Anesthesiology
V 35, No 3, Sept 1971

Methoxyflurane Metabolism and Renal Dysfunction:

Clinical Correlation in Man

Richard I. Mazze, M.D., James R. Trudell, Ph.D.,†*
*Michael J. Cousins, M.B., F.F.A.R.A.C.S.**

Serum inorganic fluoride concentration and urinary inorganic fluoride and oxalic acid excretion were found to be markedly elevated in ten patients previously shown to have methoxyflurane-induced renal dysfunction. Five patients with clinically evident renal dysfunction had a mean peak serum inorganic fluoride level (190.4 ± 20.9 μm/l) significantly higher ($P < 0.02$) than that of those with abnormalities in laboratory tests only (105.8 ± 17.0 μm/l). Similarly, patients with clinically evident renal dysfunction had a mean peak oxalic acid excretion (286.8 ± 39.3 mg/24 hours) significantly greater ($P < 0.05$) than that of those with laboratory abnormalities only (130.6 ± 51.4 mg/24 hours). That patients anesthetized with halothane had insignificant changes in serum inorganic fluoride concentration and oxalic acid excretion indicates that these substances are products of methoxyflurane metabolism. A proposed metabolic pathway to support this hypothesis is presented, as well as evidence to suggest that inorganic fluoride is the substance responsible for methoxyflurane-induced renal dysfunction. (Key words: Methoxyflurane; Metabolism; Nephrotoxicity; Inorganic fluoride; Oxalic acid.)

Since 1966, there have been several reports associating methoxyflurane anesthesia with nephrotoxicity.[1-9] In a recent controlled study we found clinical and laboratory evidence of renal dysfunction in six of 12 patients anesthetized with this agent.[10] It has been suggested that one of the metabolites of methoxyflurane, inorganic fluoride, may be related to this toxicity.[9] We are now able to report a statistical correlation between the degrees of renal dysfunction in patients from our previous study and increased concentrations of two methoxyflurane metabolites, inorganic fluoride and oxalic acid.

Methods

Twenty-two male patients scheduled for elective surgical procedures were randomly divided into two groups: 12 patients received methoxyflurane; ten patients received halothane. Atropine, 0.5 mg, was the only drug administered for premedication. In the study group, anesthesia was induced and maintained with methoxyflurane and oxygen, 6 l/min, using a Pentec vaporizer and a semiclosed circle system for carbon dioxide absorption. A vaporizer setting of 1.5 per cent was used during induction; the setting was adjusted to 0.3–0.5 per cent for maintenance. In five patients end-tidal methoxyflurane concentrations measured during the third hour of anesthesia were 0.6–1.9 (mean 1.4) times the minimum alveolar concentration (MAC) for methoxyflurane.[11] In the control group, halothane and oxygen, 6 l/min, were delivered with Vernitrol or Copper Kettle vaporizers. Vaporizer settings of 3.5 per cent during induction and 1.0–1.5 per cent during maintenance were reached. Durations of anesthesia, patients' physical status, and types of surgical operations were similar in the two groups. Details have been reported previously.[10]

Serum and 24-hour urine specimens from our initial study, refrigerated for as long as 48 hours following collection and subsequently frozen at −15 C, were available for analysis for ten of 12 patients anesthetized with methoxyflurane and five of ten patients anesthetized with halothane. A fluoride ion-specific electrode ‡ was used to determine fluoride

* Assistant Professor of Anesthesia.
† Research Associate.
Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305, and Veterans Administration Hospital, Palo Alto, California 94304. Accepted for publication April 5, 1971. Supported in part by VA Hospital, Palo Alto, California, USPHS grants FR 5353-08 and GM 12527, and the Post-Graduate Medical Foundation, University of Sydney, Australia.

‡ Orion Research Incorporated, 11 Blackstone Street, Cambridge, Massachusetts 02139.

TABLE 1. Serum Inorganic Fluoride Concentrations Following Methoxyflurane Anesthesia—Patients* with Laboratory Abnormalities in Renal Function

	Serum Inorganic Fluoride (μmoles/liter)						
	Postoperative Day 1	Postoperative Day 2	Postoperative Day 3	Postoperative Day 4	Postoperative Day 5	Postoperative Day 6	Postoperative Day 7
Patient 5	76†	46	15	6	4	2	1
Patient 6	87†	82	60	34	25	17	10
Patient 8	90	99†	70	48	46	40	35
Patient 11‡	150	172†	109	62	53	31	28
Patient 12	84	86	95†	62	56	43	26
Mean	97.4	97.0	69.8	42.4	36.8	26.6	20.0
SE	±13.4	±20.7	±16.2	±10.5	±9.8	±7.6	±6.3

* Patient numbers same as in reference 10.
† Peak value (mean 105.8 ± 17.0 μm/l).
‡ This patient had abnormalities in serum sodium, osmolality, urea nitrogen, uric acid, and uric acid clearance and weight loss similar to those noted in patients with clinically evident renal disease, but did not have polyuria or abnormalities in urine-concentrating ability.

TABLE 2. Serum Inorganic Fluoride Concentrations Following Methoxyflurane Anesthesia—Patients* with Clinically Evident Renal Dysfunction

	Serum Inorganic Fluoride (μmoles/liter)						
	Postoperative Day 1	Postoperative Day 2	Postoperative Day 3	Postoperative Day 4	Postoperative Day 5	Postoperative Day 6	Postoperative Day 7
Patient 3	112	140	160	180†	124	120	98
Patient 4	142	176†	164	127	93	59	56
Patient 7	102	146†	107	91	72	36	32
Patient 9	144	180†	120	100	72	51	42
Patient 10	210	270†	165	120	80	52	45
Mean	142.0	182.4	143.2	123.6	88.2	63.6	54.6
SE	±18.9	±23.3	±12.3	±15.5	±9.7	±14.6	±11.5

* Patient numbers same as in reference 10.
† Peak value (mean 190.4 ± 20.9 μm/l).

concentration.[12] § Urinary oxalate assay was carried out in a Warburg respirometer using the oxalic acid decarboxylase method.[13] ¶

For purposes of analysis, patients anesthetized with methoxyflurane who had abnormalities in laboratory tests only were placed in one subgroup. These patients had hypernatremia, serum hyperosmolality, elevated BUN, increased serum creatinine, increased serum uric acid, and decreased uric acid clearance. Patients who had the above-mentioned abnormalities as well as polyuria with delayed return of ability to concentrate urine, marked weight losses, and thirst were considered to have clinical renal dysfunction and were placed in another subgroup.

Results

All patients anesthetized with methoxyflurane had highly significant increases in serum inorganic fluoride concentration (tables 1 and 2, fig. 1). Patients with clinically evident re-

§ The effect of storage on fluoride concentration was determined in serum and urine samples from patients anesthetized with methoxyflurane but not included in the study. Fluoride concentration increased as much as 3 per cent after refrigeration at 4 C for 48 hours, with no further change following frozen storage.

¶ Oxalic acid determinations repeated on several specimens after 60 days of frozen storage showed no significant change.

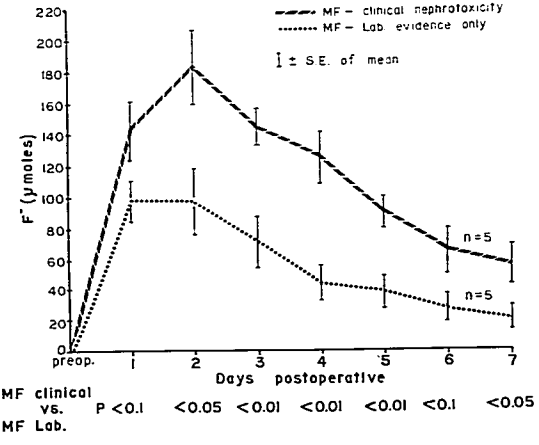

FIG. 1. Mean daily serum inorganic fluoride concentrations. Patients anesthetized with methoxyflurane were divided into two subgroups, those with laboratory abnormalities in renal function and those with laboratory abnormalities as well as clinically evident renal dysfunction (see text). Preoperative inorganic fluoride concentration was approximately 1 μm/l in all patients, with no change noted following halothane anesthesia. F^- (μmoles) = inorganic fluoride, μmoles/l.

nal dysfunction had significantly greater ($P < 0.02$) elevations in mean peak serum inorganic fluoride concentrations (190.4 ± 20.9 μm/l) than patients with abnormalities in laboratory tests only (105.8 ± 17.0 μm/l). In contrast to these findings, postoperative serum inorganic fluoride concentration remained approximately 1 μm/l in every patient anesthetized with halothane.

Mean 24-hour urinary inorganic fluoride excretion was markedly increased in every patient anesthetized with methoxyflurane, from 67 μm/day preoperatively to 4,760 μm on the first day following anesthesia (fig. 2). There was no difference between urinary fluoride excretion values for the patients in the two methoxyflurane subgroups. Patients anesthetized with halothane had only slight increases in urinary inorganic fluoride excretion.

Oxalic acid excretion was increased in all patients anesthetized with methoxyflurane (tables 3 and 4). Once again, patients with clinically evident renal dysfunction had significantly greater ($P < 0.05$) mean peak oxalic acid excretion (286.8 ± 39.3 mg/24 hours) than those with only laboratory abnormalities in renal function (130.6 ± 51.4 mg/24 hours). Patients anesthetized with halothane excreted 30.0 ± 6.0 mg/24 hours of oxalic acid in the urine preoperatively, with no significant change after anesthesia.

Discussion

Taves[9] reported increased concentrations of inorganic fluoride in the serum and urine of a patient who had renal dysfunction following methoxyflurane anesthesia. Frascino[14] reported oxalic acid crystals in renal biopsy specimens and increased urinary oxalic acid excretion in several patients with postoperative renal insufficiency after methoxyflurane anesthesia. This finding, however, was not attributed to methoxyflurane biotransformation. Holaday *et al.*[15] recently identified dichloroacetic acid and methoxydifluoroacetic acid in the urine of patients anesthetized with methoxyflurane; they have postulated the formation of oxalic acid as one metabolite of methoxyflurane. Our study demonstrated increases in serum and urinary inorganic fluoride and urinary oxalic acid in all patients studied after methoxyflurane anesthesia, with no such increases in patients anesthetized with halothane. This strongly suggests derivation of both of these substances from metabolism of methoxyflurane.

The observed correlation between markedly elevated serum inorganic fluoride and urinary oxalic acid excretion and clinically evident renal dysfunction suggests that renal dysfunction in these cases is related to the degree of metabolism. It is not known which of the metabolites, alone or in combination, is re-

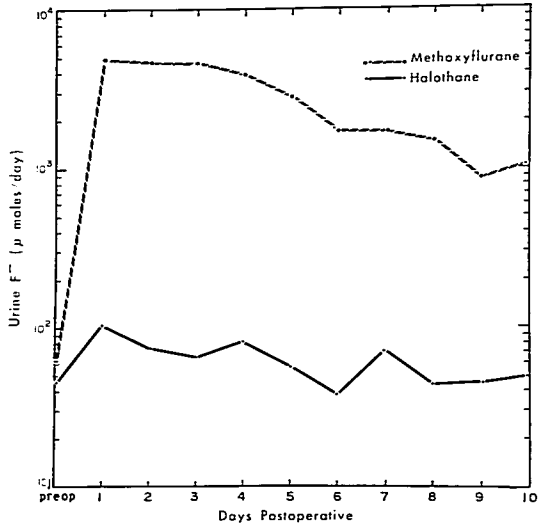

FIG. 2. Mean 24-hour urinary inorganic fluoride excretion following methoxyflurane (n = 10) and halothane (n = 5) anesthesia. There was no difference between the two methoxyflurane subgroups. Note that the graph is a semilogarithmic plot.

sponsible for renal dysfunction, nor is the possibility that molecular methoxyflurane is the offending agent excluded. High levels of oxalic acid in people who have abnormal oxalic acid metabolism may result in chronic renal disease, with kidney stone formation.[16] Acute oxalic acid intoxication results in classical anuric or oliguric renal failure rather than the polyuric renal insufficiency seen in the present study.[17] Therefore, oxalic acid-induced renal dysfunction following methoxyflurane anesthesia seems unlikely.

Evidence that inorganic fluoride may produce a renal lesion is not clear, but we suspect that inorganic fluoride may be the nephrotoxic substance. Prior to the present study, a statistical correlation between inorganic fluoride level and renal dysfunction in man had not been reported. Apart from Taves' report, we found only two documented cases of fluoride-

TABLE 3. Urinary Oxalic Acid Excretion Following Methoxyflurane Anesthesia—Patients* with Laboratory Abnormalities in Renal Function

	Urinary Oxalic Acid Excretion (mg/24 hours)	
	Preoperative Average	Peak Postoperative Excretion
Patient 5	21	68
Patient 6	7	81
Patient 8	15	63
Patient 11	7	334
Patient 12	37	107
Mean	17.4	130.6
SE	±5.6	±51.4

* Patient numbers same as in reference 10.

TABLE 4. Urinary Oxalic Acid Excretion Following Methoxyflurane Anesthesia—Patients* with Clinically Evident Renal Dysfunction

	Urinary Oxalic Acid Excretion (mg/24 hours)	
	Preoperative Average	Peak Postoperative Excretion
Patient 3	33	442
Patient 4	8	261
Patient 7	12	179
Patient 9	14	301
Patient 10	40	319
Mean	21.4	286.8
SE	±6.3	±39.4

* Patient numbers same as in reference 10.

induced renal dysfunction unrelated to methoxyflurane, and in neither was fluoride concentration measured or renal function evaluated.[18, 19] The most compelling arguments for fluoride-induced renal dysfunction are the consistent elevation of serum inorganic fluoride concentrations in all patients studied, with the greatest increases seen in patients with clinically evident renal dysfunction; the occurrence of polyuric renal insufficiency in rats fed a diet high in sodium fluoride[20]; and the known potent inhibitory effects of inorganic fluoride on many enzyme systems,[21] including those thought to be involved in the action of antidiuretic hormone.[22]

To explain the increases in inorganic fluoride concentration and oxalic acid excretion following methoxyflurane anesthesia, we propose two complementary metabolic routes for biotransformation of methoxyflurane (fig. 3). These are based on our findings, other metabolites identified previously,[9, 15, 23, 24] and enzyme systems known to exist in man.

Pathway I: It has been shown that methoxyflurane can be O-demethylated in the liver by a microsomal enzyme system requiring TPNH and molecular oxygen.[24] Enzymatic cleavage of a carbon–fluorine bond is very rare because of its high bond energy. The initial cleavage of methyl ether would provide an energetically favorable route to dehydrofluorination.[25] The proposed product, 2,2-dichloro-1,1-difluoroethanol (*1*), is chemically unstable and would spontaneously decompose to 2,2-dichloroacetyl fluoride (*2*), with the liberation of a molecule of hydrogen fluoride. Acetyl fluoride would be further hydrolyzed by cellular water to dichloroacetic acid (*3*),[15] with the liberation of another molecule of hydrogen fluoride. Some of the dichloroacetic acid may be excreted, while a second part may be oxidatively dechlorinated by dehalogenases known to exist in the liver,[26] producing glyoxalic acid (*4*),[27] which is enzymatically oxidized to oxalic acid (*5*).

Pathway II: Methoxyflurane is enzymatically

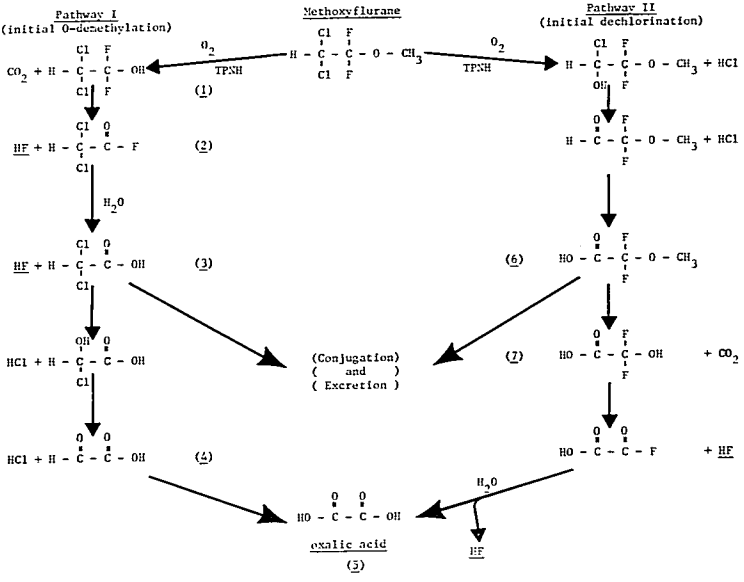

FIG. 3. Proposed metabolic pathways for biotransformation of methoxyflurane.

dechlorinated by a process requiring TPNH and molecular oxygen.[24] A portion of the proposed product, 2,2-difluoromethoxyacetic acid (6),[15] may be excreted, while some may be O-demethylated by liver enzymes producing the unstable difluorohydroxyacetic acid (7). Subsequent dehydrofluorination and hydrolysis result in oxalic acid (5) formation.

Though the elevated concentration of urinary oxalic acid may be due to interference with intermediary metabolism, it is almost certain that its increase and also that of inorganic fluoride result from the biotransformation of methoxyflurane. It also appears that one or both of these substances, but probably inorganic fluoride, may be responsible for the renal dysfunction seen after administration of methoxyflurane.

The authors thank Mrs. S. Scoggins and Mr. G. L. Shue for skillful technical assistance and Drs. E. N. Cohen and J. W. Bellville for helpful discussion and support.

References

1. Crandell WB, Pappas SG, Macdonald A: Nephrotoxicity associated with methoxyflurane anesthesia. ANESTHESIOLOGY 27:591–607, 1966
2. Austin WH, Villandry PJ: Methoxyflurane and renal function. ANESTHESIOLOGY 28:637, 1967
3. Elkington SG, Goffinet JA, Conn HO: Renal and hepatic injury associated with methoxyflurane anesthesia. Ann Intern Med 69: 1229–1236, 1968
4. Kuzucu EY: Methoxyflurane, tetracycline, and renal failure. JAMA 211:1162–1164, 1970
5. Lapointe A, Belle-Binda N: Nephrotoxicite associeé au methoxyflurane. Canad Anaesth Soc J 17:145–156, 1970
6. Lebowitz MH: Nephrogenic diabetes insipidus following methoxyflurane anesthesia: A report of two cases. Anesth Analg Curr Res 48:233–236, 1969
7. Mignault G, Labrecque B, Hamel S: Methoxyflurane et nephrotoxicite: Etude de la fonction renale de 22 malades anesthesies au methoxyflurane. Canad Anaesth Soc J 17:331–340, 1970
8. Pezzi PJ, Frobese AS, Greenberg SR: Methoxyflurane and renal toxicity. Lancet 1: 823, 1966
9. Taves DR, Fry BW, Freeman RB, *et al.*: Toxicity following methoxyflurane anesthesia. II. Fluoride concentration in nephrotoxicity. JAMA 214:91–95, 1970
10. Mazze RI, Shue GL, Jackson SH: Renal dysfunction associated with methoxyflurane anesthesia: A randomized prospective clinical evaluation. JAMA 216:278–288, 1971
11. Saidman LJ, Eger EI II, Munson ES, *et al.*: Minimum alveolar concentrations of methoxyflurane, halothane, ether and cyclopropane in man: Correlation with theories of anesthesia. ANESTHESIOLOGY 28:994–1002, 1967
12. Fry BW, Taves DR: Serum fluoride analysis with the fluoride electrode. J Lab Clin Med 75:1020–1025, 1970
13. Ribeiro ME, Elliott JS: Direct enzymatic determination of urinary oxalate. Invest Urol 2:78–81, 1964
14. Frascino JA, Vanamee P, Rosen PP: Renal oxalosis and azotemia after methoxyflurane anesthesia. New Eng J Med 283:676–679, 1970
15. Holaday DA, Rudofsky S, Treuhaft PS: The metabolic degradation of methoxyflurane in man. ANESTHESIOLOGY 33:579–593, 1970
16. Hockaday TDR, Clayton JE, Frederick EW, *et al.*: Primary hyperoxaluria. Medicine (Balt) 43:315–345, 1964
17. Jeghers H, Murphy R: Practical aspects of oxalate metabolism. New Eng J Med 233: 208–215, 1945
18. Goldemberg L: Tratamiento de la enfermedad de Basedow 'y del hipertiroidismo por fluor. Rev Soc Med Int Soc Tisiolog 6:217–242, 1931
19. Maes J, Dufax J, Vanderbroucke J: Chronische fluorintoxicatie. Presentatie van een geval met nierensufficientie. Acta Clin Belg 15: 65–80, 1960
20. Bond AM, Murray MM: Kidney function and structure in chronic fluorosis. Brit J Exp Path 33:168–176, 1952
21. Wiseman A: Effect of inorganic fluoride on enzymes, Handbook of Experimental Pharmacology, Pharmacology of Fluorides. Edited by FA Smith. New York, Springer-Verlag, 1970, pp 48–93
22. Orloff J, Handler JS: The cellular mode of action of antidiuretic hormone. Amer J Med 36:686–697, 1964
23. Van Dyke RA, Chenoweth MB: Metabolism of volatile anesthetics. ANESTHESIOLOGY 26:348–357, 1965
24. Van Dyke RA: Metabolism of volatile anesthetics. III. Induction of microsomal dechlorinating and ether-cleaving enzymes. J Pharmacol Exp Ther 154:364–369, 1966
25. Pauling L: The Nature of the Chemical Bond. Third edition. Ithaca, New York, Cornell University Press, 1960, p 83
26. Gillette JR: Metabolism of drugs and other foreign compounds by enzymatic mechanisms. Fortschr Arzneimittelforsch 6:11–73, 1965
27. Goldman P: The carbon–fluorine bond in compounds of biological interest. Science 164:1123–1130, 1969