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A Simplified Gas Chromatographic Method for Quantifying the Sevoflurane Metabolite Hexafluoroisopropanol

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Background: **The results of sevoflurane biotransformation (fluoromethyl-1,1,1,3,3,3,-hexafluoro-2-propyl ether) to inorganic fluoride have been examined. However, these investigations have lacked a simplified assay for determining the primary organic metabolite, hexafluoroisopropanol. Previous attempts have involved extensive extraction steps, complicated derivatization techniques, or sophisticated detectors.**

Methods: **After enzymatic hydrolysis of conjugates, hexafluoroisopropanol is detected readily using a head space gas chromatographic analysis with a flame ionization detector.**

Results: **The gas chromatographic technique was linear from 10 to 800 μM with a correlation coefficient of 0.999. The detection limit was 10 μM in urine and 25 μM in blood.**

Conclusions: **This simplified approach does not require the extraction, derivatization, or mass spectrometric detectors of previous methods. As sevoflurane utilization and research increases, this assay should allow for a variety of laboratory and clinical disposition studies to be performed. (Key words: Anesthestic gases: sevoflurane. Biotransformation: metabolite. Hexafluoroisopropanol. Instrumentation: gas chromatography.)**

SEVOFLURANE (fluoromethyl-1,1,1,3,3,3,-hexafluoro-2-propyl ether), a halogenated inhalational anesthetic currently undergoing clinical evaluation, undergoes oxidative defluorination (fig. 1) to produce the organic metabolite hexafluoroisopropanol (HFIP).[1] HFIP can be toxic but only at very high concentrations.[2,3] HFIP primarily is excreted in the urine as a glucuronide conjugate, but its level of conjugation has not been examined in various patient groups, particularly those who may be deficient in conjugation enzymes.[4] Although there are a few sevoflurane disposition studies in which HFIP was detected,[1–5] additional studies have been hampered because of the difficulty in detecting HFIP. In fact, the prevailing method for the quantification of HFIP in the pharmaceutical industry has been limited to a gas chromatography-mass spectrometry technique.§,‖ The routine access and economics of maintaining gas chromatography-mass spectrometry has made such analysis prohibitive for most anesthesia research laboratories.

Assay techniques involving gas chromatography[1,4] have been accomplished, but these require extensive extraction steps using organic solvents that result in much longer analysis times or require complicated derivatization techniques. We have developed a simplified procedure using a gas chromatography head space technique with flame-ionization detection.

Methods and Materials

Sevoflurane was supplied by Maruishi Pharmaceutical Company (Osaka, Japan). HFIP (99%) was obtained from PCR (Gainesville, FL). Citric acid, sodium citrate, β-glucuronidase (Type H-1 from Helix pomatia), and dextrose were obtained from Sigma Chemical Company (St. Louis, MO). One-milliliter microreaction vials, Teflon-coated septum, and gas-tight syringes were purchased from Supelco (Bellefonte, PA). Glass screw-top test tubes capped with Teflon-lined caps were acquired from Baxter Scientific Products (Tempe, AZ). Micro-

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§ Narukami T, Imai M, Chow T, Tamura T, Satoh N: Blood concentrations of HFIP and HFA following a bolus dose of HFIP in the rats. Maruishi Pharmaceutical Co., Ltd. Central Research Laboratory, Osaka, Japan, 1990.

‖ Imai M, Tashima T, Tamura T, Satoh N: Phase I clinical study: Identification of urinary metabolite of sevoflurane in man by GC-MS. Maruishi Pharmaceutical Co., Ltd. Central Research Laboratory, Osaka, Japan, 1990.

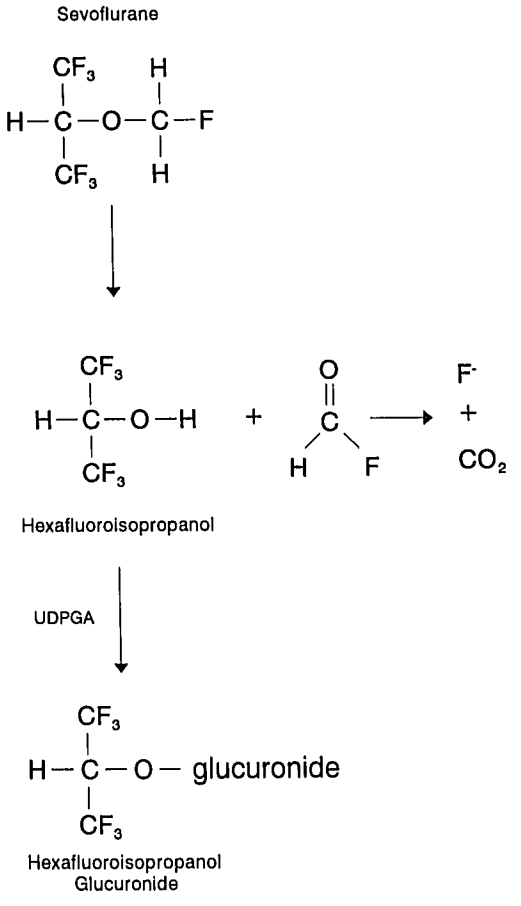

Fig. 1. Proposed biotransformation of sevoflurane. UDPGA = uridine diphosphate glucuronic acid.

centrifuge tubes (1 ml) were purchased from Tekmar Company (Cincinnati, OH).

Blood anticoagulated with heparin and urine samples were collected from patients anesthetized with sevoflurane. The protocol was approved by the Institutional Review Board Human Subjects Committee of the University of Arizona, and written informed consent was obtained from all patients. Two-milliliter venous blood samples were collected by way of a heparin lock catheter in the forearm and placed in 5-ml glass screw-top test tubes capped with Teflon-lined caps. One-milliliter human urine samples were collected *via* a Foley catheter and placed in 1-ml microcentrifuge tubes. Samples were frozen (−20° C) for 3–6 months, until analyzed. Initial samples were analyzed 6 months later to verify freezing had no affect on the concentration of HFIP.

Standards

A stock solution containing known amounts of HFIP was prepared in deionized water. Standards (10–800 μM) were prepared by adding aliquots (0.25 ml) of serial dilutions in microreaction vials containing 0.5 ml citric acid-dextrose solution. The citric acid-dextrose solution was comprised of 25 mM citric acid, 46 mM sodium citrate, and 82 mM dextrose. The concentration *versus* detector response was plotted to establish a standard curve. Sample concentrations were determined by linear regression.

Blank blood and urine samples were spiked with 100 μM of HFIP to ensure equivalent concentrations of HFIP were partitioned into the head space of the microreaction vial. This resulted in peak areas of 15,723 ± 429 (average ± SD, n = 10). These peak areas correlate to the 100 μM standard.

When samples exceeded calibration curve limits, a diluted duplicate sample was analyzed, and the resulting concentration was multiplied by the dilution factor.

Procedure

A 0.25-ml aliquot of standards or samples (blood or urine) were added to 1-ml reaction vials containing 0.5 ml of citric acid dextrose solution. The microreaction vials were capped with Teflon silicone septums and vortexed for 30 s. Because HFIP forms a glucuronide conjugate,[1] all samples and standards were treated with 1,000 U of β-glucuronidase (EC number 3.2.1.31) and placed in a 37° C water bath for 12 h. This amount of β-glucuronidase and length of incubation were found to be optimal for cleavage of the conjugate. A higher concentration of β-glucuronidase (10,000 U) required a shorter incubation period (8 h) with comparable results to the lesser concentration. However, the time saved during incubation is lost by the cost of β-glucuronidase.

The vials were placed in a block heater (Thermolyne Dri-bath, Dubuque, IA) at 60° C for 15 min. Just before analysis, the vial was removed from the heating block and vortexed for 10 s. A 0.5-ml aliquot of the head space vapor was slowly (15–20 s) drawn into a heated (60° C) gas-tight syringe. Care was taken to ensure no liquid was withdrawn. The vapor phase was compressed to 0.3 ml in the syringe before injection.

The sample was injected into a Hewlett Packard (Palo Alto, CA) 5890 series II gas chromatograph equipped with a flame ionization detector and a Hewlett Packard 3396 series II integrator. A 3 m × 2 mm-ID glass column packed with 10% Carbowax 20 M on 100/120

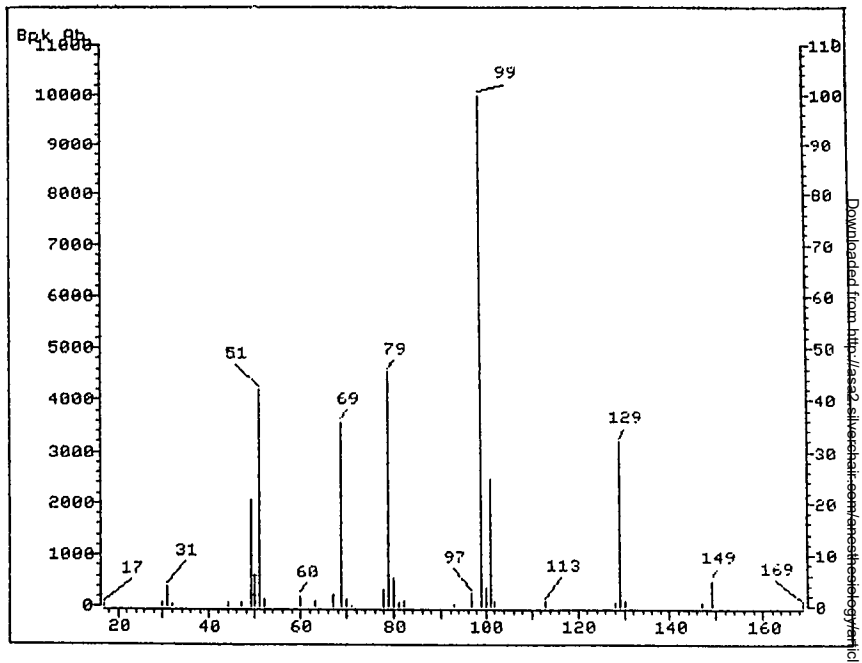

Fig. 2. Mass spectra of hexafluoroisopropanol. National Bureau of Standards Wiley database, New York, New York. Bpk Ab = base peak absorbance.

mesh Chromosorb (Supelco) was used for the separation. The injector, detector, and column temperatures were maintained at 150°, 190°, and 125° C, respectively. The helium carrier gas had a flow rate of 25 ml/min. The column head pressure was 28–30 psi. A temperature gradient was not required because all analytes eluted within 5 min. Because trace amounts of fluorocarbons are known to penetrate Teflon components of gas-tight syringes, the syringes were disassembled between experiments and placed in a lyophilizer to remove any contaminants.

Gas chromatography-mass spectrometry was used to confirm the detection of HFIP. Gas chromatography-mass spectrometry analysis was performed on a Hewlett Packard 5890 gas chromatograph with a Hewlett Packard 5970 mass spectrophotometer detector and Hewlett Packard RTE-6 data system. The above gas chromatography conditions were duplicated for the mass spectrophotometer detector. Mass spectrophotometer conditions were electron impact ionization, at 70 eV, with an ion source temperature of 200° C. The mass spectra resulted in a parent molecular ion of 99 m/z with ion fragments of 79 (48%), 51 (42%), 69 (35%), and 129 (35%) m/z. These are the expected parent ion and fragment ions for HFIP found in the National Bureau of Standards Wiley database (fig. 2).

Table 1. Hexafluoroisopropanol Calibration Curve for Gas Chromatographic Analysis: Peak Area

	Concentration (μM)						
	10	20	50	100	200	500	800
Average	1,369	2,505	7,078	15,848	30,914	67,615	105,205
SD	169	345	839	1,339	3,277	6,915	2,338
%CV	12.3	13.8	11.9	8.4	10.6	10.2	2.2

Hexafluoroisopropanol calibration curves (n = 12) in citric acid dextrose solution. r = 0.999; slope = 163; y-intercept = 1,442. Integrator attenuation was 0, peak width 0.04 min, and chart speed 1 cm/min.

Results

Typical calibration curves of HFIP, in buffer (table 1), show a linear relationship between the flame-ionization detector response to the concentration of HFIP (10–800 μM). The correlation coefficients routinely were greater than 0.999. Because the standard curve peak areas were not found to vary from day to day (table 1), internal standards were deemed unnecessary.

A typical chromatogram (fig. 3) of the head space injection technique from a urine sample collected 12 h after sevoflurane anesthesia illustrates the complete separation and baseline resolution of HFIP. A typical chromatogram of human venous blood collected at 4 h after anesthesia (fig. 4), contains greater noise and

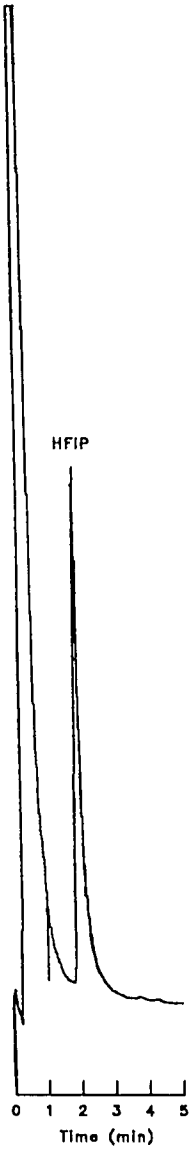

Fig. 3. Chromatogram from a human urine sample obtained 12 h after sevoflurane anesthesia. The patient was exposed to 1.69 MAC h sevoflurane anesthesia. Hexafluoroisopropanol (HFIP) represents 2,160 μM. Integrator attenuation was zero, peak width 0.04 min, and chart speed 1 cm/min.

more peaks when compared to the chromatograms obtained from urine. The higher background present in the analysis of blood samples decreases the detection limit of HFIP in the blood. The detection limits were 10 μM for urine and 25 μM for blood.

The gas chromatography head space technique can easily detect HFIP appearing in urine after sevoflurane anesthesia. Figure 5 shows results obtained from a patient who was exposed to sevoflurane for 1.12 MAC h. Peak HFIP production, 2,160 μM, occurred 4 h after anesthesia.

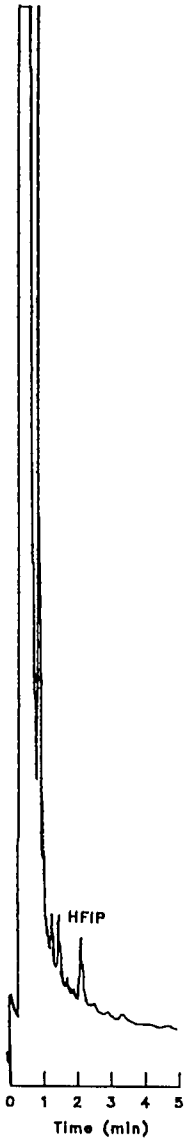

Fig. 4. Chromatogram from a human venous blood sample obtained 4 h after sevoflurane anesthesia. Patient was exposed to 1.69 MAC h sevoflurane anesthesia. Hexafluoroisopropanol (HFIP) represents 39 μM. Integrator attenuation was zero, peak width 0.04 min, and chart speed 1 cm/min.

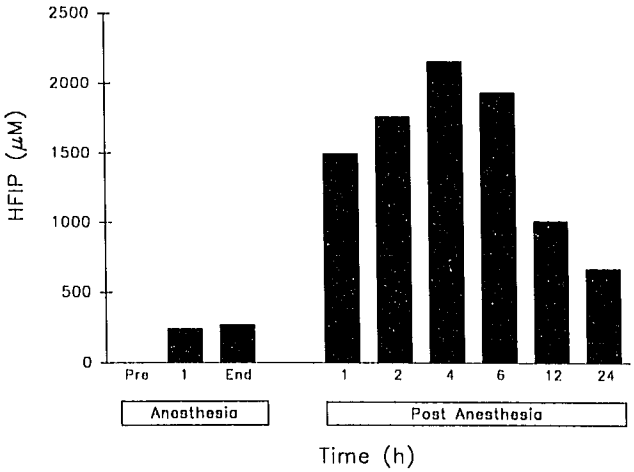

Fig. 5. Appearance of total hexafluoroisopropanol (HFIP) in urine of a patient receiving 1.12 MAC h sevoflurane anesthesia. Peak HFIP production, 2,160 μM, occurred 4 h after anesthesia. Values are an average of three determinations.

Discussion

The plasma and urine samples obtained from patients receiving sevoflurane anesthesia were analyzed for HFIP. Our results correlated with existing values using different instruments for detection.[1,4,5]

The current technique was the result of prolonged efforts to develop a simplified analytical technique for HFIP. Numerous column packings (SE-30, Porapak Q, Supelco, diisodecyl phthalate on chromosorb P), derivatization and/or extraction techniques (acid hydrolysis, ethyl acetate, n-heptane, ether) and other detectors (electron capture, thermal conductivity) were evaluated. However, two problems haunted these other techniques. Either the separation was nonexistent (or fragmented) or there was no linear relationship between detector response and HFIP concentration. The head space approach previously used to detect the primary metabolite of halothane, trifluoroacetic acid, proved to be the solution.[6] The major difficulty entailed in this method is that collection of the head space vapor into the gas-tight syringe requires a systematic routine to ensure reproducible and accurate gas chromatography results (table 1). However, the lack of a solvent peak allows for as many as ten analyses per hour.

The detection limits of the present procedure are not as sensitive as mass spectrometers, but the accessibility, ease of use, and lower costs make this technique more attractive.

As sevoflurane utilization and research increases, this assay should allow for a variety of laboratory and clinical disposition studies to be performed.

References

1. Holaday DA, Smith FR: Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers. ANESTHESIOLOGY 54:100–106, 1981

2. Ghantous HN, Fernando J, Gandolfi AJ, Brendel K: Sevoflurane is biotransformed by guinea pig liver slices but causes minimal cytotoxicity. Anesth Analg 75:436–440, 1992

3. Martis L, Lynch S, Napoli MD, Woods EF: Biotransformation of sevoflurane in dogs and rats. Anesth Analg 60:186–191, 1981

4. Jiaxiang N, Sato N, Fujii K, Yuge O: Urinary excretion of hexafluoroisopropanol glucuronide and fluoride in patients after sevoflurane anesthesia. J Pharm Pharmacol 45:67–69, 1993

5. Fujii K, Morio M, Kikuchi H, Nakatani K, Ikeda K: Ion chromatographical analysis of a glucuronide as a sevoflurane metabolite. Hiroshima J Anaesth 23:3–7, 1987

6. Maiorino RM, Gandolfi AJ, Sipes IG: Gas chromatographic method for the halothane metabolites, trifluoroacetic acid and bromide in biological fluids. J Analyt Toxicol 4:250–254, 1980