Can the Metabolism of an Inhaled Anesthetic Delay Recovery? A Comparison of Sevoflurane with a Sister Anesthetic, $CHF_2OCH(CF_3)_2$ in Rats

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Running head: A comparison of two anesthetics

Implications:

Our results suggest that metabolism of inhaled anesthetics might lead to the production of active metabolites that would delay recovery from anesthesia, but that this is not an issue for sevoflurane in rats.

Abstract

Background:

Among presently used inhaled anesthetics, desflurane and isoflurane remain structurally unaltered in their transit through the body while sevoflurane undergoes a small bioconversion to an anesthetic alcohol, hexafluoroisopropanol (HFIP). An experimental sister compound to sevoflurane, called Compound 15 might be less stable, permitting a greater conversion and a consequent delay in recovery from anesthesia. We tested the hypothesis that metabolism of Compound 15 or sevoflurane to HFIP can delay recovery from anesthesia.

Methods:

Using conventional methods, we compared sevoflurane versus Compound 15 in their solubilities, resistance to degradation by carbon dioxide absorbents, MAC values in rats, and times to recovery of righting versus walking on a rotating rod (Rota-Rod). Finally, we determined whether a preanesthetic experience with sevoflurane would decrease the MAC of desflurane determined subsequently.

Results:

We found that rats given sevoflurane took longer to right themselves than rats given Compound 15 but more rapidly recovered the ability to walk on the Rota-Rod. Pre-exposure to sevoflurane did not alter the MAC of desflurane determined subsequently. We also found that within an hour of halting administration of sevoflurane, the concentration of sevoflurane decreased to less than 2% of MAC. At 5 hours we found no presence of HFIP in rat blood or brain.

Conclusions:

We conclude that the formation of a metabolite by degradation of the inhaled anesthetic Compound 15 may delay awakening in rats, but that such a delay does not apply to sevoflurane.

Introduction

Recovery from anesthesia with sevoflurane takes longer than recovery from a less soluble anesthetic such as desflurane. Sometimes this difference persists depending on the endpoint examined. For example, Rortgen et al. found that digit span was impaired for at least 6-8 hours after sevoflurane but not desflurane anesthesia.[1] Sometimes patients given sevoflurane do not feel normal for 24 hours after sevoflurane anesthesia.[2] The conventional explanation for this difference is that the solubility of sevoflurane in blood and tissues exceeds that of desflurane. That is, desflurane is eliminated more rapidly. And context sensitive decrement times may play a role, too.[3]

Could there be an additional explanation? Could sevoflurane, but not desflurane, be degraded to an anesthetically active metabolite? Certainly we know that compounds other than inhaled anesthetics can degrade to active depressant compounds (midazolam, morphine).[4,5] Moreover, degradation-based delays in recovery from inhaled anesthetics have been predicted. For example halothane metabolism leads to the formation of the depressant ion, bromide.[6] Others have shown that sevoflurane is metabolized to hexafluoroisopropanol,[7-9] (HFIP) an anesthetically active compound with a MAC of 0.0044 percent of 1 atmosphere.[10] A sense of the smallness of this value is given by comparison with the MAC for sevoflurane in rats of 2.4% of 1 atmosphere (i.e., a value 550 times greater).[11] HFIP has a partition coefficient 267 times that of sevoflurane,[10] and thus, unlike sevoflurane, it will be eliminated slowly, potentially leading to a prolonged effect. However, HFIP is in large part conjugated with glucuronide, inactivating its anesthetic properties.[7,9]

Combine these thoughts with a third. Compound 15 [$CHF_2OCH(CF_3)_2$], so named because it was 15th in a series of study compounds (EIE, Personal communication), is structurally similar to sevoflurane [$CH_2FOCH(CF_3)_2$]. Speers et al. noted that this compound was a “good anesthetic” and provided details of

synthesis, but no more.[12] However, it is less soluble than sevoflurane and less stable (data to be shown). Because it is less soluble, it should be eliminated more rapidly and awakening should occur more rapidly – unless degradation produces a greater amount of an anesthetically active metabolite.

We hypothesized that the pre-exposure of rats to sevoflurane would decrease the MAC of another anesthetic, desflurane, due to the conversion of sevoflurane into HFIP. We hypothesized further that the initial awakening from Compound 15 anesthesia would be more rapid than from sevoflurane anesthesia, but that more subtle forms of awakening would be delayed with Compound 15 relative to sevoflurane. The basis for this reasoning is as follows. Consider that each anesthetic, Compound 15 and sevoflurane is really two anesthetics – the parent compound and its metabolite, HFIP. The parent compounds are eliminated during recovery at rates dictated by their solubilities (see below for solubility data). Thus initial recovery from Compound 15 will be faster than recovery from sevoflurane. As such recoveries are being accomplished, the residual HFIP will be sustained because it has a great solubility,[10] and thus it wil exert a progressively increasing role in hindering recovery. Since we hypothesize that Compound 15 is less stable, its administration will produce more HFIP and that HFIP will relatively delay later recovery.

Materials and Methods

With approval of the Committee on Animal Research of the University of California, San Francisco, we studied 77 Long-Evans or Sprague-Dawley specific-pathogen-free, male rats (Crl:CD(SD)BR) weighing 300-450 g obtained from Charles River Laboratories (Hollister, California). Each animal was caged with up to as many as 2 additional rats, and all had continuous access to standard rat chow and tap water before study.

Anesthetic concentrations were analyzed using gas chromatography as described previously.[13] The chromatograph was calibrated with either primary or secondary standards.

First Series of Experiments (Compound 15)

Compound 15 was synthesized by DL, using a previously described method of synthesis.[12] Purity exceeded 99%.

Partition coefficients of Compound 15 were determined for four specimens of human (EIE) blood, and four determinations each of saline and olive oil, all at 37°C using the headspace equilibration method (blood and saline); or using a technique involving transfer of a small aliquot of solvent (olive oil) containing a known partial pressure of anesthestic to a large flask of known volume, and, after tonometry, determinating the concentration of anesthetic in the gas phase. The methods and calculations have been described previously.[10,14,15]

The first obstacle to in vivo studies was the known vulnerability of Compound 15 to degradation by soda lime (Ross Terrell, personal communication). To circumvent this we used Amsorb®[16] to remove carbon dioxide. We demonstrated that 100 g of Amsorb® placed in a 600 mL flask at 40°C did not

degrade Compound 15 whereas 100 g of soda lime did (Fig. 1). Thus Amsorb® was used in all in vivo studies of Compound 15.

We determined MAC of compound 15 using conventional methods, including a tail clamp for stimulation.[10,14,15] Because little Compound 15 was available, all studies were conducted in a closed system. Once MAC was determined, we measured the effect of 30, 60, and 120 minutes of anesthesia at 1.25 MAC with Compound 15 on recovery of the righting reflex (rat placed supine after removal from the anesthetic chamber and time measured to the moment when the rat repeatedly turned prone) and the ability to walk on a rotating rod (Rota-Rod, rotating at 4 cycles per minute) for 1 minute without falling off. All rats had been trained to walk on the Rota-Rod (Stoelting Co. Wood Dale, IL) before anesthesia.

Second Series of Experiments (Desflurane with and without Pre-exposure to Sevoflurane)

Experiment 1. Twelve rats were anesthetized with desflurane in oxygen and MAC measured at target times of 1, 4, 5 and 8 hours (actual times always were greater than the target times) after induction of anesthesia, using tail clamp. We applied the tail clamp progressively more proximally with each set of determinations. Rectal temperatures were maintained between 35.5-38°C, using external warming. Infrared analysis was used to monitor anesthetic and carbon dioxide concentrations. Anesthetic concentration was held constant at approximately 60% of MAC for 30 min before stimulation for the first determination of responsiveness. All rats moved at this concentration. Successive concentration increases of approximately 15% of MAC were made and held for 20 min before the next application of the tail clamp. Gas chromatography was used to measure the concentration of anesthetic at the end of each equilibration. This process continued until the highest concentration permitting and the lowest concentration preventing movement were reached

(MAC was the midpoint between these concentrations). Rats were allowed to recover for a week, during which time they were weighed repeatedly.

Experiment 2. After a week of recovery, Experiment 1 was repeated with the following differences. Anesthesia was induced and maintained with sevoflurane for the first two MAC determinations. After the second MAC determination, desflurane was substituted for sevoflurane, and desflurane MAC was determined at “5” and “8” hours.

In addition, at 5 (3 rats), and 15, 30, 60, 120, 180, and 240 minutes (6 rats) after discontinuing administration of sevoflurane, gas samples were taken from each chamber and analyzed for sevoflurane by gas chromatography. Alas, for the two study groups of 6 rats (12 total study animals), 1 died after receiving sevoflurane. This rat developed what appeared to be obstructed ventilation. All data for this rat are excluded, leaving data for the remaining 11 rats. The sevoflurane concentration analyzed by gas chromatography was corrected to the concentration in the alveoli by making the assumption that the alveolar carbon dioxide equaled 40 mmHg and that the carbon dioxide value obtained by infrared analysis indicated the dilution of that partial pressure. For example, a chamber value of 8 mmHg indicated a 5-fold dilution, and thus the corrected concentration would equal the measured sevoflurane concentration times 5.

After obtaining the final MAC determination, anesthesia with desflurane was continued and the abdomen entered. Cannulation of the abdominal aorta allowed removal of 7 to 11 mL of arterial blood into heparinized 50 mL syringes capped with 3-way stopcocks. An equal volume of room air was added and the syringe equilibrated by rotating in an oven at 37°C for approximately an hour. The concentration of sevoflurane in the gas phase was analyzed using gas chromatography, the gas completely ejected, and fresh gas added to restore the gas volume. This new gas was equilibrated and analyzed as above. The

concentration measurements then were used to calculate the partial pressure originally in the blood (head-space method).[14]

Immediately after extracting the aortic blood, the brain was removed, weighed and placed in a 100 mL gas-tight syringe containing 6 mL of glass beads. The syringe was capped with a 3-way stopcock. The plunger of the syringe was inserted to the 40 mL mark. An empty 50 mL glass syringe was connected to the 3-way stopcock, after which the gas-tight syringe plunger was advanced to crush the brain into the glass beads, the extruded gas being captured by the 50 mL glass syringe. The extruded gas was re-introduced into the gas-tight syringe and additional gas introduced via the 3-way stopcock so that the plunger marker equaled 40 mL. Each such syringe was equilibrated in the oven at 37°C for approximately an hour. The gas concentration was analyzed for sevoflurane after this equilibration.

In addition, after calibration of the gas chromatograph with primary standards of HFIP, the analysis of the blood and brain for sevoflurane continued at the lowest attenuation (times 1). At this attenuation, we could measure 0.000074% HFIP (less than 2% of MAC of HFIP).

Results

Values for Compound 15 relative to sevoflurane are shown in Table 1. Compound 15 had slightly less than 3 times the vapor pressure and 3.5 times the MAC of sevoflurane. Solubility of Compound 15 was half to a third that of sevoflurane.

Rats given 1.25 MAC Compound 15 righted themselves more rapidly than those given 1.25 MAC sevoflurane (Fig. 2). However, times to walk on the Rota-Rod were longer after Compound 15 than after sevoflurane (significantly so after 2 hours of anesthesia with Compound 15; $P<0.01$ by a two-tailed t-test). Not shown on the graph was the finding that 2/4 rats given Compound 15 for 60 minutes could not walk on the Rota-Rod after 30 minutes of recovery, and after 120 minutes of anesthesia with Compound 15, none of the 7 rats given Compound 15 could walk on the Rota-Rod at 30 min of recovery. However, all could walk on the Rota-Rod the following day. And all 7 rats anesthetized with 1.25 MAC sevoflurane for 2 hours could walk on the Rota-Rod by 13 to 22 minutes after cessation of anesthetic administration.

After the first anesthesia with desflurane, all rats appeared to recover rapidly (righted themselves within 5 minutes). In the ensuing week, all rats gained weight and appeared healthy.

The second anesthetic, 5 hours of anesthesia with sevoflurane followed by 5 hours of anesthesia with desflurane, was uneventful except that one rat developed labored breathing and died soon after completion of anesthesia with sevoflurane. Elimination of sevoflurane after approximately 5 hours of anesthesia with sevoflurane, took a multiexponential course (Fig. 3). By approximately 2 hours of elimination, the residual alveolar concentration was estimated to be approximately 2% of MAC and by 4 hours it was less than 1% of MAC. Consistent with these results, the measurements of sevoflurane in blood

and brain suggested sevoflurane partial pressures equal to 1%-3% of MAC. Neither blood nor brain revealed any presence of HFIP.

Over the course of 10 hours, desflurane MAC remained essentially unchanged (Fig. 4). When these rats were given 5 hours of anesthesia with sevoflurane at approximately 1 MAC, the subsequent MAC values for desflurane did not differ from those produced in the absence of pre-equilibration with sevoflurane (Fig. 4).

Discussion

In sum, we found that Compound 15 was less potent and less soluble than sevoflurane, the latter suggesting that it should allow a more rapid recovery from anesthesia. Consistent with this prediction, recovery of righting occurred more rapidly with Compound 15. However, a residual effect of Compound 15 was apparent in a delayed recovery of the capacity to walk on the Rota-Rod. While all rats given sevoflurane for 2 hours could walk on the Rota-Rod by 30 minutes after cessation of sevoflurane administration, none of those rats given Compound 15 for 2 hours could do so.

How might these results [quicker initial recovery (righting) with Compound 15; but slower later recovery (Rota-Rod) with Compound 15] be explained? Consistent with our hypothesis, we would suggest that Compound 15 is less resistant than sevoflurane to degradation to HFIP. A lesser resistance to degradation by monovalent bases is clear in Fig. 1. We do not know if this, however, extends to metabolism by the liver, but if it did, it might explain the present findings. These results do not appear to be consequent to a toxic effect since all rats given Compound 15 could walk on the Rota-Rod the next day after anesthesia. Given the 3-fold smaller solubility of Compound 15 (Table 1), the results would be inconsistent with a slower elimination of Compound 15.

Could Compound 15 have a peculiar phenotypic response, perhaps a greater capacity to produce depression of motor coordination and function relative to its capacity to prevent movement in response to noxious stimulation (MAC)? For example, might it profoundly depress neuromuscular transmission? If this were a potent factor, though, it would seem inconsistent with survival – with continued diaphragmatic function – at MAC concentrations. Perhaps Compound 15 produces greater impairment of the vestibular apparatus – but rats given Compound 15 righted themselves more rapidly than rats given sevoflurane (Fig. 2).

Although our data are consistent with our hypothesis regarding Compound 15, our hypothesis concerning sevoflurane and a residual effect from its administration are not supported. If there is metabolism of sevoflurane to HFIP, such metabolism does not leave a measureable effect. It does not decrease the MAC of desflurane given immediately after a long (5 hour) anesthetic with sevoflurane (Fig. 4). There is not even a trend suggesting a possible effect. The results showing the rapid washout of sevoflurane are consistent with absence of an effect from residual sevoflurane – there isn't enough to meaningfully add to the anesthetic effect of desflurane (Fig. 3). The absence of HFIP in the blood or brain is again consistent with the absence of a finding of an effect on MAC (Fig. 4).

What we have found applies, of course, to rats. But we don't know about the application to humans. If humans produce more HFIP or are less able to conjugate what they produce with glucuronide, then the capacity of metabolism to delay recovery from sevoflurane remains a possibility. Our results with Compound 15 would be consistent with such a possibility. Kharasch's finding[7,9] that a small but measureable amount of free HFIP is present in the blood of humans given sevoflurane is consistent with such a possibility.

In summary, we present two studies. One with the experimental anesthetic, Compound 15, indirectly suggests that metabolism of an anesthetic can produce a metabolite that delays recovery from anesthesia. The second study suggests that such a delay is not a concern with sevoflurane, but this positive finding must be qualified to note that it may singularly apply to rats.

Table 1. Characteristics of Compound 15 versus Sevoflurane

Item	Compound 15	Sevoflurane[17]
Structure	CHF_2-O-$CH(CF_3)_2$	CH_2F-O-$CH(CF_3)_2$
GMW (g)	218	200
Density @ ~ 20°C (g/mL)	1.52	1.52
Vapor pressure @ ~ 20°C (mm Hg)	440	157
MAC in rats (% atm)*	8.34±0.85%* (N = 5)	2.4%
Blood/gas partition coef. (human)**	0.214±0.005	0.65
Saline/gas partition coef.	0.086±0.003 (N = 4)	0.34
Olive oil/gas partition coef.	25.7±0.8 (N = 4)	47

*Mean±SD. **At 37°C. Structure, density, and vapor pressure for Compound 15 comes from information supplied on data sheet, 4-26-66, supplied by Ross Terrell.

Figures

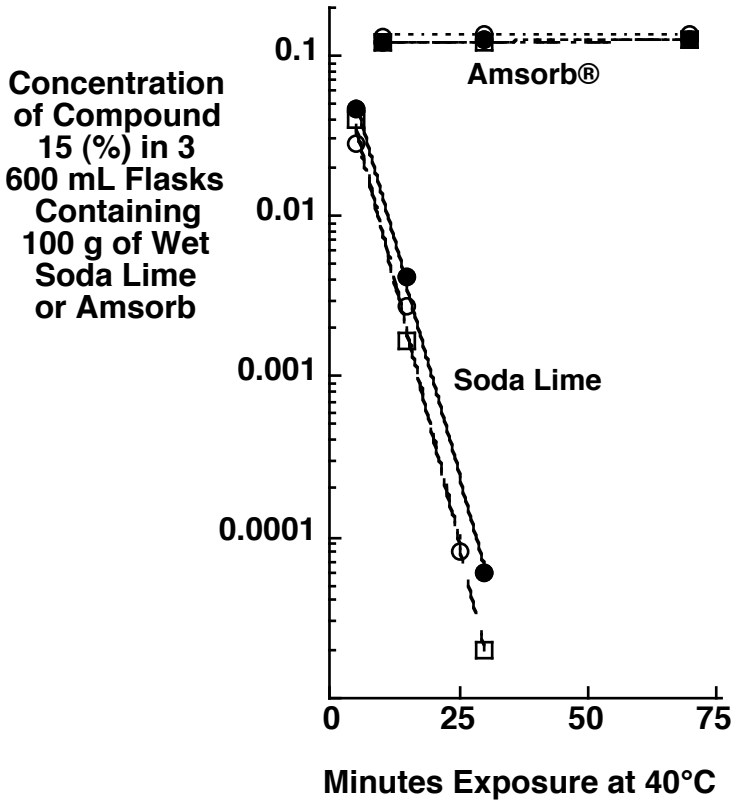

Fig. 1.

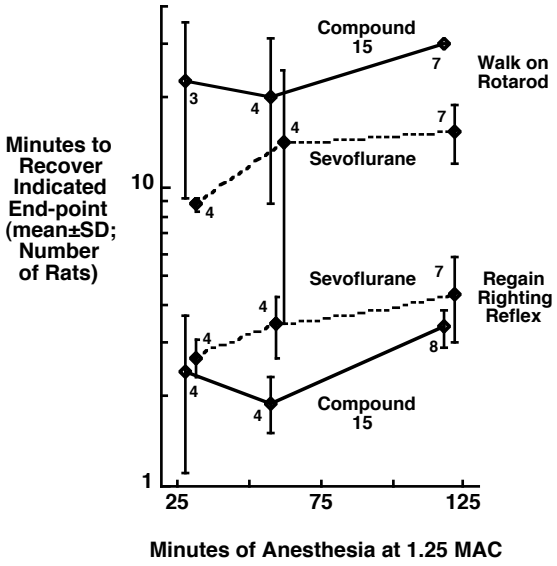
Minutes to Recover Indicated End-point (mean±SD; Number of Rats)
10
1
25
75
125
Minutes of Anesthesia at 1.25 MAC
Compound 15
Walk on Rotarod
Sevoflurane
Sevoflurane
Regain Righting Reflex
Compound 15
3
4
7
4
4
7
4
4
4
7
8
4

Fig. 2.

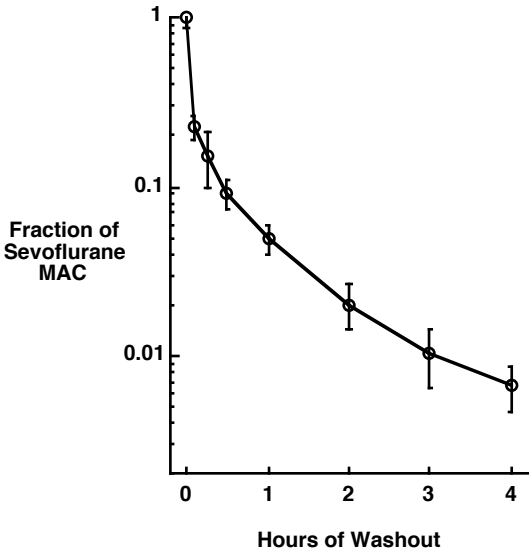

Fig. 3

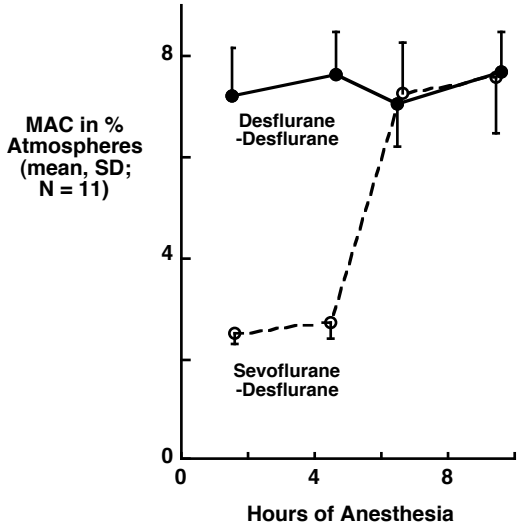
MAC in %
Atmospheres
(mean, SD;
N = 11)
8
4
0
0
4
8
Hours of Anesthesia
Desflurane
-Desflurane
Sevoflurane
-Desflurane

Fig. 4

Figure Legends

Figure 1.
Amsorb® and soda lime radically differ in their capacities to degrade Compound 15. The concentrations of Compound 15 in 3 flasks of 600 ml containing 100 g of soda lime at 40°C decreased by 3 orders of magnitude in 25 minutes（the three descending lines）. However, the concentrations of Compound 15 in another 3 flasks of 600 ml containing 100 g of Amsorb® at 40°C were stable (the three horizontal lines).

Figure 2.
Compound 15 and sevoflurane at 1.25 MAC differentially affected the recovery time for the righting reflex and walking on the Rota-Rod. Rats given Compound 15 (solid line) righted themselves more rapidly than those given sevoflurane (dash line; significant by t-test for 1 hour of anesthesia – $P<0.01$). However, the recovery time to walk on the Rota-Rod was longer after anesthesia with Compound 15 (solid line) than with sevoflurane (dash line; significant by t-test for 2 hours of anesthesia – $P<0.01$).

Figure 3.
The washout of sevoflurane after 5 hours of anesthesia with sevoflurane followed a multiexponential course. The residual alveolar concentration by 2 hours of washout is approximately 0.02 MAC, and it is less than 0.01 MAC by 4 hours of washout.

Figure 4.
Five hours of pre-exposure to 1 MAC sevoflurane (dash line) did not alter the MAC of desflurane in the subsequent 5 hours, the comparator being the MAC of desflurane determined repeatedly over the course of 10 hours (solid line).

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