. The regression line was $Y = 141 + 2.25 X$, $r = 0.83$, $P < 0.05$ where Y represents total amounts of urinary excreted fluoride ion for 6 days in μ moles; X, the blood concentration of isoflurane in μ mole/l; and r, the correlation coefficient.

ganic fluorine might be TFA. The mean half-life of organic fluorine in our study (38–47 h) was shorter than that of TFA released by the metabolism of halothane (55 h), and longer than that of TFA released as a metabolite of fluroxene (24 h).¹⁴ As in the case of fluoride ion, these values were correlated with the lipid solubility of the anesthetic. This suggests that the larger amounts of residual anesthetic agent in fatty tissue might be subjected to the metabolism for a longer period of time, although the rate of metabolism might also be influenced.

In pharmacokinetic terms, 98% of a substance would be expected to be eliminated from the body within four elimination half-lives.⁸ The period of measurement in our study was sufficiently long such that the cumulative amounts of two metabolites excreted can be regarded as more than 98% of the theoretically expected total excretion.

Note that the cumulative amount of fluoride ion excreted in urine after 1 h inhalation of isoflurane increased dose-dependently in all three groups. The organic fluorine excretion increased in groups I and II, but not so prominently in group III. If we calculate the ratio of the cumulative amount of organic fluorine/fluoride ion excreted in urine, this ratio in group III shows 0.71, which is almost the same as that reported by Ho-

laday's group (0.86),¹ where the ratio in groups I and II is about 2. In Holaday's study, the alveolar concentration of isoflurane was kept at 1.3 MAC, and the end-tidal concentration of isoflurane in our group III was almost 1 MAC. The difference in the foregoing ratio might be derived from the difference in the concentration of isoflurane inhaled, although the mechanism is unknown.

In our study, 1.30 ± 0.495 mmol of fluoride ion was excreted per 1 MAC \times hour inhalation. This is approximately four times higher ($P < 0.001$) than the amount of fluoride ion after 1.3 MAC \times 2.17 h reported by Holaday *et al.*¹ (0.260 ± 0.216 mmol). Although the reasons for this difference are not known, possibilities include differences in analytical methods and/or a difference in patient population.

In conclusion, the urinary excretion of fluoride ion was dose-dependent following exposure to end-tidal concentrations of 0.3, 0.6, and 1.15% for 1 h. Although the serum concentrations of fluoride ion and of organic fluorine remained very low, as already reported, isoflurane might be biotransformed much more than reported previously.

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