Assessment of Low-flow Sevoflurane and Isoflurane Effects on Renal Function Using Sensitive Markers of Tubular Toxicity

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Background: Carbon dioxide absorbents degrade sevoflurane, particularly at low gas flow rates, to fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (compound A). Compound A causes renal proximal tubular injury in rats but has had no effect on blood urea nitrogen (BUN) or creatinine concentrations in patients. This investigation compared the effects of low-flow sevoflurane and isoflurane on renal tubular function in surgical patients using conventional (BUN and creatinine) and finer indices of renal injury, specifically those biomarkers sensitive for compound A toxicity in rats (glucosuria, proteinuria, and enzymuria [N-acetyl-β-D-glucosaminidase (NAG) and α-glutathione-S-transferase (αGST)]).

Methods: Consecutively patients with normal preoperative renal function at two institutions were randomized to receive sevoflurane (n = 36) or isoflurane (n = 37) in oxygen and air. Total gas flow was 1 L/min, opioid doses were minimized, and barium hydroxide lime was used to maximize anesthetic degradation. Inspiratory and expiratory compound A concentrations were quantified every 30–60 min. Blood and urine were obtained before and 24–72 h after anesthesia for laboratory evaluation.

Results: Sevoflurane and isoflurane groups were similar with respect to age, weight, sex, American Society of Anesthesiologists status, anesthetic duration (3.7 or 3.9 h), and anesthetic exposure (3.6 or 3.5 minimum alveolar concentration [MAC] hour). Maximum inspired compound A concentration (mean ± standard deviation) was 27 ± 13 ppm (range, 10–67 ppm). Areas under the inspired and expired compound A concentration versus time curves (AUC) were 79 ± 54-ppm-h (range, 10–223 ppm-h) and 53 ± 40 ppm-h (range, 6–159 ppm-h), respectively. There was no significant difference between anesthetic groups in postoperative serum creatinine or BUN, or urinary excretion of protein, glucose, NAG, proximal tubular αGST, or distal tubular πGST. There was no significant correlation between compound A exposure (AUC) and protein, glucose, NAG, αGST, or πGST excretion. Postoperative alanine and aspartate aminotransferase concentrations were not different between the anesthetic groups, and there were no significant correlations between compound A exposure and alanine or aspartate aminotransferase concentrations.

Conclusions: The renal tubular and hepatic effects of low-flow sevoflurane and isoflurane were similar as assessed using both conventional measures of hepatic and renal function and more sensitive biochemical markers of renal tubular cell necrosis. Moderate duration low-flow sevoflurane anesthesia, during which compound A formation occurs, appears to be as safe as low-flow isoflurane anesthesia. (Key words: Anesthesia, low-flow. Anesthetics, volatile: isoflurane; sevoflurane. Carbon dioxide, absorption: Baralyme. Complications, inhalation anesthesia. Degraders: Compound A; PIFE. Toxicity: Compound A; hepatic; renal.)

All currently used volatile anesthetics are degraded by the strong bases in carbon dioxide absorbents to by-products.
that are potentially toxic. Desflurane, enflurane, and isoflurane are degraded by desiccated absorbents to carbon monoxide, which can significantly elevate carboxyhemoglobin concentrations. Halothane and sevoflurane are degraded to difluorovinyl products, which are nephrotoxic in rats.\textsuperscript{1-4} Desflurane, enflurane, and isoflurane are not known to undergo degradation to nephrotoxic difluorovinyl products. The sevoflurane degradation product of greatest concern is fluoromethyl-2,2-difluoro-1-(trifluoromethyl) vinyl ether, which is also called compound A.\textsuperscript{5} In rats, compound A nephrotoxicity is characterized histologically as corticomedullary tubular necrosis localized to the proximal tubule. The biochemical manifestations of this proximal tubular lesion include elevations in serum BUN and creatinine, glucosuria and proteinuria, and increased urinary excretion of N-acetyl-\(\beta\)-D-glucosaminidase (NAG) and alpha glutathione-S-transferase (\(\alpha\)GST), site-specific tubular cell enzymes.\textsuperscript{3,4,6-8}

Compound A is detected in low concentrations (less than 10 ppm) in the inspiratory limb of anesthesia circuits during high flow (more than 2 l/min) anesthesia. Factors predisposing circuits toward greater compound A concentrations include higher sevoflurane concentrations, use of barium hydroxide lime compared with soda lime, higher carbon dioxide production, higher absorbent temperature, and lower fresh gas flow rates that result in higher absorbent temperatures.\textsuperscript{9} Most clinically relevant are low-flow and closed circuit anesthesia, during which higher compound A concentrations occur. The effects of low-flow (1 l/min) or closed-circuit sevoflurane anesthesia on renal function in humans have been examined.\textsuperscript{10-14} Maximum inspired compound A concentrations averaged 8-24 ppm and 20-32 ppm with soda lime and barium hydroxide lime, respectively, in these investigations, in which there were no significant differences in postoperative renal function. Nevertheless, renal function was only monitored by serum creatinine and BUN concentrations and serum osmolarity. We have shown previously, in rats, that high compound A concentrations caused necrosis, glucosuria, proteinuria, increased urinary \(\alpha\)GST excretion, and increased serum BUN and creatinine concentrations.\textsuperscript{7,8} In contrast, lower compound A concentrations, near the threshold for renal toxicity, caused comparatively less glucosuria, proteinuria, and increased urinary \(\alpha\)GST excretion. These occurred in the absence of changes in BUN and creatinine.\textsuperscript{7,8} Thus glucosuria, proteinuria, and increased urinary \(\alpha\)GST excretion may be more sensitive monitors of sevoflurane and compound A effects on renal function in humans than are serum BUN and creatinine. The purpose of this investigation was to use more sensitive markers of renal function to determine the effects of low-flow (\(\leq 1\) l/min) sevoflurane anesthesia on renal function in humans undergoing surgery of typical duration (2-4 h). The effects of sevoflurane were compared with those of low-flow isoflurane anesthesia.

**Materials and Methods**

**Patient Selection and Clinical Protocol**

Seventy-three American Society of Anesthesiologists physical status 1-3 patients undergoing anesthesia for elective surgery with planned duration exceeding 2 h were studied at two investigational sites (University of Washington, \(n = 42\); University of Arizona, \(n = 31\)). The investigational protocol was reviewed by the Food and Drug Administration and approved by the human subjects committees of both institutions, and all patients provided written informed consent. Eligible patients were aged at least 18 yr and had no history of hepatic disease or renal insufficiency. Patients with a previous known abnormality in serum creatinine (more than 1.5 mg/dl), aspartate aminotransferase (AST), or alanine aminotransferase (ALT) concentrations were not enrolled. Patients undergoing renal or ureteral surgery or procedures that compromise renal blood flow (cardiac and aortic surgery) were also excluded, as were those having undergone general anesthesia within 2 weeks or who were treated with any experimental drug within 28 days of surgery. Women of childbearing potential were anesthetized only after a urine pregnancy test was performed and found to be negative. Patients were randomized by blocks to receive sevoflurane or isoflurane. Separate randomization schemes were used at each institution.

The anesthetic protocol was designed to result in high compound A concentrations. Fresh barium hydroxide lime (Baradyme; Allied Healthcare Products, St. Louis, MO) was used for every case. After midazolam (1 mg given intravenously) premedication, anesthesia was induced with thiopental or propofol and fentanyl (50-100 \(\mu\)g). A neuromuscular blocking drug was administered to facilitate tracheal intubation. After tracheal intubation, anesthesia was maintained with sevoflurane (generally 0.8-2.5\% end-tidal) or isoflurane (0.5-1.4\% end-tidal) in oxygen (\(\leq 30\%)\) and air at a total flow rate of 5 l/min for 5 min. Nitrous oxide was not used in any patient. After 5 min, the fresh gas flow rate was reduced
to 1 l/min for the duration of the procedure. Hemodynamic stability was maintained by adjusting the inspired anesthetic concentration or with small doses of fentanyl. No neuroaxial opioids or local anesthetics were used during operation. End-tidal anesthetic concentrations were monitored continuously (Capnomac; Datex Medical Instrumentation, Tewksbury, MA) via a sampling port located at the Y piece of the anesthesia circuit and recorded at 15-min intervals while patients were intubated. In patients anesthetized with sevoflurane, respiratory gas samples were obtained from the inspiratory and expiratory limbs of the anesthesia circuit using gas-tight syringes adjacent to the inspiratory and expiratory valves, immediately after intubation before the introduction of sevoflurane, at 0.5-h intervals for the first 2 h of low-flow anesthesia, hourly thereafter, and at the end of low-flow anesthesia.

Venous and urine samples were obtained the morning of surgery, and at 24 h (range, 20–28 h) and 72 h (range, 68–76 h) after the end of surgery, for routine laboratory analysis. Blood for serum fluoride determination was obtained before and 0, 2, 24, and 72 h after the end of anesthesia. Urine was collected in 24-h intervals for 72 h after anesthesia (0–24 and 48–72 h) and also at 24–48 h at one investigational site. The volume was measured and an aliquot was frozen for analysis of glucose, protein, creatinine, and NAG. Another aliquot was added to stabilization buffer (Biotrin International, Dublin, Ireland) and frozen for later analysis of GST.

Chemical Analysis

Compound A concentrations were determined by gas chromatography with flame ionization detection using the same validated assay at each institution, as described previously. Briefly, a 1.5-ml gas sample at ambient pressure and 0.5 ml of internal standard (approximately 1.6 ppm n-butylchloride) were mixed in a gas-tight syringe. The mixed gases were injected into the gas chromatography unit, which consisted of a Hewlett-Packard 5890 Series II (Hewlett-Packard, Palo Alto, CA) with packed column injector, flame ionization detector, and a glass 6 foot x 2 mm inner diameter packed column with a liquid phase of 1% AT-1000 on 60/80 mesh Graphpac GB (Alltech Associates, Deerfield, IL). The head pressure of the helium carrier gas was 35.5 psi. The injector, oven, and detector were held at 145°C, 140°C, and 225°C, respectively. Compound A and n-butylchloride eluted at 4.5 and 9.6 min, respectively. Compound A concentrations were determined using standard curves of peak area ratios of compound A to n-butylchloride versus the amount of compound A added. Strict validation criteria were used to ensure comparable results from both investigational sites. Each site obtained similar analyte retention times, peak widths, area response ratios, and area reproducibilities (< 3% coefficient of variation). For each patient sample, the absolute area of the internal standard was required to be within 10% of those measured for the calibration standards.

Serum and urine samples from both investigational sites were combined for analysis. Serum fluoride concentrations were determined by ion-selective electrode. Glucose, protein, and creatinine concentrations in 0- to 24-h and 48- to 72-h urine samples were determined by a commercial laboratory using an autoanalyzer (hexokinase, benzethonium chloride, and picric acid methods, respectively). Concentrations of NAG and GST were determined (University of Washington) in all 24-h urine specimens. Urine NAG activity (units/milliliter) was determined colorimetrically using a commercially available method (Boehringer-Mannheim, Mannheim, Germany). Urine α-GST and π-GST concentrations were quantified by enzyme immunoassay with antibodies against the human antigens using a commercially available method (Biotrin International). The interday coefficients of variation were 7% for α-GST at 5 ng/ml and π-GST at 30 ng/ml. All laboratory analyses were conducted by persons who were not investigators and who were naive to the purpose of the investigation and blinded to sample identity. Urine analyte concentrations were multiplied by the 24-h urine volume to obtain 24-h excretion. Twenty-four-hour NAG and GST elimination values are expressed as amounts per milligram of urine creatinine. Creatinine clearance was calculated from 0- to 24-h and 48- to 72-h urine creatinine excretion and the 24-h and 72-h postoperative serum creatinine concentrations.

Data Analysis

Anesthetic exposure was calculated as the product of end-tidal concentration and time, determined in 15-min intervals. Total exposure is expressed as minimum alveolar concentration (MAC)-hours (uncorrected for age; sevoflurane MAC = 2.05%, isoflurane MAC = 1.15%). Compound A exposure was similarly calculated as the product of inspiratory or expiratory concentration and time, determined in 15-min intervals.

Patients' demographic data were analyzed using Student's unpaired t test. Correlations with Compound A
exposure and serum fluoride concentration were evaluated by linear regression analysis with influential data-point analysis. Serum chemistries and urine glucose, protein, and NAG and GST excretion in the two groups were compared by two-way repeated measures analysis of variance. Serum ALT and AST data were also examined by chi-square analysis before and after exclusion of patients with abnormal preoperative values. Statistical significance was assigned at P < 0.05. Results are expressed as means ± standard deviation.

**Results**

Table 1 shows patient demographic data. Patients anesthetized with sevoflurane and isoflurane were similar with respect to age, weight, sex, ASA physical status, case mix, duration of anesthesia, anesthetic exposure (MAC-hour), and fentanyl dose (1 ± 1 μg·kg⁻¹·h⁻¹). Low-flow conditions were maintained throughout the duration of anesthesia in both groups.

**Compound A Concentrations**

Figure 1A shows inspiratory compound A concentrations for all patients anesthetized with sevoflurane. The highest measured inspiratory compound A concentration was 67 ppm. Average inspired and expired compound A concentrations are shown in Figure 1B. The mean inspired compound A concentrations were 15-30 ppm throughout the period of anesthetic exposure, and the mean maximum was 27 ± 13 ppm. Compound A concentrations remained relatively constant in some patients and increased in others. The three patients with the highest and steadily increasing compound A concentrations (fig. 1A) received substantially higher sevoflurane concentrations (3%, 3.1%, and 2.6%) compared with all other sevoflurane patients (mean, 1.9%). Total compound A exposure was calculated from the areas under the curve (AUC) of compound A concentration versus time (table 1). The mean inspiratory compound A exposure was 79 ± 54 ppm-h, and the mean expiratory compound A AUC was 53 ± 40 ppm-h. A better approximation of compound A deposition during anesthesia was obtained from the AUC of inspired minus expired compound A concentrations (27 ± 16 ppm-h).

Factors influencing compound A production and exposure were determined (fig. 2A, B). The best predictor of compound A exposure, measured as inspiratory compound A AUC, was total sevoflurane exposure (fig. 2A). This also was the best predictor of compound A deposition during anesthesia (fig. 2D). There were excellent correlations between inspired compound A AUC or in-
was observed ($r = 0.67$), which was highly leveraged by the three patients with the highest sevoflurane concentrations and not significant in their absence. Compound A exposure, deposition, and maximum inspired concentration were also significantly correlated with patient weight (figs. 2C, 2F, and 2I), although there was considerable scatter of the data and the correlation coefficients were substantially lower ($r = 0.45 - 0.60$). The slopes of the regression lines predicted an increase of $23 \pm 6$ ppm-h in compound A exposure (AUCinsp), $5 \pm 2$ ppm-h in compound A retention (AUCexp-insp), and $5 \pm 1$ ppm-h in maximum inspired compound A concentration for every 10-kg increase in body weight. Weight accounted for only $20 - 36\%$ of the variability in compound A exposure and concentration. Thus sevoflurane concentration was the best predictor of maximum inspired compound A concentration, and sevoflurane exposure (MAC-hour) was the best predictor of compound A exposure and deposition.

**Plasma Fluoride Concentrations**

Sevoflurane, isoflurane, and compound A are metabolized with the release of inorganic fluoride. As expected, serum fluoride concentrations were substantially higher after sevoflurane compared with isoflurane anesthesia (fig. 3). The maximum fluoride concentration in patients receiving sevoflurane was $44 \pm 17 \mu M$ (range, 14 - 92 µM) and exceeded 50 µM in ten patients (average, $67 \pm 12 \mu M$).

**Renal and Hepatic Function**

Renal effects of low-flow anesthesia were measured by serum BUN and creatinine concentrations (fig. 4A and B). Blood urea nitrogen and creatinine concentrations decreased after operation in both sevoflurane and isoflurane patients, there was no significant difference between groups at any time after anesthesia, and no patient had a serum creatinine concentration exceeding the upper limit of the reference range (1.4 mg/dl). A more sensitive analysis defines a maximal permissible increase in postoperative creatinine concentration. The numbers of patients receiving sevoflurane or isoflurane who had serum creatinine values $\geq 0.2$ mg/dl greater than the preanesthesia value were 3 versus 4 at 24 h and 3 versus 1 at 72 h. The differences between anesthetic groups were not significant. Creatinine clearance values in patients receiving sevoflurane and isoflurane were $114 \pm 44$ and $120 \pm 36$ ml/min at 24 h, and $100 \pm 48$ and $110 \pm 61$ ml/min at 72 h (not significantly different between groups).

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"Inspired sevoflurane concentrations were not routinely recorded."
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Renal effects of low-flow anesthesia were also measured by urinary excretion of protein and glucose, which are more sensitive than BUN and creatinine in detecting compound A nephrotoxicity in rats.2 Twenty-four-hour protein excretion was not different in patients anesthetized with low-flow sevoflurane or...
isoflurane when measured 0–24 or 48–72 h after operation (fig. 5A). Although mean values were not different between groups, a more sensitive analysis examined the relation between protein excretion and compound A exposure in patients receiving sevoflurane to localize potential outliers exhibiting toxicity. There was no significant relation between either 0- to 24 h or 48- to 72-h protein excretion and compound A exposure (measured as the area under the inspired compound A concentration versus time curve; figs. 6A and B). Patients with the highest compound A exposures did not have the highest protein excretion. Twenty-four-hour glucose excretion was similar in patients anesthetized with sevoflurane and isoflurane 0–24 and 48–72 h after anesthesia (fig. 5B). Further, there was no significant relation between either 0- to 24 h or 48- to 72 h glucose excretion and inspired compound A AUC (figs. 6C and D). There was also no correlation, with either sevoflurane or isoflurane, between 24-h protein and glucose excretion values (data not shown). There was also no correlation, in patients receiving sevoflurane, between 24-h protein and glucose excretion and peak serum fluoride concentration (data not shown).

Frequently NAG is used as a marker of proximal tubular cell necrosis, and urine NAG excretion was increased after compound A nephrotoxicity in rats. In this investigation, 24-h NAG excretion on postanesthesia days 1, 2, and 3 was not significantly different between patients receiving sevoflurane or isoflurane (fig. 7). In the sevoflurane group, daily NAG excretion was compared with the compound A exposure and with serum fluoride concentrations. There was no correlation between urinary NAG excretion and inspired compound A AUC either 0 to 24 or 48 to 72 h after operation (figs. 8A and B) or 24 to 48 h after operation (data not shown). There was no correlation between NAG excretion and maximum serum fluoride concentration after 0 to 24 or 48 to 72 h (figs. 8C and D).

Urinary αGST and πGST excretion were also used to evaluate the renal tubular effects of low-flow anesthesia because αGST and πGST are localized exclusively in

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Fig. 3. Serum fluoride concentrations after sevoflurane (open bars) or isoflurane (solid bars) anesthesia. The number of patients with fluoride concentrations exceeding 50 μM is shown within the bar.

Fig. 4. Serum blood urea nitrogen (A) and creatinine (B) concentrations before and after low-flow anesthesia. Heavy lines depict mean values. There were no significant differences between anesthetic groups. Blood urea nitrogen concentrations at 24 and 72 h were significantly lower (P < 0.05) than preanesthetic values. No patient exceeded the upper limit of the creatinine reference range (1.4 mg/dl).
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Fig. 5. Excretion of protein (A) and glucose (B) in urine collected 0–24 h and 48–72 h after anesthesia. Individual and mean values (± SD) are shown. The dotted line represents the upper limit of the reference range. There were no significant differences between the sevoflurane and isoflurane groups.

Proximal and distal tubules, respectively, in human kidneys, and αGST is the most sensitive marker of compound A nephrotoxicity in rats. Neither daily excretion of αGST (fig. 9A) nor πGST (fig. 9B) were significantly different in the patients anesthetized with sevoflurane or isoflurane either 0–24 h, 24–48 h, or 48–72 h after operation. In the sevoflurane patients, αGST and πGST excretion were compared with compound A exposure, measured as the inspired compound A AUC. There was no significant correlation between urinary excretion of either αGST (figs. 10A and B) or πGST (figs. 10C and D) and inspired compound A AUC, measured either 0–24 or 48–72 h after operation. There was also no significant correlation 24–48 h after operation (data not shown). There was no correlation, in patients receiving sevoflurane, between αGST or πGST excretion and peak serum fluoride concentration on any postoperative day (data not shown).

Hepatic effects of anesthesia were measured by serum AST, ALT, lactate dehydrogenase, and alkaline phosphatase concentrations. Abnormal postoperative AST or ALT values were observed in patients receiving sevoflurane or isoflurane, which is consistent with previous observations, but there was no significant difference between groups (by analysis of variance or chi-square analysis) at either 24 or 72 h after anesthesia (figs. 11A and B). There was no significant correlation between compound A exposure and either serum AST or ALT concentrations 24 or 72 h after anesthesia nor between compound A exposure and the difference between postanesthesia and preanesthesia values for either AST or ALT (results not shown). No significant differences were observed between anesthetic groups for either lactate dehydrogenase or alkaline phosphatase concentrations at 24 or 72 h after anesthesia (results not shown).

DISCUSSION

Results of this investigation show that the renal tubular effects of low-flow sevoflurane anesthesia, with compound A formation, were not significantly different from those of low-flow isoflurane. Renal effects were similar, as assessed using serum creatinine and BUN concentrations and conventional (urine protein and glucose excretion) and experimental (NAG and GST excretion) biomarkers of proximal tubular reabsorptive function and tubular cell necrosis. Similarly, the hepatic effects of low-flow sevoflurane and isoflurane anesthesia were not significantly different. Low-flow sevoflurane anesthesia in patients having surgery, with inspired compound A concentrations ≤ 67 ppm and compound A exposures ≤ 225 ppm-h, had no demonstrable adverse renal or hepatic effects compared with low-flow isoflurane anesthesia.

Compound A Concentrations

The experimental protocol was designed to produce high compound A concentrations to more rigorously evaluate potential low-flow sevoflurane toxicity. Flow rates were ≤ 1 l/min because compound A formation is inversely proportional to gas flow rates; for example, inspiratory compound A concentrations were approximately ten times higher at 1 l/min than at 6 l/min.25
Higher sevoflurane concentrations were targeted because inspired compound A concentrations are directly proportional to sevoflurane concentration.\textsuperscript{3,5,24} Thus nitrous oxide was avoided, and opioid concentrations were minimized to increase sevoflurane requirements. Barium hydroxide lime was used because compound A formation is greater than with soda lime.\textsuperscript{10,24} Finally, fresh carbon dioxide absorbent was used for every case, because fresh barium hydroxide lime produces higher compound A concentrations.\textsuperscript{10} The protocol was also designed to study patients undergoing procedures of routine duration.

Under these conditions, the mean maximum inspiratory compound A concentration was 27 ± 13 ppm, which is comparable to compound A concentrations reported previously.\textsuperscript{10–14} These reports expressed compound A formation in concentration (parts per million). However, it is well known that for inhaled toxins, toxicity is more closely related to exposure, expressed as the product of concentration and time, than to concentration alone.\textsuperscript{25,26} Therefore, we also calculated compound A exposure as parts per million, from the AUC. Average exposure, based on inhaled concentrations, was 79 ± 54 ppm-h, and the average deposition during anesthesia was 27 ± 16 ppm-h, based on the inspired-expired concentration difference.\textsuperscript{17} The best predictor of maximum compound A concentration (parts per million) was sevoflurane concentration (MAC), as observed previously.\textsuperscript{12} The best predictor of both compound A exposure and amount retained (ppm-h) was sevoflurane.

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MAC-hour. The mean exposure in the present investigation was comparable to 67 ppm-h for 5-h sevoflurane, 10 50 ppm-h for 3-h sevoflurane, 23 and 65 ppm-h for 4-h sevoflurane, 13 as calculated from data reported in investigations using barium hydroxide lime. Greater compound A exposures have also been reported, such as 120 ppm-h for 7-h sevoflurane (7.4 MAC-h), 11 250 ppm-h for 16-h sevoflurane (11.5 MAC-h), 12 260 ppm-h for 3-h (13.1 MAC-h), 14 and 260 ppm-h for 16-h sevoflurane (11.4 MAC-h) 24 using soda lime, and 330 ppm-h for 18-h sevoflurane (10.7 MAC-h) using barium hydroxide lime. 24

Previous investigations of compound A formation and toxicity during low-flow sevoflurane anesthesia were performed in Japan, where patients are smaller. Because compound A formation is temperature dependent, 27 and the higher absorbent temperatures resulting from greater carbon dioxide production in larger patients might be expected to enhance compound A formation, the relevance of these studies to larger patients has been questioned. However, the 27 ppm mean maximum inspired compound A concentration we observed in patients averaging 78 kg is not substantially different from the 20 to 25 ppm in patients averaging 53 to 62 kg, observed by Bito et al. 9,11,12,14,25 Although extrapolations based on compound A concentrations from Japanese investigations and the relation in figure 21 would have predicted higher compound A concentrations in larger North American patients, these were not observed in the present or previous 10 investigations. Thus body weight appears not to be the predominant factor determining compound A exposure, which is consistent with the observation that it accounted for only 29% of the variability in compound A concentration. Calculated according to body weight, Japanese patients have undergone greater compound A exposures than those reported presently.

Human compound A exposures may be compared with those required to elicit nephrotoxicity in rats. The threshold for tubular cell necrosis was 150–300 ppm-h in rats weighing 120–180 g, 28 340 ppm-h in rats weighing 180–250 g, 6 and 300 ppm-h in those weighing 240 g. 7 Using conventional allometric methods for cross-species scaling, 29,30 as proposed for adoption by the Environmental Protection Agency and Food and Drug Administration, 7 the corresponding compound A exposure threshold in an 80-kg human would be several times greater. Actual human exposures are less than this projected effect threshold.

Nephrotoxicity
Effects of low-flow sevoflurane and isoflurane anesthesia on renal tubular function and structural integrity were assessed using conventional clinical indicators of renal function (serum creatinine and BUN), specific measures of proximal tubular reabsorptive function (urine protein and glucose excretion), and established (NAG excretion) and new (GST excretion) experimental markers of tubular cell necrosis. Severe compound A nephrotoxicity in rats results in elevated serum creatinine and BUN concentrations. 3,6 Serum creatinine and BUN concentrations did not differ in patients anesthetized with low-flow sevoflurane and isoflurane. This result is similar to all previous investigations of low-flow or closed-circuit sevoflurane, in which serum creatinine and BUN concentrations were unchanged. 10–14,31 A single measurement of BUN or creatinine is an accepted index of renal function reflecting the preceding 12–24 h, and an increase, in the absence of increased production, suggests a decrement in renal function.

Compound A nephrotoxicity in rats also results in glucosuria and proteinuria, probably resulting from loss of proximal tubular reabsorptive function, consistent...
with necrosis that has been localized to tubular cells.\textsuperscript{4,6,7,32} Glucosuria and proteinuria in rats were more sensitive biochemical markers of mild compound A toxicity than were serum creatinine and BUN concentrations.\textsuperscript{7,8} In this investigation, neither postoperative urine protein nor glucose excretion differed between patients receiving low-flow sevoflurane or isoflurane, and there was no correlation between glucose or protein excretion and compound A exposure, further suggesting that low-flow sevoflurane and isoflurane had similar effects on proximal tubular reabsorptive function.

Compound A proximal tubular cell necrosis in rats also resulted in increased urinary excretion of NAG and αGST.\textsuperscript{6,7} Urinary NAG is a proximal tubular cell lysosomal enzyme released into the tubular lumen and excreted in urine after cell necrosis.\textsuperscript{33,34} Excretion of NAG is an established biomarker sensitive for proximal tubular cell necrosis and is increased by experimental nephrotoxins in animals and by nephotoxic antibiotics.
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Fig. 9. Urinary excretion of α-glutathione-S-transferase (A) and π-glutathione-S-transferase (B) 0–24 hr, 24–48 hr, and 48–72 hr after anesthesia. Individual and mean (± SD) values are shown. There were no significant differences between sevoflurane and isoflurane patients.

in humans. Such excretion also has been used clinically to evaluate the renal tubular effects of high-flow (≥ 5 l/min) sevoflurane.35–40 Our results show that urinary NAG excretion was similar after low-flow sevoflurane and isoflurane on all three postoperative days, and there was no correlation between NAG excretion and compound A exposure, suggesting similar low-flow sevoflurane and isoflurane effects on proximal tubular cell integrity.

Glutathione-S-transferase is a family of enzymes, consisting of cytosolic α, π, μ, and θ isoforms and a microsomal form, and constituting as much as 2% of renal tubular cell cytoplasmic protein.41 Isoforms of GST are selectively expressed in human kidney, with α-GST localized exclusively in proximal tubules and π-GST found exclusively in distal tubules.42–45 Because circulating GST is effectively filtered at the glomerulus, urinary GST excretion is thought to represent tubular cell membrane permeability and leakage into tubular fluid. Recent developments permitting selective quantitation of individual GST isoforms have shown that α-GST and π-GST are sensitive biomarkers of experimental nephrotoxicity in rats46 47 and promising selective biomarkers in humans. In humans, acute tubular necrosis and renal infarction increased urinary α-GST and π-GST excretion, cyclosporine toxicity selectively increased only α-GST, and rejection after renal transplantation increased only π-GST.48 In rats, urinary excretion of α-GST was the most sensitive urine or plasma biomarker of mild proximal tubular cell necrosis caused by compound A.49 50 In the present investigation, urinary α-GST and π-GST excretion in patients anesthetized with low-flow sevoflurane or isoflurane were not significantly different, and there was no correlation between GST excretion and compound A exposure. These results suggest no significant effect of compound A formation during low-flow sevoflurane, compared with that of low-flow isoflurane, on proximal and distal tubular cell integrity.

There was considerable variability in urine protein, glucose, and NAG and GST excretion among patients anesthetized with isoflurane and sevoflurane. Individual patients did show elevations in one or more parameters. There were no correlations, however, between urinary excretion of protein, glucose, NAG, or GST, suggesting the lack of a common etiologic factor such as proximal tubular cell necrosis. Further, in patients anesthetized with sevoflurane, there was no correlation between compound A exposure and urine protein, glucose, NAG, or GST excretion. These results provide evidence against a specific proximal tubular injury in patients exposed to compound A during low-flow sevoflurane, compared with isoflurane anesthesia.

Nephrotoxicity may also be caused by inorganic fluoride, such as that resulting from methoxyflurane metabolism. One clinical investigation of sevoflurane anesthesia reported a greater average NAG excretion in patients with plasma fluoride concentrations greater than 50 μM compared with those less than 50 μM, influenced by a single patient with a high value.51 Fluoride release during low-flow sevoflurane may result from metabolism.

Fig. 10. Relation between urine excretion of α-glutathione-S-transferase (A, B) or γ-glutathione-S-transferase (C, D) and compound A exposure in patients anesthetized with low-flow sevoflurane. Results are shown for 0- to 24-h (A, C) and 48- to 72 h (B, D) postoperative collections.

of sevoflurane, compound A, or both.\textsuperscript{18, 20, 46} We found no evidence of increased NAG excretion or nephrotoxicity despite serum fluoride concentrations greater than 50 μM in ten patients (maximum, 92 μM), which is consistent with other reports.\textsuperscript{37} This result further supports the concept that serum fluoride concentrations alone do not predict anesthetic nephrotoxicity, and that the 50-μM fluoride toxicity threshold for methoxyflurane does not apply nonselectively to all anesthetics.\textsuperscript{38}

Several differences exist between laboratory investigations demonstrating compound A nephrotoxicity in rats\textsuperscript{3, 6, 8, 28, 49} and the current and previous\textsuperscript{10-14} investigations showing no specific nephrotoxicity after low-flow sevoflurane anesthesia in humans. As described previously, weight-adjusted compound A exposures in rats have exceeded those in humans. Rats have received compound A alone, whereas human compound A exposures occur with coadministration of sevoflurane in large excess. Metabolism of compound A by cytochrome P450 is inhibited by sevoflurane in excess.\textsuperscript{18}
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species by the renal cysteine conjugate β-lvase,\textsuperscript{46,51} which is 8–30 times more active in rat compared with human kidneys.\textsuperscript{46,52,53}

**Limitations**

The present investigation studied a relatively small number of patients. Most were admitted to the hospital the morning of surgery, and thus preoperative 24-h urine collections could not be obtained reliably. Comparisons were made between anesthetic groups, and pre-post anesthesia analysis was not possible. Surgical procedures of a target duration of 2–4 h were studied, sevoflurane exposure averaged 3.9 and 3.6 MAC-h with a maximum of 8.5 MAC-h, and compound A exposure averaged 79 ppm/h. Additional investigations using sensitive biomarkers are required to assess the renal effects of longer duration low-flow sevoflurane anesthesia and greater compound A exposure.

In addition, the NAG and GST results must be interpreted carefully. The current diagnostic value of enzymuria as a sensitive clinical marker of tubular necrosis in humans lies in a negative result (absence of enzymuria). The negative predictive power of enzymuria is high, because the absence of enzymuria denotes the absence of cell necrosis. The significance of a positive result (increased enzymuria) is questionable because the clinical predictive value of enzymuria is unknown.\textsuperscript{54} Clearly, nephrotoxin administration in experimental animals can cause tubular cell necrosis and enzymuria, and the severity of injury is reflected in the degree of enzymuria. The converse in humans, that enzymuria denotes tubular cell necrosis, however, is unproved. Although attractive and widely used, enzymuria (more specifically NAG excretion) as a marker of nephrotoxicity in humans is controversial.\textsuperscript{54–56} Problems include specificity and the lack of clinical validation. Even the established marker, NAG, is nonspecific and markedly affected by various factors such as surgery, circadian rhythm, nephrotoxic antibiotics, hypertensive episodes, prostatic hypertrophy, nonsteroidal antiinflammatory drugs, radiopaque dyes, and contamination from seminal fluid.\textsuperscript{54,57} The newer experimental GST markers appear more promising, because GST isoforms are more specific and respond to site-specific tubular lesions.\textsuperscript{55} Thus, although the positive predictive value of enzymuria in humans remains controversial, the absence of increased NAG and GST excretion after low-flow sevoflurane, compared with low-flow isoflurane, supports the absence of anesthetic-specific tubular cell necrosis.

In addition, human exposures to compound A have occurred via an endotracheal tube, which bypasses the upper respiratory tract, whereas rats were not intubated. A significant proportion of total haloalkane absorption can occur in the upper respiratory tract.\textsuperscript{59} Finally, compound A undergoes extensive metabolism and there are species differences in the activities of enzymes responsible for compound A biotransformation to toxic metabolites.\textsuperscript{8,18,20,46,51} For example, compound A–cysteine conjugates are metabolized to toxic

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Conclusions

There were no significant differences, using conventional and sensitive biomarkers of renal tubular function and cellular integrity, between the renal effects of sevoflurane and isoflurane in surgical patients undergoing low-flow anesthesia for as long as 7 h. There were no significant differences in liver enzymes between patients anesthetized with low-flow sevoflurane or isoflurane. Low-flow sevoflurane anesthesia, during which compound A formation occurs, appears to be as safe as low-flow isoflurane anesthesia.

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References


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