New Insights into the Mechanism of Methoxyflurane Nephrotoxicity and Implications for Anesthetic Development (Part 2)

Identification of Nephrotoxic Metabolites

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Background: Methoxyflurane nephrotoxicity results from its metabolism, which occurs by both dechlorination (to methoxydifluoroacetic acid [MDFA]) and O-demethylation (to fluoride and dichloroacetic acid [DCAA]). Inorganic fluoride can be toxic, but it remains unknown why other anesthetics, commensurately increasing systemic fluoride concentrations, are not toxic. Fluoride is one of many methoxyflurane metabolites and may itself cause toxicity and/or reflect formation of other toxic metabolite(s). This investigation evaluated the disposition and renal effects of known methoxyflurane metabolites.

Methods: Rats were given by intraperitoneal injection the methoxyflurane metabolites MDFA, DCAA, or sodium fluoride (0.22, 0.45, 0.9, or 1.8 mmol/kg followed by 0.11, 0.22, 0.45, or 0.9 mmol/kg on the next 3 days) at doses relevant to metabolite exposure after methoxyflurane anesthesia, or DCAA and fluoride in combination. Renal histology and function (blood urea nitrogen, urine volume, urine osmolality) and metabolite excretion in urine were assessed.

Results: Methoxyflurane metabolite excretion in urine after injection approximated that after methoxyflurane anesthesia, confirming the appropriateness of metabolite doses. Neither MDFA nor DCAA alone had any effects on renal function parameters or necrosis. Fluoride at low doses (0.22, then 0.11 mmol/kg) decreased osmolality, whereas higher doses (0.45, then 0.22 mmol/kg) also caused diuresis but not significant necrosis. Fluoride and DCAA together caused significantly greater tubular cell necrosis than fluoride alone.

Conclusions: Methoxyflurane nephrotoxicity seems to result from O-demethylation, which forms both fluoride and DCAA. Because their coformation is unique to methoxyflurane compared with other volatile anesthetics and they are more toxic than fluoride alone, this suggests a new hypothesis of methoxyflurane nephrotoxicity. This may explain why increased fluoride formation from methoxyflurane, but not other anesthetics, is associated with toxicity. These results may have implications for the interpretation of clinical anesthetic defluorination, use of volatile anesthetics, and the laboratory methods used to evaluate potential anesthetic toxicity.

THE volatile anesthetic methoxyflurane causes renal failure in animals and humans.3 The potential for anesthetic nephrotoxicity has influenced the screening, development, and clinical use of every potential and approved volatile anesthetic since the 1960s. It has been well established that the nephrotoxicity of methoxyflurane was related to its metabolism. The most easily measured metabolite was inorganic fluoride, and methoxyflurane toxicity was associated with increased plasma fluoride concentrations.

Based on the association between renal failure and methoxyflurane defluorination, it was concluded that methoxyflurane nephrotoxicity was caused by inorganic fluoride. Furthermore, it was assumed that any increase in plasma fluoride, from any anesthetic, might cause renal toxicity. The once-classically accepted “fluoride hypothesis,” subsequently expanded to fluorinated volatile anesthetics in general (albeit without evidence), was that anesthetics undergo hepatic defluorination, inorganic fluoride is released and disseminated systemically and acts as a specific renal toxin, and that the threshold for renal toxicity is a plasma fluoride concentration greater than 50 μM. Nevertheless, it is now unambiguously clear that hepatic defluorination and systemic migration of fluoride to the kidney is not the mechanism of anesthetic nephrotoxicity, that a generic plasma fluoride concentration greater than 50 μM has no causal implications for renal toxicity, and that the 50-μM fluoride threshold for nephrotoxicity has been relegated to historical artifact.2,4 Rather, anesthetic nephrotoxicity seems agent (methoxyflurane) specific, and the mechanisms remain enigmatic.

Why then is methoxyflurane nephrotoxic, how is toxicity related to metabolism, why was methoxyflurane associated with a plasma fluoride of greater than 50 μM, and why are other anesthetics resulting in even higher fluoride concentrations not associated with nephrotoxicity? And, given that methoxyflurane disappeared from clinical use decades ago, why are these questions important? One potential explanation for methoxyflurane nephrotoxicity is that intrarenal production of fluoride (or other toxins) may be the actual determinant of anesthetic toxicity. Another (not necessarily exclusive) explanation is that a different methoxyflurane metabolite (unique to methoxyflurane) may be the actual toxin. If the toxic metabolite were coformed with inorganic fluoride, fluoride might simply be a "reporter" for toxin formation. Methoxyflurane undergoes defluorination by two different routes, dechlorination to 2,2-difluoro-2-

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methoxyacetic acid (methoxydifluoroacetic acid [MDFA]) (which may degrade to fluoride) and O-demethylation to fluoro- and dichloroacetic acid (DCAA) (which yields oxalic acid and fluoride). Nevertheless, the metabolic pathway responsible for methoxyflurane nephrotoxicity has never been identified.

In an accompanying article, experiments were conducted to quantify formation of methoxyflurane metabolites and evaluate the effect of up- or down-regulation of methoxyflurane metabolism on both metabolism and toxicity. Results showed that the O-demethylation pathway mediated methoxyflurane nephrotoxicity in rats. The purpose of this current investigation was to identify which methoxyflurane metabolites resulting from O-demethylation mediate methoxyflurane nephrotoxicity in rats.

Materials and Methods

Chemicals

Methoxydifluoroacetic acid was synthesized as described previously and was 99% pure. DCAA and sodium fluoride (NaF) were from Sigma Chemical Company (St. Louis, MO). Trifluoroacetic acid, Magtrieve, and benzophenone hydrazone were purchased from Aldrich (Milwaukee, WI). Chlorodifluoroacetic acid was from Fluka (Milwaukee, WI).

Animals and Treatments

All experiments were approved by the University of Washington Animal Care and Use Committee, Seattle, Washington, and conducted in accordance with American Association for Accreditation of Laboratory Animal Care guidelines. Male Fischer 344 rats (12 weeks old, 250–300 g) were purchased from Harlan (San Diego, CA). Rats were housed in individual metabolic cages, provided food and water ad libitum, and maintained on a 12-h light–dark cycle. They were allowed to acclimate to the metabolism cages for at least 48 h before any experimental treatment. Before any treatment, a baseline 24-h urine collection and blood sample were obtained via saphenous vein puncture were performed by a standard curve prepared using samples of known

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Concentrations of fluoride in plasma and urine, and blood urea nitrogen and creatinine in plasma, and urine osmolality were determined as described previously. MDFA and DCAA concentrations in urine were determined by gas chromatography–mass spectrometry, as described in the accompanying article. Formic acid concentrations in urine were determined using an existing method, modified for 96-well plates. Briefly, urine (20 μl) was added to 500 μl buffered diaphorase (1 mg/ml NAD, 800 mU/ml diaphorase, 1 mg/ml p-iodonitrotetrazolium violet, in 0.1 M phosphate buffer, pH 7.4). Formate dehydrogenase (20 μl, 3 U/ml) was added to each sample, and they were mixed and left at room temperature for 60 min. Absorbance (500 nm) was then determined spectrophotometrically (SpectraMax 340; Molecular Devices Corporation, Sunnyvale CA). Concentrations in unknown samples were determined using a standard curve prepared using samples of known concentration (0–200 μg/ml). Kidneys were fixed in 10% neutral-buffered formalin and processed, and histopathologic analysis was performed by a veterinary pathologist who was blinded to animal treatments as described previously. The semiquantitative severity score ranged from 0 to 4 (normal, minimal, slight, moderate, and marked, respectively) to reflect the degree and distribution of any tubular necrosis.

Statistical Analysis

Data were initially analyzed using a two-way repeated-measures analysis of variance (time and treatment), fol-
allowed by the Student-Newman-Keuls test for multiple comparisons, using SigmaStat (Systat, Point Richmond, CA). Because within-treatment between-day (days 1–4) differences were rare and group sizes were small, post-treatment means for day 1–4 renal function parameters or cumulative 4-day urine metabolite excretions were calculated. One-way analysis of variance (between treatments) followed by Student-Newman-Keuls tests for multiple comparisons was then performed. Results are reported as mean ± SD. Statistical significance was assigned at $P < 0.05$.

Results

Experiments were conducted to assess the effects of the known stable metabolites of methoxyflurane (MDFA, DCAA, and inorganic fluoride), alone and together, on renal function and to assess their disposition. Preliminary experiments were conducted to evaluate the excretion of methoxyflurane metabolites in urine, to guide their subsequent dosing. After 3 h of 0.5% methoxyflurane, 4-day urinary excretion of MDFA and inorganic fluoride was 236 ± 46 and 102 ± 25 μmol, respectively (mean ± SD, n = 12), with excretion on day 1 (or occasionally day 2) approximately twice that on subsequent days. For example, urine fluoride excretion averaged 110, 76, 40, and 28 μmol/kg on days 1–4. Approximately one half of fluoride is cleared into bone at the concentrations liberated by methoxyflurane metabolism,$^{10}$ hence fluoride formation might be estimated at twice the amount recovered in urine. No published information was available about the disposition of MDFA. Hence, these preliminary investigations, together with previous reports on fluoride excretion,$^{11,12}$ were used to guide an initial dosing regimen for methoxyflurane metabolites of 0.225 mmol/kg on day 1 followed by 0.112 mmol/kg on days 2–4. This was used as an initial estimate of the minimum amount of metabolite formed, and doses were then escalated. Thus also studied were 0.45, 0.9, or 1.8 mmol/kg on day 1 followed by 0.225, 0.45, or 0.9 mmol/kg on days 2–4. Total metabolite doses in the four groups were approximately 0.14, 0.28, 0.56, and 1.12 mmol per animal.

Methoxydifluoroacetic acid had no effect on renal function, assessed by urine volume, urine osmolality, and blood urea nitrogen, even at the highest dose studied (fig. 1). Light microscopy showed no significant renal effects of MDFA, even at the highest dose (not shown). MDFA and fluoride were measured in urine (fig. 2). Total 4-day MDFA recovery was 51 ± 6, 93 ± 19, 258 ± 78, and 454 ± 141 μmol at the four doses studied. Thus, based on urine recovery, MDFA exposure was equal to or greater than that after methoxyflurane anesthesia. Approximately 40% of the MDFA dose was recovered in urine. Because the excretion rate of MDFA is unknown, a longer collection period may have increased recovery. Total 4-day inorganic fluoride recovery was 21 ± 2, 34 ± 2, 84 ± 13, and 116 ± 28 μmol, or approximately 6% of the MDFA dose (assuming liberation of both MDFA fluoride molecules). Based on 50% recovery of fluoride (the remainder taken up by bone), approximately 12% of MDFA seemed metabolized or degraded to inorganic fluoride.

Dichloroacetic acid alone had little or no effect on renal function, assessed by urine volume, osmolality, and blood urea nitrogen, even at the highest dose studied (fig. 3). Light microscopy showed no significant renal effects of DCAA, even at the highest dose (fig. 4). DCAA was only detected in urine at the highest doses injected. Total 4-day DCAA recovery was 0 ± 0, 6 ± 1, 70 ± 13, and 279 ± 51 μmol at the four doses studied. DCAA recovery was dose dependent, averaging 0, 2, 12, and
24% at the four doses, indicating a saturable elimination pathway. Because no DCAA was detected in urine after methoxyflurane anesthesia, and DCAA was detected in urine at doses greater than 0.28 mmol, it can be deduced that DCAA exposure after methoxyflurane is less than approximately 0.28 mmol. Thus, based on urine recovery, DCAA exposure was equal to or greater than that after methoxyflurane anesthesia.

Effects of inorganic fluoride, alone and in combination with DCAA, were assessed by biochemistry and histology (figs. 5 and 6). Fluoride alone at both doses resulted in decreased urine osmolality and resulted in significant diuresis but only at the high dose. Urine output averaged 5.1 ± 2.1 ml/day predose, and 5.7 ± 1.3 and 11.4 ± 2.6 ml/day (P < 0.05 vs. baseline) after the low and high fluoride doses. Blood urea nitrogen was unchanged. Kidneys from rats given fluoride alone showed no to mild histologic changes, characterized principally by swelling of midcortical tubular epithelial cells (fig. 4). Rare necrosis of widely scattered individual tubular epithelial cells was observed; however, the degree was variable and did not reach overall statistical significance. Total 4-day urine inorganic fluoride recovery was 125 ± 24 and 245 ± 76 μmol at the two doses administered (fig. 7). Thus, fluoride recovery was equal to or greater than that after methoxyflurane anesthesia.

Administration of DCAA, in addition to fluoride, exacerbated some effects of fluoride alone. On day 4, urine output was greater than baseline in rats treated with 0.22 ms NaF and DCAA but not in those receiving NaF alone, and was greater in rats treated with 0.45 ms NaF and DCAA compared with NaF alone (not shown). Nevertheless, cumulative 4-day urine volume in rats receiving DCAA with fluoride was not significantly greater than in those treated with fluoride alone. In contrast, histologic lesions in rats given both DCAA and fluoride were significantly greater than those occurring after fluoride alone (fig. 6). Histologic changes involved midcortical tubular epithelial cells, but with combined fluoride and DCAA treatment, moderate, multifocal necrosis, tubular cast formation, and sometimes tubular cell regeneration accompanied the degenerative changes. Thus, adminis-
tration of increasing DCAA doses concomitantly with a constant dose of inorganic fluoride resulted in increasing renal tubular necrosis. Coadministration of fluoride and DCAA did not alter their disposition. Fluoride and DCAA excretion when administered together were not different than when administered individually (figs. 3 and 7).

Dichloroacetic acid can increase urine formic acid excretion, and long-term exposure to formate can cause nephrotoxicity. Formate exposure as a potential mechanism of methoxyflurane nephrotoxicity has never been explored. Therefore, urine formate concentrations after exposure of rats to methoxyflurane and its metabolites were determined (fig. 8). DCAA increased urine formate excretion; however, formate excretion was similar in animals given methoxyflurane, methoxyflurane after enzyme induction with phenobarbital, or the methoxyflurane metabolites MDFA or inorganic fluoride. Formate excretion after DCAA was unaffected by coadministration of inorganic fluoride.

Discussion

Methoxyflurane causes clinical dose-dependent polyuric or, occasionally, oliguric as well as anuric renal insufficiency. Since the first reports of methoxyflurane nephrotoxicity, this feared anesthetic complication has influenced clinical anesthetic use and the development of all new fluorinated anesthetics.

Mazze et al. provided incontrovertible evidence that methoxyflurane nephrotoxicity is intrinsically linked to methoxyflurane metabolism. In humans, severity of the renal concentrating defect was proportional to methoxyflurane metabolism. In rats, up- or down-modulation of methoxyflurane metabolism correspondingly changed renal toxicity. Methoxyflurane deuteration diminished metabolism and prevented polyuria. Methoxyflurane yields many metabolites, only one of which, inorganic fluoride, was readily measurable during the investigations of methoxyflurane toxicity. In rats, both renal tox-
icity and serum fluoride concentrations increased with methoxyflurane dose.11,17 Enzyme induction with phenobarbital increased defluorination, serum fluoride, and renal toxicity.12,18,19 Conversely, both enzyme inhibition and methoxyflurane deuteration diminished methoxyflurane defluorination, serum fluoride, and toxicity.12,16 In rats, fluoride injection (albeit at large doses) reproduced renal changes caused by methoxyflurane.17 Fluoride injection in rats caused renal toxicity, although the resultant serum fluoride concentrations were quite high (> 400 μM), and greater than those after methoxyflurane.17,22 Injection of fluoride which produced concentrations approximating those after methoxyflurane anesthesia had no effect on renal function.24 In humans, renal dysfunction was related to peak serum fluoride concentrations.20,21 No effects were reported at fluoride less than 40 μM, subclinical toxicity at 50–80 μM, mild clinical toxicity at 90–120 μM, and overt nephrotoxicity at 80–175 μM fluoride.22 It was concluded that nephropathy was caused by methoxyflurane metabolism to inorganic fluoride, the ultimate renal toxin.

The classic fluoride hypothesis was that methoxyflurane was hepatically defluorinated and nephrotoxicity was caused by systemic fluoride with a toxic threshold of greater than 50 μM in serum. This theory was subsequently generalized (albeit without supporting data) to all fluorinated anesthetics.4 The 50-μM fluoride threshold evolved as a number of mythic proportions. New anesthetics were scrutinized for their potential to undergo hepatic defluorination, generate greater than 50 μM systemic fluoride, and, supposedly, cause renal toxicity. If defluorinated, they were often rejected for further development.

Nevertheless, there is now accumulated evidence that does not support the generalized fluoride hypothesis. In rats, methoxyflurane produced serum fluoride greater than 50 μM in several strains, but only Fisher 344 rats developed toxicity.17 Fluoride injection in rats caused renal toxicity, although the resultant serum fluoride concentrations were quite high (> 400 μM), and greater than those after methoxyflurane.17,22 Injection of fluoride which produced concentrations approximating those after methoxyflurane anesthesia had no effect on renal function.24 In humans, the absence of renal toxicity after enflurane, isoflurane, and sevoflurane despite plasma fluoride greater than 50 μM is well documented. Sevoflurane can produce plasma fluoride greater than 50 μM without adverse renal effects,25 and peak plasma fluoride as high as 110 μM after sevoflurane or 130 μM after enflurane caused no renal toxicity.26,27 Prolonged isoflurane anesthesia (> 50 μM fluoride for 2–3 days) or
sedation (as high as 93 μM fluoride for days) had no effect on renal function. Nephrotoxicity was not observed in cases of fluoride intoxication; indeed, one victim had a plasma fluoride of 480 μM without renal effects. Together these data suggest that neither peak plasma fluoride concentration nor the duration of fluoride increase can adequately explain anesthetic nephrotoxicity. Owing to the need for a mechanistic understanding of anesthetic toxicity and the implications for anesthetics development, it was relevant to reevaluate the mechanism of methoxyflurane nephrotoxicity.

Methoxyflurane is metabolized by two independent pathways, yielding putative unstable intermediates and stable metabolites. Dechlorination forms MDFA, which may degrade to oxalate and inorganic fluoride. O-demethylation forms two molecules of inorganic fluoride and one of DCAA, which then forms oxalate. An accompanying article suggests that the O-demethylation pathway, rather than the dechlorination pathway, is the predominant route of methoxyflurane toxification. The current investigation assessed the renal effects of the known stable methoxyflurane metabolites themselves (MDFA, DCAA, and inorganic fluoride), alone and in combination. Metabolite elimination was evaluated, and compared with that after methoxyflurane anesthesia, to verify that relevant metabolite doses were administered. Oxalic acid effects were not evaluated, because oxalate was previously discounted as the nephrotoxic methoxyflurane metabolite.

Methylenefluoroacetic acid is an alkoxyacetic acid and might theoretically be nephrotoxic, because such acids adversely affect mitochondrial respiration and renal function. MDFA, however, had no effect on renal histology or function and did not reproduce methoxyflurane toxicity. This is consistent with previous observations that the dechlorination pathway (which forms MDFA) seems not to mediate methoxyflurane nephrotoxicity. The minimal amount of MDFA defluorination observed herein is consistent with previous reports. Because fluoride is not formed with or significantly from MDFA, MDFA formation would not account for the association between increased fluoride and renal dysfunction after methoxyflurane. Together these results suggest that methoxyflurane dechlorination metabolites do not mediate nephrotoxicity.

Dichloroacetic acid might theoretically be nephrotoxic in vivo, because it is a renal mitochondrial toxin that inhibits state 3 respiration and some haloacetic acids can adversely affect renal function. DCAA alone, however, had no significant effect on renal histology or function and did not reproduce methoxyflurane toxicity. Thus, although fluoroacetic acids can cause nephrotoxicity, the chloroacetic acid DCAA did not, consistent with previous results.

It is well established that inorganic fluoride, in sufficiently high doses, is nephrotoxic. Fluoride injection seemed to mimic the injury caused by methoxyflurane. At issue, however, which affects the conclusion reached in these studies that fluoride caused methoxyflurane toxicity, is the relevance of the fluoride...
dose and resultant serum concentrations (> 400 
\mu M).\textsuperscript{11,25} Although fluoride injection \textit{in vivo} was reported to alter renal function at 50 \mu M serum fluoride,\textsuperscript{36} others found that fluoride at less than 200 \mu M had little effect on urine output.\textsuperscript{24} In isolated perfused rat kidneys, the threshold for renal function changes was 500 \mu M.\textsuperscript{40} In renal tubular cells \textit{in vitro}, the threshold for cytotoxicity was 5,000 \mu M fluoride.\textsuperscript{42–44}

In the current investigation, fluoride doses were targeted to more closely approximate fluoride exposure during methoxyflurane anesthesia. Fluoride injection (0.22 mmol/kg, then 0.11 mmol/kg for 3 days) resulting in a 4-day urinary excretion of 125 ± 25 \mu mol, which approximated the excretion of 102 ± 25 \mu mol after 3 h of 0.5\% methoxyflurane, caused no significant increase in urine volume or renal necrosis. Only a higher dose (0.45 mmol/kg, then 0.22 mmol/kg for 3 days), resulting in a urinary fluoride excretion of 245 ± 76 \mu mol, increased urine output. The current results are similar to the effects of high fluoride doses (> 0.6 mmol/kg) in rats,\textsuperscript{13,4,45} which may be required for toxicity.

In contrast, when inorganic fluoride was administered together with DCAA, renal necrosis was greater than with fluoride alone. Although DCAA coadministration with fluoride dose-dependently increased renal necrosis, diuresis was not commensurately increased. Nonetheless, although mild renal injury can cause diuresis, more severe renal injury can cause oliguria. The greater necrosis of DCAA plus fluoride is consistent with the previous observation that the O-demethylation pathway (which forms DCAA and fluoride) seems to mediate methoxyflurane nephrotoxicity.\textsuperscript{46} Together these results suggest a new hypothesis, that methoxyflurane O-demethylation and the metabolites DCAA and inorganic fluoride together mediate methoxyflurane nephrotoxicity.

Results suggesting that the renal toxicity of DCAA and fluoride together exceeds that of the metabolites alone, particularly fluoride, are novel and potentially important. This would explain all observations regarding methoxyflurane nephrotoxicity, and the differing clinical effects of methoxyflurane and other anesthetics. Coformation of the coordinate toxins is consistent with the role of metabolism in methoxyflurane nephrotoxicity. Associations between methoxyflurane toxicity and fluoride concentrations could be explained, in part, because coformation of fluoride and DCAA would mean that fluoride formation also reflects that of DCAA. Modulation (enzyme induction or inhibition, methoxyflurane deuteration) of methoxyflurane metabolism and toxicity would be “seen” as a change in fluoride but might represent a change in both fluoride and DCAA formation. Altered DCAA formation would not be apparent because of its further metabolism and undetectability in urine at relevant methoxyflurane doses. In contrast, whereas fluoride is a ubiquitous metabolite of all fluorinated volatile anesthetics, only methoxyflurane yields DCAA and the coordinate formation of DCAA and fluoride. Hence, without coformation of DCAA, which is unique to methoxyflurane, anesthetic metabolism causing plasma fluoride greater than 50 \mu M might not affect renal function. Thus, fluoride alone from sevoflurane, enfurane, and isoflurane would not be expected to cause toxicity, consistent with clinical observations.

Another potential explanation for methoxyflurane nephrotoxicity is that intrarenal, in addition to hepatic, formation of fluoride (or other metabolites) may contribute to toxicity. Human kidney microsomes substantially defluorinated methoxyflurane but not sevoflurane.\textsuperscript{3} Fluoride nephropathy seems to depend more on intrarenal than systemic concentrations.\textsuperscript{38,39} Renal parenchymal fluoride concentrations are threefold to sevenfold higher than plasma.\textsuperscript{46} Fluoride injection in rats \textit{in vivo} caused a renal defect when serum concentrations were only 20 \mu M while urinary concentrations (suggested to reflect intrarenal concentrations) were greater than 700 \mu M.\textsuperscript{39} Similarly, diuresis occurred when intrarenal fluoride was 100–200 \mu M, but plasma fluoride was only 32 \mu M.\textsuperscript{38} In isolated kidneys, perfusate fluoride up to 500 \mu M had no effect on renal function.\textsuperscript{40} \textit{In vitro}, fluoride concentrations required to elicit toxicity (2–10 mM) in rat proximal tubules\textsuperscript{47} and cortical membranes\textsuperscript{48} and human collecting duct cells\textsuperscript{42} far exceed those achieved systemically. The current results are also consistent with the intrarenal metabolism hypothesis. Intrarenal methoxyflurane O-demethylation to fluoride would also form DCAA. Greater effects of fluoride and DCAA compared with fluoride alone, combined with their intrarenal coformation from methoxyflurane (but not other anesthetics), are also consistent with the toxicity of only methoxyflurane.

Because methoxyflurane disappeared from clinical use decades ago, why are methoxyflurane nephrotoxicity and its uniqueness among all other defluorinated anesthetics of interest? There may be implications for finally understanding the mechanism of anesthetic nephrotoxicity. There may also be relevance for the development of new anesthetics. As a consequence of the fluoride hypothesis, new anesthetics were scrutinized for their hepatic metabolism and, if sufficiently defluorinated (and hence purportedly potentially nephrotoxic), often rejected for further development. If coordinate DCAA and fluoride formation, particularly intrarenal rather than hepatic, is the mechanism of methoxyflurane toxicity, defluorination as a screen for potential nephrotoxicity merits reevaluation. The “ideal anesthetic” may have been inappropriately rejected in the past or may be in the future.

This investigation has limitations. Results with small numbers of animals should be interpreted cautiously. DCAA and fluoride together, compared with fluoride alone, affected necrosis but not all measures of renal function. The mechanism by which DCAA apparently...
potentiates fluoride effects remain unknown. Other, unstable potential intermediates arising from methoxyflurane O-demethylation were not evaluated and may also contribute to methoxyflurane nephrotoxicity. The new hypothesis regarding methoxyflurane nephrotoxicity merits further evaluation.

In summary, results of this investigation suggest that metabolites arising from methoxyflurane O-demethylation may be the putative nephrotoxins. Specifically, renal necrosis from fluoride and DCAA together was greater than from fluoride alone. Because DCAA and fluoride are formed from methoxyflurane, which is unique to methoxyflurane compared with other volatile anesthetics, this suggests a new hypothesis for methoxyflurane nephrotoxicity. It would also explain why increased fluoride formation from methoxyflurane (also reflecting formation of the toxin DCAA) but not from other anesthetics (not metabolized to DCAA) is associated with renal toxicity. This hypothesis may have implications for the interpretation of clinical anesthetic defluoridation, use of volatile anesthetics, and the laboratory methods used to evaluate potential anesthetic toxicity.

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