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CASE REPORT

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The Distribution of Sevoflurane in a Sevoflurane Induced Death

ABSTRACT: The distribution of sevoflurane (fluoromethyl 2,2,2,-trifluoro-1-(trifluoromethyl) ethyl ether) in blood, urine, liver, kidney, vitreous humor, and tracheal aspirate is presented from a subject with a sevoflurane induced death. Sevoflurane is a nonflammable general anesthetic administered by inhalation of vaporized liquid. Although general inhalation anesthetics have the potential to be fatal if not properly administered, the incidence of abuse is minute in comparison to other illicit drugs (1). Currently, there are no citations in the literature defining the body distribution of sevoflurane in a sevoflurane induced death. The decedent was found lying in a bed with an oxygen mask containing a gauze pad secured to his face. Three empty bottles and one partially full bottle of Ultane™ (sevoflurane) were found with the body in addition to two pill boxes containing a variety of prescription and non-prescription drugs. Serum, urine and gastric contents from the deceased were screened for numerous drugs and metabolites using a combination of thin layer chromatographic, colorimetric and immunoassay techniques. Analysis of biological specimens from the deceased revealed the presence of: amphetamine, caffeine, pseudoephedrine, nicotine, nicotine metabolite, and valproic acid. Sevoflurane concentrations were determined by headspace gas chromatography with flame ionization detection and revealed concentrations of 26.2 μg/mL in the blood, 105 μg/mL in the urine, 31.9 μg/mL in the tracheal aspirate, 86.7 μg/mL in the vitreous humor, 30.8 mg/kg in the liver, and 12.8 mg/kg in the kidney. The decedent had pathologies consistent with respiratory suppression including pulmonary atelectasis, pulmonary edema, and neck vein distention. The official cause of death was respiratory suppression by sevoflurane and the manner of death was unclear.

KEYWORDS: forensic science, ultane, partitions, anesthetic, postmortem

Sevoflurane (Ultane®) is a liquid anesthetic indicated for general anesthesia and is part of the "flurane" family of anesthetics that includes isoflurane, desflurane, and enflurane. It is administered by vaporization and typically given concomitantly with other anesthetics to achieve the desired level of anesthesia (2,3). The molecular targets of anesthetics are believed to be intracellular proteins, including the γ-aminobutyric acid A receptor, rather than the phospholipid bilayer (4). Volatile anesthetics are known to directly affect not only the central nervous system, but also the cardiovascular, pulmonary, and neuromuscular systems in a dose-dependant manner (5). Physical, chemical and pharmacodynamic data of sevoflurane are given in Table 1.

Sevoflurane is known to suppress electroencephalographic activity at a minimal alveolar concentration (MAC) of greater than 1.0 and is not associated with seizure activity up to a MAC of 2.5 (6–8). Unlike isoflurane, sevoflurane is not associated with tachycardia upon administration (9). A dose-dependant decrease in blood pressure is observed with sevoflurane induced anesthesia and is comparable to desflurane and isoflurane (10). Ventilatory depression is noted with sevoflurane administration which resulted in an increased ventilation rate, decreased tidal volume, elevation of $paCO_2$ and therefore a decreased blood pH (respiratory acidosis) (6,11). A post-junctional neuromuscular blockade enhancement is also observed when sevoflurane is used (12).

The frequency of inhalant abuse in terms of a general class of substances abused is relatively low when compared to substances that are administered by other routes (1). Inhalants most commonly abused are glue and paint (toluene containing substances), halogenated hydrocarbons, and nitrous oxide. General anesthetic abuse is extremely rare, possibly because of its limited accessibility and volatility. Consequently, the distribution of these compounds in biological fluids and tissues when death was a result of misuse is not well documented.

Case History

A 44-year-old white male was found dead, lying in a hotel room bed with an oxygen mask containing a gauze pad secured to his face. Medications found at the scene included: amphetamine sulfate (Adderal®), topiramate (Topamax®), atenolol (Tenormin®), loratadine/pseudoephedrine (Claritin-D®), zolpidem (Ambien®), divalproex sodium (Depakote-ER®), aspirin, olanzapine (Zyprexa®), celecoxib (Celebrex®), doxazosin mesylate (Cardura®), amantadine (Symmetrel®), levothyroxine (Unithroid®), and assorted

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TABLE 1—*Physical, chemical and pharmacodynamic data of sevoflurane**.

Specific gravity @ 20°C (g/mL)	1.52
Vapor pressure @ 20°C (mm Hg)	157
Boiling point at 760 mm Hg (°C)	58.6
Potency (MAC, % atm.)	1.7−2.05
Partition coefficients	
Oil : gas	47.2−53.4
Blood : gas	0.68
Tissue : blood†	
Brain	1.7
Heart	1.78
Liver	1.85
Kidney	1.15
Muscle	3.13
Fat	47.5

*From ref. 6.
†In vitro solubility analysis.

vitamins/nutritional supplements. One partially full 250 mL bottle and three empty 250 mL bottles of sevoflurane (Ultane®) were located on the nightstand. All of the medications were stored in two "weekly" pill boxes. We were unable to determine if the medications in the decedent's possession were prescribed to him, or if he had been following the prescribed dosage regimen. The victim was identified by a co-worker to be employed as an anesthetist, allowing him access to sevoflurane.

Materials and Methods

Specimen Collection

One tube of blood was collected from the left femoral vein and seven tubes of blood were collected from the right femoral vein by venipuncture and stored in sterile plain Vacutainer® tubes or Vacutainer® tubes containing potassium oxalate and sodium fluoride. Vitreous humor was collected by optic puncture and stored in a sterile plain 15 mL Vacutainer® tube. Urine was collected by bladder puncture and transferred directly to a plain Vacutainer® tube. Gastric contents were stored in a plastic container. Tracheal aspirate was collected by puncturing the trachea with a 20 gauge, 1.5 in. double ended hypodermic needle with hub and the gaseous contents were directly aspirated into a plain 15 mL Vacutainer™ tube. Blood, urine, vitreous humor, and tracheal aspirate specimens that were to be analyzed for sevoflurane were kept sealed and stored at 4°C until analysis. Liver and kidney sections were excised and stored at −80°C until analysis.

Analytical Methods

Biological specimens (serum, gastric contents, and urine) were screened for various acidic, basic and neutral drugs and metabolites including: narcotics, over-the-counter analgesics, barbiturates, benzodiazepines, cannabinoids, cocaine, phencyclidine, phenothiazines, sympathomimetic amines, and tricylic antidepressants by a combination of thin layer chromatographic, specific colorimetric, and enzyme immunoassay procedures.

The quantitation of sevoflurane in the biological specimens was performed by a modification of a previously published method for enflurane quantitation (13). A 76.0 mg/mL solution of sevoflurane was prepared by adding 0.5 mL of sevoflurane (Abbott, Abbott Park, IL) chilled to 4°C in an ice bath to a 10 mL volumetric flask. The flask was diluted to the mark with dimethyl sulfoxide (DMSO, Sigma Chemical, St. Louis, MO) that was chilled to approximately 20°C in a water bath. A 3.04 mg/mL solution of sevoflurane was prepared by adding a 2.0 mL aliquot of the 76.0 mg/mL solution in a 50 mL volumetric flask and diluting to the marked with chilled (20°C) DMSO. Immediately before analysis, 5.0 mL of the 3.04 mg/mL solution was added to a 50 mL volumetric flask and diluted to the mark with 18 MΩ water that was chilled in an ice bath to yield a 0.304 mg/mL working standard solution.

Thirteen standards ranging from 0 to 109 μg/mL sevoflurane were prepared by adding 0.00 to 0.9 mL of working standard solution to 7 mL blood collection tubes that were positioned in ice. To these tubes, 1.5 to 0.6 mL of 18 MΩ water and 1 mL of adult bovine serum (Sigma) were added so that the final volume was 2.5 mL. Before sealing the tubes, 20 μL of 1-propanol (Sigma) was added to all of the tubes as an internal standard. The tubes were sealed with a rubber septum, vortexed for 15 s, and allowed to equilibrate at 25°C for 1 h before analysis.

To urine, blood and vitreous humor specimens, two-10 μL aliquots of 1-propanol was added to the original Vacutainer® tubes by a 10 μL syringe (Hamilton Microliter™ #701, Reno, NV.). In order to quantitate the sevoflurane in the vial containing the tracheal aspirate with the prepared standards, we had to partition the analyte and internal standard between a liquid and gas phase. This was accomplished by adding 1.0 mL of adult bovine serum and 1.5 mL of 18 MΩ water with a 5 mL syringe (Becton-Dickinson, Franklin Lakes, NJ.). Two-10 μL aliquots of 1-propanol was also added to the container by a 10 μL syringe (Hamilton Microliter™). Tubes containing blood, urine, vitreous humor and tracheal aspirate were vortexed for 15 s and allowed to equilibrate to 25°C for 1 h before analysis. Frozen liver and kidney tissue was cut into approximately 0.5 square centimeter sections and a known weight of each tissue (∼1.0 g, and assuming 1 g/mL density) was placed into a 7 mL blood collection tube. To the tubes containing the tissue, 1.5 mL of 18 MΩ water was added along with 20 μL of 1-propanol. The tubes were sonicated (FS30, Fisher) for 1 h and allowed to equilibrate at 25°C overnight.

Standards and specimens were chromatographed on a Perkin-Elmer 8500 gas chromatograph (Perkin-Elmer, Wellesley, MA) with a 6′ × 2 mm glass column packed with 0.2% Carbowax 1500 on 80/100 Carbopac C (Supelco, St. Louis, MO). The injector temperature was set at 120°C, the column temperature was held constant at 100°C, the flame ionization detector was set at 200°C. The flow rate of the helium carrier gas was 20 mL/min. Recorder (Hewlett-Packard, Palo Alto, CA) parameters were as follows: zero = 10, chart speed = 0.5 in./min, attenuation = 6, peak width = 0.16, threshold = 2. A syringe (Gastight, Hamilton™) was used to inject a sample headspace volume of 0.25 mL. The retention time of sevoflurane was 2.92 minutes (a 1.87 relative retention time to 1-propanol).

The resulting chromatograms allowed for integration of peak areas and subsequent calculation of the peak area ratio of analyte/internal standard. The peak area ratios obtained from the spiked calibration standards were plotted against their respective concentrations. A linear regression was performed on the twelve points to obtain a standard curve with a correlation coefficient >0.980. Sevoflurane concentration for any given specimen was obtained by subtracting the intercept from the PAR and dividing by the slope of the standard curve and correcting for volume. In order to adjust the concentration for volume differences between the specimens and the standards and to avoid volatilization and loss of sevoflurane, the actual volume of specimen was measured in a 10 mL graduated cylinder (Fisherbrand™, Pittsburg, PA) after the analysis was performed. The volume of the specimen (in mL) was divided by 2.5 mL (the volume of the standards). The resulting quotient

was multiplied by the concentration determined from the standard curve.

The quantitation of amphetamine and methamphetamine in urine and blood specimens was performed by a modification of a previously published gas chromatographic/mass spectrometric method (14). Briefly, standards ranging from 0 to 1.00 μg/mL of amphetamine (Glaxo-SmithKline, Research Triangle Park, NC) and methamphetamine (Sigma) were prepared in both urine and serum matrices. Deuterated (d_5) amphetamine (Cerilliant, Round Rock, TX) was used as an internal standard. The forensic blood specimen and standards were extracted on a solid phase extraction cartridge (ZSDAU020, United Chemical Technologies, Bristol, PA) followed by derivatization with heptafluorobutyric anhydride (Sigma). The reconstituted specimens were analyzed on a Hewlett-Packard 5890 gas chromatograph with a 5972 mass selective ion detector (Hewlett Packard). Concentrations of the specimens were determined as a function of the peak area ratios of the following quantitation (qualifier) ions: amphetamine, 240 (91,118); methamphetamine, 254 (118,210); d_5-amphetamine 244 (91,123). Plasma valproic acid concentration was determined by fluorescence polarized immunoassay (FPIA, Abbott) on a AXSYM analyzer following standard assay protocol (15).

Results

Toxicological Findings

Analytical results are given in Tables 2 and 3. The serum drug screen was negative for ethanol and positive for nicotine and metabolite. The gastric drug screen revealed the presence of amphetamine, nicotine and metabolite. The urine drug screen was negative for ethanol and positive for amphetamine, caffeine, pseudoephedrine, nicotine and metabolite. Quantitative analysis of sevoflurane in various specimens revealed the following concentrations: blood, 26.2 μg/mL; urine 105.2 μg/mL; tracheal aspirate 31.9 μg/mL; liver, 30.8 mg/kg; kidney, 12.8 mg/kg; vitreous humor 86.7 μg/mL. The plasma contained 60.8 μg/mL of valproic acid. Blood and urine analysis for amphetamine and methamphetamine revealed amphetamine concentrations of 0.275 μg/mL and 7.02 μg/mL, respectively. The vitreous humor potassium concentration was 9.2 mmol/L.

TABLE 2—*Analytical results.*

Specimen	Result
Drug screen	
Serum	nicotine and metabolite
Urine	amphetamine, caffeine, pseudoephedrine, nicotine and metabolite
Gastric	nicotine and metabolite, amphetamine
Drug quantitation (in μg/mL)	
valproic acid	60.8 (femoral plasma)
amphetamine	0.275 (femoral blood) 7.02 (urine)

TABLE 3—*Distribution of Sevoflurane in biological specimens.*

Specimen	Sevoflurane Concentration (μg/mL)	Specimen/Blood Partition
Blood	26.2	1.00
Urine	105	4.01
Tracheal aspirate	31.9	1.22
Vitreous	86.7	3.31
Liver	30.8*	1.17
Kidney	12.8*	0.488

* In mg/kg.

Pathological Findings

The body was that of a well-developed, well-nourished, 95.5 kg adult male. He had several well healed scars over sporadic areas of the body, including arcuate longitudinal surgical scars on both elbows and two areas of healing abrasions on the left knee. The body presented a band of increasing cyanosis across the upper chest toward the head, and neck vein distension. There was trace pitting pretibial edema. There were areas of pulmonary atelectasis and edema. The right and left lung had masses of 830 and 655 g, respectively. Both lungs exuded a moderate amount of pink-tan froth on cut surfaces. Microscopic examination revealed a small focus of left lung hemorrhage, but lack of crystalline material with polarized light. There was moderate atherosclerotic change in the coronary arteries and mild thickening of the left and right ventricles. No anatomic origin for a seizure focus was found. Serology assays revealed the decedent's blood was non-reactive for HIV-1 or HIV-2 antibodies and negative for hepatitis B and hepatitis C.

Discussion

Respiratory suppression is encountered with most general anesthetics, and unless respiratory function is artificially maintained, death can result (3). The edema, atelectasis, and frothing of the lungs are consistent pathologies associated with a death caused by sevoflurane induced respiratory suppression. The amphetamine concentration in the blood did not appear to contribute to the individual's death. Although the therapeutic range of amphetamine is documented to be 0.030 to 0.110 μg/mL, toxicity is not known to occur until blood concentrations of 0.500 μg/mL are obtained (16,17). The valproic acid concentration in the victim's plasma was within a therapeutic range of 50–100 mg/L (16). Medical reasons for the use of these medications are unknown.

Comparison of sevoflurane liver and kidney/blood partitions between the in vitro analysis reported in the literature and this particular forensic case, revealed the forensic coefficients are markedly reduced relative to the in vitro coefficients (6). It would be expected that postmortem redistribution of sevoflurane occurs in a manner to increase the blood concentration and/or reduce the tissue concentration of the anesthetic after the victim ceases to inhale the sevoflurane as defined by Henry's Law. The ratio of the tracheal aspirate to blood was greater than 1.0 and is suggestive of active inhalation of sevoflurane at the time of death. Tight cellular junctions of the eye allow for an extended period of time in which the histological integrity of the sclera in postmortem specimens is maintained, this allows for the analysis of vitreous humor which would reflect higher blood concentrations of sevoflurane achieved nearer to the time of death and prior to postmortem redistribution or elimination. The trapping of anesthetic in the vitreous humor at a time of higher blood sevoflurane concentrations would explain the elevated sevoflurane vitreous humor to blood ratio (3.31) being greater than unity. Sevoflurane metabolites, inorganic fluoride and hexafluoroisopropanol, but not the parent compound have been quantitated in the urine in clinical cases of therapeutic sevoflurane use (18). Although the sevoflurane metabolites are more water soluble than the parent compound, only 5% of a given dose is metabolized (6). Our data show concentrations of sevoflurane in the urine to be four

times greater than that found in the blood. Given the lipophilicity of sevoflurane and a previously reported clinically effective peak blood concentration of 134 μg/mL, we calculate that the blood concentration in the victim was at least 105 μg/mL to produce the urine sevoflurane concentration measured in the deceased (16).

The clinical administration of sevoflurane is performed with artificial ventilation and sevoflurane is typically given concomitantly with other medications to achieve the desired plane of anesthesia. The purpose of using multiple anesthetics and other medications is to decrease the side effects of each anesthetic by reducing the quantity of each drug given and to obtain all of the desired components of anesthesia, i.e., muscle relaxation, loss of consciousness, decreased secretions etc. An acute overdose of sevoflurane alone will modify total peripheral resistance and therefore change the perfusion to various compartments within the body (19). One would also expect the integrity of the tissue/blood compartments within a postmortem individual to be compromised. Due to the pharmacodynamics and pharmacokinetics of sevoflurane, as well as the distribution/redistribution postmortem effects, the in vitro partition coefficients of sevoflurane may differ from the postmortem partition coefficients of sevoflurane in which the cause of death was an acute overdose.

Conclusion

The deceased exhibited pathologies of pulmonary distress and anoxia, consistent with a sevoflurane induced respiratory suppression and subsequent death. The postmortem distribution of sevoflurane and elucidation of tissue/blood partition coefficients in an individual with an acute sevoflurane overdose are novel findings. Postmortem interval, redistribution, and the inherent problems associated with a volatile substance present in biological matrices should be considered when interpreting the quantitated concentrations of sevoflurane.

References

1. http://www.samhsa.gov/oas/nhsda/2k1nhsda/vol1/CHAPTER2.HTM#fig2.1 06/2003.
2. Gilman AG, Hardman JG, Limbird LE, editors. The pharmacological basis of therapeutics, 10th ed. New York: McGraw-Hill, 2001.
3. Berry AJ, Knos GB, editors. Anesthesiology. Baltimore: Williams and Wilkins, 1995.
4. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994 Feb;376:607–14.
5. Frink EJ, Brown BR. Sevoflurane. Baillieres Clin Anaesth 1993 Dec; 7(4):899–913.
6. Patel SS, Goa KL. Sevoflurane, a review of its pharmacodynamic and pharmacokinetic properties and its clinical use in general anaesthesia. Drugs 1996;51:658–700. [PubMed]
7. Osawa M, Shingu K, Murakawa M, Adachi T, Kurata J, Seo N, et al. Effects of sevoflurane on central nervous system electrical activity on cats. Anesth Analg 1994 Jul;79(1):52–7. [PubMed]
8. Scheller MS, Nakakimura K, Fleischer JE, Zornow MH. Cerebral effects of sevoflurane in the dog: comparison with isoflurane and enflurane. Br J Anaesth 1990 Sep;65(3):388–92. [PubMed]
9. Kikura M, Ikeda K. Comparison of effects of sevoflurane/nitrous oxide and enflurane/nitrous oxide on myocardial contractility in humans: load independent and noninvasive assessment with transesophageal echocardiography. Anesthesiology 1993 Aug;79(2):235–43. [PubMed]
10. Malan TP Jr, DiNardo JA, Isner RJ, Frink EJ Jr, Goldberg M, Fenster PE, et al. Cardiovascular effects of sevoflurane compared with those of isoflurane in volunteers. Anesthesiology 1995 Nov;83(5):918–28. [PubMed]
11. Doi M, Ikeda K. Respiratory effects of sevoflurane. Anesth Analg 1987 Mar;66:241–4. [PubMed]
12. Gissen AJ, Karis JH, Nastuk WL. Effects of halothane on neuromuscular transmission. JAMA 1966 Sep;197(10):770–4. [PubMed]
13. Jacob B, Heller C, Daldrup T, et al. Fatal accident enflurane intoxication. J Forensic Sci 1989 Nov;34(6):1408–12. [PubMed]
14. Stout PR, Horn CK, Klette KL. Rapid simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine in urine by solid-phase extraction and GC-MS: a method optimized for high-volume laboratories. J Anal Toxicol 2002 Jul-Aug;26(5):253–61. [PubMed]
15. AxSYM valproic acid assay protocol #34-3028/R8. Abbott Park: Abbott Laboratories, 2003.
16. Baselt RC. Disposition of toxic drugs and chemicals in man, 6th ed., California: Chemical Toxicology Institute 2002;963–4.
17. Winek CL, Wahba WW, Winek CL Jr, Balzer TW. Drug and chemical blood-level data 2001. Forensic Sci Int 2001 Nov 1;122(2–3):107–23. [PubMed]
18. Buratti M, Valla C, Xaiz D, Brambilla G, Colombi A. Determination of hexafluoroisopropanol, a sevoflurane urinary metabolite, by 9-fluorenylmethyl chloroformate derivatization. J Chromatogr B Analyt Technol Biomed Life Sci 2002 Sep 5;776(2):237–43. [PubMed]
19. Kawana S, Wachi J, Nakayama M, Namiki A. Comparison of haemodynamic changes induced by sevoflurane and halothane in pediatric patients. Can J Anaesth 1995 Jul;42(7):603–7. [PubMed]

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