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COMMUNICATIONS

Urinary excretion of hexafluoroisopropanol glucuronide and fluoride in patients after sevoflurane anaesthesia

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Abstract—The excretion of sevoflurane metabolites in the urine collected every 12 h after sevoflurane anaesthesia was measured by ion exchange chromatography. A metabolite, which was converted on incubation with glucuronidase to hexafluoroisopropanol was detected in the urine. The maximum excretion was found in the first 12 h after anaesthesia, none was found in the last collection 3 days after anaesthesia. The excretion half-life for the metabolite was calculated to be 55 h. A significant increase in the urinary excretion of organic and inorganic fluoride was also observed during the first 12 h after anaesthesia. The cumulative organic and inorganic fluoride excretion in the 3 days after sevoflurane anaesthesia was 1588 and 856 μmol, respectively (ratio = 1·85). The excreted half-lives for organic and inorganic fluoride were calculated to be 4028 and 2069 min, respectively. Our study showed that a hexafluoroisopropanol glucuronide is excreted in the urine, and the major part of urinary metabolites of sevoflurane, organic and inorganic fluoride, are excreted within 2 days of sevoflurane inhalation in man.

Sevoflurane (fluoromethyl-2,2,2-trifluoro-1-[trifluoromethyl]-ethylether) is a new volatile inhalational anaesthetic with some advantages such as a low blood/gas partition coefficient of 0·63. Holaday & Smith (1981) found a metabolite in the urine of patients following sevoflurane anaesthesia and suggested that it was likely to be a glucuronide of hexafluoroisopropanol. A glucuronide conjugate of hexafluoroisopropanol was indirectly identified as a sevoflurane metabolite after pretreating samples with β-glucuronidase (Maris et al 1981). In this study, the urinary excretion of the main metabolite, hexafluoroisopropanol glucuronide, organic fluoride and inorganic fluoride were measured by ion exchange chromatography in patients following sevoflurane anaesthesia.

Materials and methods

Patients. Six patients 30 ± 15 years old (weight 65 ± 9 kg, height 167 ± 9 cm), with no hepatic or renal abnormalities were studied. Patients were undergoing thyroid tumour resection, arthroplasty, tympanolplasty, lumbar disc resection, neoplasty of a tibial fracture or perineal flap surgery. All patients received 0·5 mg atropine sulphate intramuscularly, 45–65 min before anaesthesia. Fentanyl (1 mg kg^{-1}), pancuronium bromide (0·1 mg kg^{-1}) and flunitrazepam (0·02 mg kg^{-1}) were administered intravenously for anaesthetic induction. Anaesthesia was maintained for 1 h with 1·5% sevoflurane and 70% N_2O in O_2. After the termination of sevoflurane administration, anaesthesia was maintained by intravenous fentanyl and flunitrazepam, as required. Urine samples were collected during the 12 h period before anaesthesia and at 12 h intervals after anaesthesia for 3 days.

Apparatus. Hexafluoroisopropanol was measured using a gas chromatograph, Shimadzu GC-4B, equipped with a flame ionization detector and a glass column packed with dioctylphtharate 80–100 mesh.

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Urinary hexafluoroisopropanol glucuronide, and fluoride ion were measured using an Ion Chromatographic Analyzer IC-100 (Yokogawa Electric Co., Japan) equipped with a suppressor and an electro-conductive detector. An anion exchange resin (SAX-1) was packed into a 25 cm × 4·6 mm column as the separator.

Hydrogen carbonate buffer (4 mM $NaHCO_3$ and 4 mM Na_2CO_3) was used as eluent for hexafluoroisopropanol glucuronide. For the fluoride ion, 5 mM sodium tetraborate was used as an eluent at a flow rate of 2 mL min^{-1}. Dodecylbenzenesulphonic acid, 50 mM, was used at a flow rate of 2 mL min^{-1}, as a scavenger.

Detection of fluoride ions was performed using sodium fluoride. Since a standard form of hexafluoroisopropanol glucuronide was unavailable, its concentration was calculated using trifluoroacetic acid, which has a similar retention time, in the conditions used.

Sample preparation. A 2 mL sample and 5 mL of buffer (pH 5·0) were incubated with β-glucuronidase for 1 h at 37°C. Ether (2 mL) was added to the mixture, and 1 μL of the ether layer was applied to the gas chromatograph for assay of hexafluoroisopropanol.

Urine samples were diluted 100-fold with deionized water and ultrafiltered for 10 min at 2000 rev min^{-1} for deproteinization. A 100 μL sample of the ultrafiltrate was injected into the analyser through a cation exchange filter for the assay of hexafluoroisopropanol glucuronide and inorganic fluoride. For organic fluoride, a 100 μL sample of urine was prepared by the oxygen-flask combustion method, and a final 10 mL sample which included total inorganic fluoride was obtained. A 100 μL sample of the ultrafiltrate was injected into the analyser through a cation exchange filter to measure total fluoride. The difference between the total and the inorganic fluoride levels was defined as the organic fluoride level.

Trifluoroacetic acid was used to obtain the recovery rate by our oxygen-flask combustion method.

Calculations. The urinary excretion rates of hexafluoroisopropanol glucuronide, organic fluoride, and inorganic fluoride were calculated from the urine volumes and their urinary concentrations. These excretion rates were then plotted against the midpoint of the urine collection times on semilogarithmic paper. Elimination half-lives ($t_{\frac{1}{2}}$) and rate constants (k) were calculated from the slopes of the regression lines using the first three data points (until 30 h). The mean and standard deviation of the data were calculated. Student's *t*-test and one-factor analysis of variance were used to assess the significance of differences ($P < 0·05$). The values obtained before anaesthesia were subtracted in order to show the true excretion rate and amounts of organic fluoride and inorganic fluoride excreted following sevoflurane anaesthesia.

Results

Determination of hexafluoroisopropanol glucuronide. Fig. 1 shows the ion exchange chromatogram from the urine of a

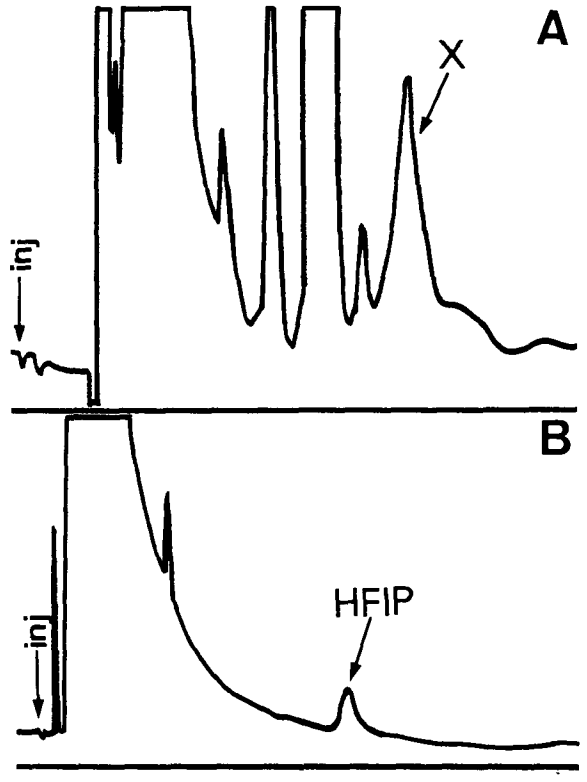

FIG. 1. Ion chromatogram of the urine of a patient after sevoflurane anaesthesia (A) and gas chromatogram of the ether extract from the incubation mixture (B): the substance X was detected after sevoflurane anaesthesia (A), but not before anaesthesia. The substance X disappeared on incubation with β-glucuronidase. After incubation, hexafluoroisopropanol (HFIP) was detected in the ether extract from the incubation mixture by gas chromatography (B).

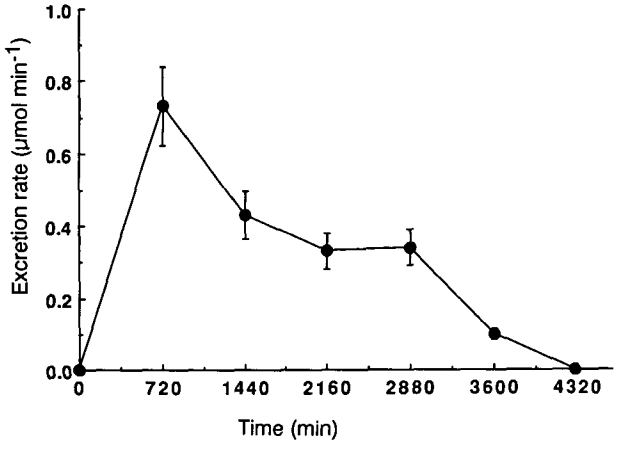

FIG. 2. The excretion rate of urinary hexafluoroisopropanol glucuronide at different time intervals after sevoflurane anaesthesia for 3 days. Each point represents the mean ± s.d. for six patients. Time interval 0 represents the 12 h before anaesthesia.

patient after sevoflurane anaesthesia. A peak was observed at 9·4 min. This peak disappeared on incubation with β-glucuronidase, and hexafluoroisopropanol was formed. Thus the substance detected by ion exchange chromatography was concluded to be hexafluoroisopropanol glucuronide.

Table 1. Elimination half-lives ($t_{\frac{1}{2}}$) and elimination rate constants (k) of urinary hexafluoroisopropanol glucuronide, organic fluoride and inorganic fluoride after inhalation of sevoflurane.

	Hexafluoroisopropanol glucuronide	Organic fluoride	Inorganic fluoride
$t_{\frac{1}{2}}$ (min)	3295 ± 572	4028 ± 1345	2069 ± 382
k	0·0001628	0·0001923	0·0002372

Values represent the mean ± s.d. for 6 patients. $t_{\frac{1}{2}}$ and k were calculated from the slope of the regression lines. $t_{\frac{1}{2}}$ is expressed by the equation: $t_{\frac{1}{2}} = 0{\cdot}639/k$.

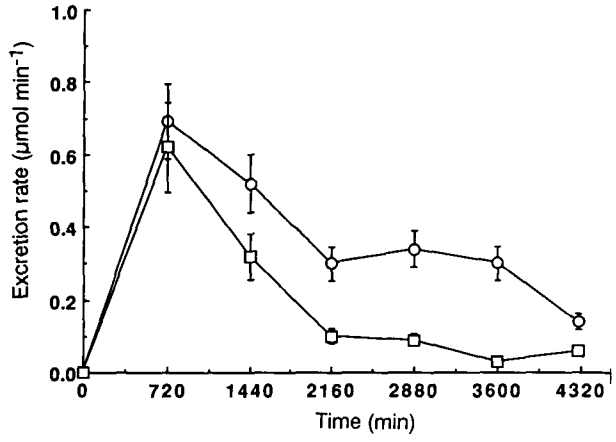

FIG. 3. Urinary excretion rate of organic fluoride (○) and inorganic fluoride (□) at different time intervals following sevoflurane anaesthesia. The values before anaesthesia were subtracted to show the excretion rate of organic fluoride and inorganic fluoride caused by sevoflurane. Each point represents the mean ± s.d. for six patients. Time interval 0 represents the 12 h before anaesthesia.

Urinary excretion of hexafluoroisopropanol glucuronide. Fig. 2 shows the urinary excretion rate of hexafluoroisopropanol after sevoflurane anaesthesia. During the 12 h before exposure to sevoflurane, no urinary hexafluoroisopropanol glucuronide was detectable in the urine. Its maximum excretion rate (0·73 ± 0·35 μmol min^{-1}) was found in the first 12 h after anaesthesia and it then decreased gradually. The total hexafluoroisopropanol glucuronide excretion in the 3 days after sevoflurane anaesthesia was 1305 μmol. The largest amount (512 ± 249 μmol) was found in the first 12 h, and none was detected in the last 12 h of this 3-day period. Elimination half-life ($t_{\frac{1}{2}}$) and rate constants (k) for the glucuronide were 3295 ± 572 min and 0·0001628, respectively (Table 1).

Urinary excretion of organic and inorganic fluoride. Fig. 3 shows the excretion rates of organic and inorganic fluoride after sevoflurane anaesthesia. A significant increase in the urinary excretion rate of organic and inorganic fluoride was observed during the first 12 h after anaesthesia. The cumulative amount of organic fluoride and inorganic fluoride excreted in the 3 days after sevoflurane anaesthesia was 1588 and 856 μmol, respectively, with the ratio being 1·85 (Table 2). Elimination half-life ($t_{\frac{1}{2}}$) and rate constants (k) for organic fluoride were 4028 ± 1345 min and 0·0001923, respectively. Elimination half-life ($t_{\frac{1}{2}}$) and rate constants (k) for inorganic fluoride were 2069 ± 382 min and 0·0002372, respectively (Table 1). Elimination half-life ($t_{\frac{1}{2}}$) and rate constant (k) for organic fluoride were similar for hexafluoroisopropanol glucuronide.

Table 2. Cumulative amount of urinary hexafluoroisopropanol glucuronide, organic fluoride and inorganic fluoride for 3 days after inhalation of sevoflurane.

Time interval (min)	Hexafluoroisopropanol glucuronide (μmol)	Organic fluoride (μmol)	Inorganic fluoride (μmol)
0	0	0	0
720	512±249	519±291	444±145
1440	297±171	352±321	228±132
2160	227±203	224±121	59±57
2880	208±57*	238±166*	65±42
3600	61±81	179±217	20±24
4320	0	76±90	40±27
Total amount	1305	1588	856

Values represent the mean±s.d. for 6 patients. Total amount indicates for the cumulative amount in 3 days for each patient. The ratio of organic fluoride/inorganic fluoride of the cumulative amount is 1·85. *$P<0.05$ compared with the inorganic fluoride group.

Discussion

Glucuronidation is known to be a major metabolic pathway for a variety of drugs and is responsible for conjugating potentially toxic lipophilic compounds with glucuronic acid, thus producing molecules with a greater hydrophilicity which are excreted more readily into the urine and bile. Such glucuronides are generally inactive and are secreted into the urine and bile by ion transport mechanisms. The excretion rates and amounts of glucuronides formed by drugs are thus useful data regarding their biotransformation. Glucuronides eliminated in the bile may be subsequently hydrolysed by intestinal or bacterial β-glucuronidase, and the liberated drugs may be reabsorbed. Such enterohepatic circulation may prolong the excretion of drugs from the body, so it is important to measure glucuronides in pharmacological studies.

Gas chromatography (GC) and GC-mass spectrometry have been used to measure glucuronides, but the isolation or preparation of samples is necessary (Holaday & Smith 1981). Ion chromatography has been mainly used to separate and detect ionized low molecular weight substances and has seldom been used for the analysis of organic compounds. In this study, we successfully detected hexafluoroisopropanol glucuronide in the urine of patients for 3 days after sevoflurane inhalation. The maximum excretion rate was observed in the first 12 h. The excretion of hexafluoroisopropanol glucuronide showed a similar trend to the excretion of organic fluoride. Hexafluoroisopropanol glucuronide may constitute a major part of the organic metabolites in human urine following sevoflurane anaesthesia. Our results also indicate that ion exchange chromatography offers the potential for the direct determination of glucuronide conjugates with little or no sample preparation. However, quantitative analysis is difficult, because a standard form of hexafluoroisopropanol glucuronide is not available.

The ratio of total amounts to organic and inorganic fluorides excreted in the urine in the 3 days after sevoflurane inhalation was 1·85. If all of the hexafluoroisopropanol glucuronide was excreted in the urine, the ratio of organic to inorganic fluorides would be about 12, because about 50% of the inorganic fluoride taken up by the body is stored in the bones, and each hexafluoroisopropanol glucuronide molecule has 6 fluoride molecules. Thus, our results suggest that the major part of hexafluoroisopropanol glucuronide was excreted via other routes.

The excretion half-lives of hexafluoroisopropanol glucuronide and organic fluoride were significantly longer than that of inorganic fluoride in our study. These prolonged half-lives might have been caused by the enterohepatic circulation of hexafluoroisopropanol glucuronide in bile excreted into the intestine. Hexafluoroisopropanol glucuronide is a unique metabolite of sevoflurane and little is known about its enterohepatic circulation or its toxicity after it is hydrolysed by intestinal or bacterial β-glucuronidase and reabsorbed. Crank et al (1970) reported that the LD50 in mice for the intraperitoneal and oral administration of hexafluoroisopropanol was 600 and 300 mg kg^{-1}, respectively. The toxicity of the hexafluoroisopropanol glucuronide following sevoflurane anaesthesia may be negligible, since the total cumulative amount measured in this study was very small compared with the LD50 of hexafluoroisopropanol.

We have clearly shown that the hexafluoroisopropanol glucuronide, a metabolite of sevoflurane, was excreted in human urine after sevoflurane anaesthesia and that ion exchange chromatography offers the potential for the direct determination of glucuronide conjugates with little or no sample preparation.

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