Hiroshima J. Med. Sci.
Vol.40, No.1, 1~7, March, 1991
HIJM 40-1

Dose-related Sevoflurane Metabolism to Inorganic Fluoride in Rabbits

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ABSTRACT

Serum concentrations and urinary excretion of inorganic fluoride (fluoride ion), a metabolite of sevofluorane, were measured by an ion-chromatographic analyzer after inhalation of three different concentrations of sevoflurane in adult, male Japanese white rabbits weighing 2.6—3.6 kg. Sevoflurane was administered at concentrations of 0% (control), 1%, 2% and 3% (Groups I, II, III and IV, respectively) through a sevoflurane vaporizer for 2 hr under controled ventilation. Blood and urine samples were collected during and after termination of sevoflurane inhalation at scheduled time intervals for 24 hr. The total volume of urine, the urinary pH and the osmolality of serum and urine were not significantly different among any of the groups. Osmolality of the serum and urine was within normal range in all groups of animals. The mean serum peak values of fluoride ion were 0.7 ± 0.5, 22.8 ± 8.7, 31.8 ± 11.0 and 41.5 ± 13.2 μM (mean $\pm$ SD) in groups I, II, III and IV, respectively. Peak values were recorded within 15 min after the termination of inhalation. The cumulative amounts of fluoride ion excreted in urine in 24 hr were calculated to be 5.0 ± 1.6, 26.1 ± 6.7, 41.4 ± 11.3 and 64.3 ± 18.0 μmol (mean $\pm$ SD) in groups I, II, III and IV, respectively. Regression analysis revealed significant correlations between the formation and excretion of fluoride ion, and the dose of sevoflurane ($r = 0.85$, $p < 0.05$ and $r = 0.89$, $p < 0.05$, respectively). The authors conclude that the formation and excretion of fluoride ion after sevoflurane anesthesia is dependent on the dose of the drug. This study also concludes that 1—3% of sevoflurane for 2 hr of inhalation is unlikely to produce renal dysfunction.

Key words: *Anesthetics, volatile: sevoflurane.*
Biotransformation: fluorometabolites.
Ions, fluoride: excretion.

Sevoflurane, fluoromethyl 2,2,2-trifluoro-1 (trifluoromethyl) ethyl ether, is a fluorinated inhalational anesthetic agent. In 1975, Wallin et al, first reported the physicochemical, pharmacological and toxicological properties of sevoflurane[18]. It has many advantages over currently used inhalational anesthetics. It is a rapid-acting and potent inhalational anesthetic having rapid uptake and elimination due to a low blood/gas partition coefficient (0.6)[9,18]. However, like other inhalational anesthetic agents, sevoflurane also exhibits some chemical and metabolic instability and undergoes biotransformation, producing fluoride ion and hexafluoroisopropanol[9]. Hexafluoroisopropanol is excreted in the urine as a glucuronide conjugate[9]. Fluoride ion, which has long been known to be toxic to the kidney, is a common metabolite of several commonly used halogenated inhalational anesthetic agents[5]. It is well established that methoxyflurane administration in man and Fischer 344 rats results in a dose-related renal concentrating defect owing to the anesthetic's metabolization to fluoride ion[2,10]. Other researchers have reported that the threshold level for nephrotoxicity is 50 μM and 33.6 μM in man using methoxyflurane and enflurane, respectively[2,12]. Holaday reported that the elevation of serum fluoride ion seemed independent of exposure duration or concentration[7]. We attempted in the present study to determine the extent of sevoflurane metabolism to fluoride ion when used at different concentrations and under control ventilation condition.

METHODS AND MATERIALS

Three to four month-old male Japanese White rabbits weighing 2.6—3.6 kg were divided at random into four groups of six rabbits each. Group I received no anesthesia; Group II, Group III and Group IV received 1%, 2% and 3% sevoflurane, respectively, for 2 hr. Four per cent sevoflurane was not used to avoid severe hypotention. At least five days were allowed for the animals to adapt to their cages. All animals were treated identically. Taking food and tap water, which contained natur-

ally fluoride ion of 41.6 mg/kg and 5.4 μM, respectively, were allowed ad libitum. Serum concentration of fluoride ion was measured and found less than 1 μM. Initially the rabbits were anesthetized with sodium pentobarbital at a dose of 25 mg/kg intravenously through the peripheral vein, and supplemented with a dose which was administered as required to maintain a stable anesthetic state. The femoral artery and vein were cannulated for arterial blood sampling and for the continuous infusion of lactated Ringer's solution (8—10 ml/kg/hr), respectively. After tracheostomy and intubation, sevoflurane was administered in oxygen through a sevoflurane vaporizer (Acoma Co. Ltd, Tokyo, Japan) via non-rebreathing circuit using fresh gas flows of 3.5 liters/min. Artificial ventilation was carried out with 100% oxygen only following sevoflurane, until the end of the experiment (24 hr) using a Harvard animal respirator (Respiration Pump Model No.607, Harvard Apparatus Co., Inc., U.S.A). Anesthesia was maintained with sodium pentobarbital at a dose of 5—6 mg/kg/hr using a micro-infusion pump (Infusion/withdrawal pump, Model No. 11-1900, Sweden). The physiological level of arterial CO_2 tension ($Paco_2$ 35—45 mmHg) was maintained by adjusting the tidal volume and rate of the respirator. Po_2, Pco_2, and pH were measured using an ABL4 blood gas analyzer (Radiometer, Copenhagen, Denmark). The urinary bladder was catheterized with a 10 Fr. Foley balloon catheter for urine sampling. Body temperature was maintained with a warming blanket at 37°C. Sevoflurane was supplied from Maruishi Pharmaceutical Co. Ltd, Osaka, Japan.

1) Measurement of the Blood Concentration and Dose of Sevoflurane

The blood concentration of sevoflurane was measured by the head space gas method at 20°C 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% dioctylphtalate and kept at 100°C. Helium (30 ml/min) was used as the carrier gas. Authentic sevoflurane added to blood obtained from rabbits was used for obtaining the calibration curve (range of the concentrations: 7.6 μM to 760 μM, with a correlation coefficient of 0.998).

The sevoflurane dose was calculated as the sum of the products of vaporizer settings and time (hr). In our study the inhalation time was constant so the dose was proportional to the vaporizer settings.

2) Collection of Blood and Urine

To prevent the contamination of fluoride ion, plastic containers and deionized water were used for dilution. Blood samples were collected before sevoflurane inhalation and at 15, 30, 60, 90, 105 and 120 min during inhalation and 15, 30 min, 1, 2, 3, 6, 12, 18 and 24 hr after the termination of inhalation. Urine samples were collected before inhalation and at time 0, 1, 2, 3, 6, 12, 18 and 24 hr after the termination of inhalation. "0" time was expressed by the time of sevoflurane inhalation termination. The urine and serum samples were frozen (at -20°C) until analysis. Serum sample was prepared in the following way: blood was taken from the rabbits, centrifuged for 10 min at 150 × g and the plasma was separeted and allowed to clot. Serum was then collected leaving the clot aside. Urinary pH and osmolality were measured within 6 hr of sample collection.

3) 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 dodecylbenzenesulfonic 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—5 μM, with a correlation coefficient of 0.9998).

4) Concentration of Fluoride Ion in Serum and Urine

For measuring the concentration of serum fluoride ion, 100 μl of serum was diluted 1:10 with deionized water and centrifuged for 10 min at 500 × g for deproteinization (molecular weight above 30,000) using an Tosoh Ultracent-30 ultrafiltration (Tosoh Corp., Tokyo, Japan). The ultrafiltrate (100 μl) was injected into the ion chromatographic analyzer through a cation exchange filter. The urine samples were prepared in the same manner, but diluted 1:100 with deionized water without deproteinization.

5) Measurement of pH and Osmolality

Urinary pH were determined with a Beckman pH meter, U.S.A (Model-PHI 70). Urinary osmolality estimations were carried out using a Auto-Osmometer 'OSMOSTAT', calibrated before each group of estimations using standard solutions of 300 mOsm/kg and 1000 mOsm/kg.

6) Pharmacokinetics[16]

All pharmacokinetic calculations were made by means of the net concentration i.e, the measured level of fluoride ion minus background level. The serum concentration-time data obtained for fluoride ion was analyzed using compartmental analysis. Bi-exponential functions were fitted to the data using least square non-linear regression analysis.

7) Statistics

Mean and standard deviations (mean ± SD) of different groups of data were calculated. Student's t-test was used to assess the significance of differences between data. Statistical significance was assumed when $p < 0.05$.

RESULTS

1) Inhaled Concentration and Blood Concentration

of Sevoflurane

Fig. 1 shows the relationship between the blood concentration of sevoflurane and the sevoflurane concentration inhaled. The blood concentration of sevoflurane was almost always constant during the inhalation. Its mean values were 124.5±8.8 μM in group II, 266.3±38.4 μM in group III and 539.8±32.1 μM in group IV. The blood concentration decreased sharply after being discontinued. After 15 min the levels were 11.0±2.0, 20.0±7.0 and 33.0±5.0 μM, respectively. Half-life of blood concentration of sevoflurane was within 15 min. In one case we collected blood samples every 2 min for 16 min after termination of sevoflurane and the blood concentration of sevoflurane was found to decrease by 50% in less than 2 min. No anesthetic was found

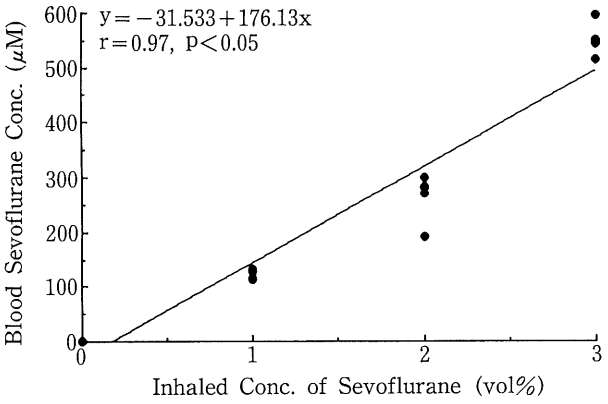

Fig. 1. Relationship between the blood concentration of sevoflurane and the inhaled concentration of sevoflurane. Linear correlation was observed. The regression line was y=−31.533+176.13x, r=0.97, p<0.05, n=18, where y represents blood concentration of sevoflurane in μM; x, the inhaled concentration of sevoflurane in vol %; r, the correlation coefficient.

in blood at 9, 14 and 20 hr after 1%, 2% and 3% of sevoflurane anesthesia, respectively.

2) Serum Concentration of Fluoride Ion

The serum concentration of fluoride ion increased significantly 15 min after the onset of inhalation (pre-exposure level<1 μM). It continued to increase until termination or 15 min after the termination of inhalation, then fell sharply. In Fig. 2, the mean peak values of 22.8±8.7, 31.8±11.0 and 41.5±13.2 μM in groups II, III and IV, respectively, were observed at time 0 or 15 min after the termination of inhalation. These values are significantly different from the control, p<0.05. The highest value obtained in one case from group IV at time 0 was 65 μM. In all other cases the levels were below 50 μM. In all groups the serum levels returned to their pre-exposure level by 24 hr. The elimination half-life was calculated from the slope of the regression line obtained from the serum concentration-time curve plotted on semi-logarithmic paper. The values were 921±347, 630±243 and 714±251 min in groups II, III and IV, respectively: not significantly different among the groups.

Fig. 3 shows the relationship between the peak serum concentration of fluoride ion and the blood concentration of sevoflurane, showing a regression line with the equation: y=8.24+0.0775x, (r=0.85; p<0.05, n=24), where y represents the peak serum concentration of fluoride ion; x, the blood concentration of sevoflurane; and r, the correlation coefficient.

3) Urinary Concentration of Fluoride Ion

Fig. 4 shows the urinary excretion rate curve following sevoflurane inhalation. Urine was collected at different time intervals and volumes measured. Regarding the excreted amount of fluoride ion in the urine, volume times concentration was calculated as the urinary excretion rate. The half-time was

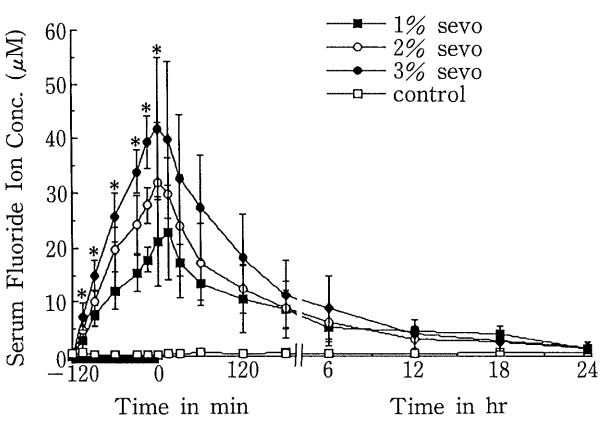

Fig. 2. Serum fluoride ion level after sevoflurane anesthesia at different concentrations. The thick line along the x-axis represents the duration of exposure. The x represents time and y, the serum fluoride ion concentration in μM. The mean±SD are shown (n=6). Asterisks indicates p<0.05 as compared to control.

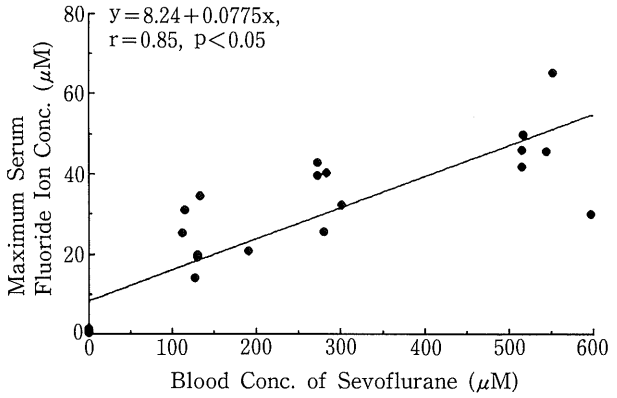

Fig. 3. Relationship between the maximum serum fluoride ion concentration and the blood concentration of sevoflurane. The increase was dose dependent. The regression line was y=8.24±0.0775x, r=0.85, n=24, p<0.05, where y represents maximum serum fluoride ion concentration in μM; x, the blood concentration of sevoflurane in μM; r, the correlation coefficient.

calculated from the slope of the regression line obtained from the excretion rate-midpoint collection time of urine. The mean values were 684±378, 414±89 and 451±79 min in groups II, III and IV, respectively: not significantly different among the groups.

The cumulative amounts of fluoride ion excreted in 24 hr were 26.1±6.7, 41.4±11.3 and 64.3±18.0 μmol in groups II, III and IV, respectively. These values are significantly different both from the control and also among the groups, $p<0.05$. Fig. 5 shows the relationship between the cumulative amounts of fluoride ion excreted and the blood concentration of sevoflurane, showing a regression line with the equation: $y=9.66+0.11x$, ($r=0.89$; $p<0.05$, $n=24$), where y represents the cumulative amounts of fluoride ion excreted; x, the blood concentration of sevoflurane; and r, the correlation coefficient.

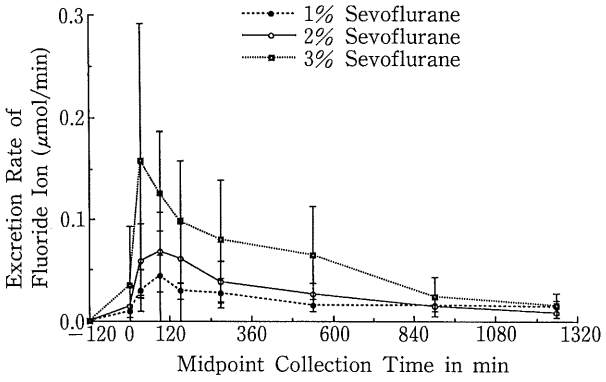

Fig. 4. Mean urinary excretion rates of fluoride ion following 2 hr sevoflurane anesthesia at different concentrations. The y represents the excretion rate of fluoride ion in μmol/min; x, the midpoint collection time of urine in min. The mean±SD are shown.

4) Serum and Urinary Osmolality, Urinary Volume and pH

Table 1 shows the mean and range of urinary volume, cumulative amount of fluoride ion excreted in urine, urinary pH and maximum concentration of serum fluoride ion. Table 2 shows the serum and urinary osmolality in the experimental groups. No significant changes were observed among the groups.

DISCUSSION

Blood-gas and oil-gas partition coefficients of sevoflurane are 0.6 and 42, respectively, which are the lowest among the inhalational anesthetics in use today[18]. The low blood-gas partition coefficient

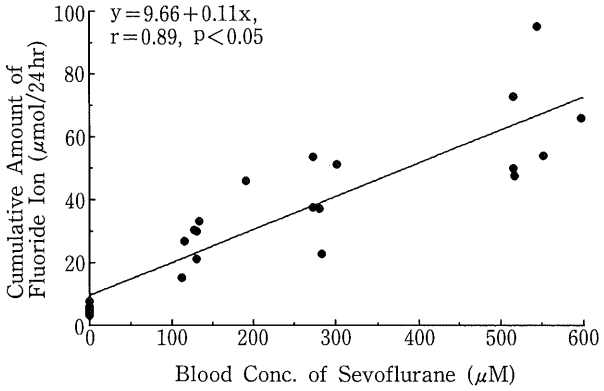

Fig. 5. Relationship between the measured cumulative amounts of fluoride ion excreted in the urine for 24 hr and the blood concentration of sevoflurane. The increase was dose-dependent. The regression line was $y=9.66+0.11x$, $r=0.89$, $p<0.05$, $n=24$, where y represents total amounts of urinary excreted fluoride ion for 24 hr in μmol; x, the blood concentration of sevoflurane in μM; and r, the correlation coefficient.

Table 1. Average and range of urinary and blood parameters

	Group-I (Control)	Group-II (1% Sevo)	Group-III (2% Sevo)	Group-IV (3% Sevo)
Maximum serum F^- concentration (μM)	0.7 ± 0.5	22.8 ± 8.7*a	31.8 ± 11.0*	41.5 ± 13.2*c
(Range)	(0.2 ~ 1.2)	(10.1 ~ 34.6)	(16.4 ~ 42.8)	(32.5 ~ 65.4)
Urinary volume (ml/24 hr)	348 ± 107	388 ± 67	437 ± 83	410 ± 97 NS
(Range)	(201 ~ 472)	(310 ~ 478)	(299 ~ 512)	(232 ~ 481)
Urinary pH: before anesth.	8.57 ± 0.20	8.77 ± 0.29	8.84 ± 0.26	8.86 ± 0.19 NS
(Range)	(8.29 ~ 9.02)	(8.22 ~ 8.99)	(8.34 ~ 9.06)	(8.68 ~ 9.20)
after anesth. (24 hr)	7.62 ± 0.34	7.84 ± 0.68	7.32 ± 1.27	7.35 ± 1.60 NS
(Range)	(7.05 ~ 8.18)	(6.79 ~ 8.69)	(5.80 ~ 9.00)	(4.95 ~ 8.22)
Cumulative amount of F^- in urine (μmol/24 hr)	5.0 ± 1.6	26.1 ± 6.7*a	41.4 ± 11.3*b	64.3 ± 18.0*c
(Range)	(3.4 ~ 7.7)	(15.2 ~ 33.1)	(22.9 ~ 53.7)	(47.7 ~ 95.1)

Each group, n=6 (mean±SD)
Sevo=Sevoflurane
NS: No significant difference.
* : Significantly different from the control, $p<0.05$.
*a, *b and *c: $p<0.05$, Group-II vs. Group-III, Group-III vs. Group-IV, and Group-II vs. Group-IV, respectively.

Table 2. Serum and urinary osmolality (mOsm/kg)

	Urinary Osmolality				Serum Osmolality			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
Pre:	1339 ± 274	1191 ± 315	1163 ± 330	1075 ± 250	291 ± 10	294 ± 16	283 ± 15	288 ± 12
Post (hr):								
0	1309 ± 398	1313 ± 374	1210 ± 246	1183 ± 271	287 ± 8	293 ± 14	286 ± 13	282 ± 9
1	1421 ± 352	1235 ± 438	1082 ± 253	1161 ± 185	286 ± 15	293 ± 13	281 ± 14	282 ± 9
2	1098 ± 272	688 ± 452	825 ± 264	721 ± 436	284 ± 14	292 ± 5	283 ± 15	285 ± 9
3	879 ± 320	515 ± 306	700 ± 318	608 ± 389	286 ± 17	297 ± 15	284 ± 11	287 ± 11
6	341 ± 185	382 ± 84	675 ± 398	378 ± 92	288 ± 8	291 ± 8	281 ± 11	284 ± 14
12	456 ± 307	541 ± 121	445 ± 73	470 ± 114	293 ± 16	289 ± 5	284 ± 13	284 ± 12
18	523 ± 282	533 ± 130	457 ± 113	526 ± 279	294 ± 17	285 ± 6	280 ± 16	283 ± 13
24	499 ± 187	474 ± 47	437 ± 81	650 ± 321	291 ± 6	295 ± 11	282 ± 13	280 ± 19

Mean ± SD,
Each group, n = 6
There is no significant difference.

and low fat solubility of sevoflurane permits very rapid clearance of sevoflurane from the body after termination of inhalation and thus allow very limited time to the drug to be metabolized.

Sevoflurane is metabolized to hexafluoroisopropanol and fluoride ion[9]. Martis et al showed that serum fluoride ion reached its peak values at 18.5 μM (n=2) and 20.0±4.8 μM (n=4) following 3% and 4% sevoflurane exposure for 2 to 3 hrs in dogs, respectively, and 2.9±0.5 μM and 2.5±0.6 μM (n=6) with 2% sevoflurane for 2 and 4 hrs in rats, respectively,[9]. In Fischer 344 rats, Cooke and co-workers observed a modest elevation of serum fluoride ion to 11 and 14 μM following 4 and 10 hrs of sevoflurane anesthesia[1]. In monkeys, serum fluoride ion concentration was recorded at 44±13 μM in venous blood samples obtained 10 min following 3 hr exposure of sevoflurane[17]. In all reported cases the serum fluoride ion returned to the pre-exposure level at the end of 24 hr.

In our study, the serum concentration of fluoride ion increased significantly after the onset of inhalation, and the mean peak serum levels were 22.8±8.7, 31.8±11.0 and 41.5±13.2 μM following administration of 1%, 2% and 3% sevoflurane, respectively, for 2 hr. The fluoride ion level returned to the pre-exposure level by 24 hr, as reported by previous researchers[1,9,17] but we observed that the fluoride ion in serum rises in a concentration-dependent manner. The discrepancy between our data and previously reported experiments may be due to species differences, analytical methods and control ventilation with intra-tracheal intubation.

Differences in metabolism of methoxyflurane by different strains of rats have been reported by Mazze et al[13]. A comparison of serum fluoride ion concentrations between the rat and the dog indicates that the amount of sevoflurane metabolized is lower in the rat than in the dog[9]. Holaday and Smith reported peak serum fluoride ion concentrations of 22.1±6.1 μM in humans anesthetized with 3% of sevoflurane for 1 hr[8]. In our study, in group IV we used 3% sevoflurane for 2 hr and the serum concentrations of fluoride ion reached 25.6±4.4 μM after 1 hr of anesthesia. These values are comparable to the serum concentrations of fluoride ion seen in human, as reported by Holaday[8].

The peak level of serum fluoride ion was consistently reached at time 0 or 15 min after the termination of inhalation. Holaday et al also reported similar data in clinical trials[6]. Tissue solubility of sevoflurane is very low, so it might be that sevoflurane was mostly metabolized during exposure[4].

The mean excretion half-time of the fluoride ion for the three groups was calculated to be 516 min (8.6 hr). This excretion half-time is shorter than the half-time of fluoride ion following isoflurane (36 hr), enflurane (37 hr), methoxyflurane (48 hr) and even sevoflurane (16 hr) reported in man[3,5,6]. The shorter half-times observed in our study may be due to a lower body fat content.

The blood sevoflurane concentration and the cumulative amounts of fluoride ion excreted in the urine increased dose-dependently. There is no report indicating that, as regards sevoflurane, neither peak serum levels nor the cumulative amounts of fluoride ion excreted in urine are dependent on the drug dose.

The nephrotoxicity of fluoride ion was clearly demonstrated in rats following intraperitoneal injection of sodium fluoride[10]. Mazze and his colleagues concluded that fluoride ion probably interfered with the renal sodium pump mechanism by acting on cellular energy-transfer system[10]. The toxicity of inhalational anesthetics depends largely on the extent of their biotransformation and the effect of metabolites in the body. It has been reported that subclinical toxicity occurs when the serum level of fluoride ion goes above 50 μM and clinical toxicity occurs when the level goes above

90 μM in man following methoxyflurane anesthesia[2]. The threshold level for fluoride ion nephrotoxicity following prolonged enflurane anesthesia in man was found to be 33.6 μM[12]. So it is obvious that the threshold level for the fluoride ion-induced nephrotoxicity is not the same for methoxyflurane and enflurane. The threshold level may be different between drugs and depends on its physico-chemical properties and/or the way of biodegradation of the parent drug. There are some other factors, like individual variations, sensitivity to the nephrotoxic effects of fluoride ion, presence of enzyme induction and concomitant use of other nephrotoxic drugs that probably increased nephrotoxicity[2]. Nephrotoxicity is actually related to the duration for which high levels of fluoride ion are maintained, as well as the absolute values (maximum serum fluoride ion concentration). In case of high doses of methoxyflurane, a high fluoride ion level (above 50 μM) was obsreved for a week or more, and in the case of prolonged enflurane anesthesia in man the level (above 20 μM) remained for approximately 18 hr[11,12]. Compared with the above two drugs, sevoflurane has a unique property in which the peak serum level does not persist for a long period.

It is reported that fluoride ion is the cause of the dose-related changes in urinary osmolality[14]. Mazze et al reported that the methoxyflurane nephrotoxicity in rats produce serum hyperosmolality, polyuria, a decrease in urinary osmolality and changes in the renal histology which is related to dose of fluoride ion[10,14,15]. On the basis of the changes in osmolality, the threshold level for nephrotoxicity of methoxyflurane and enflurane has been reported to be 50 μM and 33.6 μM[2,12]. In our study, the ranges of serum concentration were 10.1~34.6 μM, 16.4~42.8 μM and 32.5~65.4 μM, in gruops II, III and IV, respectively. However, we found no changes in the serum and urinary osmolality in any of the groups. We suggest, therefore, that the serum fluoride ion levels in our study were below the critical level. The reason may be posited that unlike methoxyflurane and enflurane the peaks do not persist for a long period of time. Moreover, the results of the 24 hr urinary volume did not show any sign of polyuria as there were no significant changes among the groups. We observed, however, that the urinary volume gradually increased with time in all the groups with the corresponding decrease in urinary osmolality which has not been seen in the case of blood osmolality. This may be due to the fluid replacement regime.

We conclude that the possibility of producing fluoride-induced nephrotoxicity after sevoflurane anesthesia is unlikely in clinical concentrations. The very short half-time for urinary excretion of the fluoride ion further ensures this possibility. However, we have to be very careful when sevoflurane is used at high concentrations for a prolong period of time because sevoflurane is metabolised to fluoride ion dose-dependently. Further investigation is required to elucidate the renal effect of fluoride ion after prolonged exposure to sevoflurane.

ACKNOWLEDGEMENT

This study was supported in part by a Research Grant from the Japanese Ministry of Education, Science and Culture.

(Received August 29, 1990)
(Accepted November 27, 1990)

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