Anesthesiology
V 38, No 6, June 1973

A Comparison of the Renal Effects of Isoflurane and Methoxyflurane in Fischer 344 Rats

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Isoflurane, although minimally metabolized to inorganic fluoride, did not cause renal functional or pathologic abnormalities in Fischer 344 rats. Mean peak serum inorganic fluoride levels were 6.5 ± 1.2 μM/l following 4.0 MAC hours of isoflurane anesthesia and 7.3 ± 0.6 μM/l following 15 MAC hours. By contrast, methoxyflurane caused dose-related polyuric nephrotoxicity. This was associated with mean peak serum inorganic fluoride levels of 56.0 ± 2.6 μM/l following 1.5 MAC hours and 79.4 ± 2.2 μM/l following 4.5 MAC hours of methoxyflurane anesthesia, respectively. In this animal model, an isoflurane exposure ten times greater than a nephrotoxic exposure to methoxyflurane did not result in sufficient metabolism to inorganic fluoride to cause nephrotoxicity. (Key words: Isoflurane; Methoxyflurane; Nephrotoxicity; Metabolism; Inorganic fluoride.)

ISOFLURANE (Forane,¶ Compound 469, 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) must be considered a possible nephrotoxic agent because it is a fluorinated compound.[1, 2] The Fischer 344 rat has been employed as a model for studies of fluoride nephrotoxicity.[3, 4, 5] Therefore, a controlled study was carried out in this rat strain to determine whether isoflurane is metabolized to inorganic fluoride and, if so, whether this metabolism alters renal function.

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Received from the Departments of Anesthesia and Pathology, Stanford University School of Medicine, Stanford, California 94305, and Veterans Administration Hospital, Palo Alto, California 94304. Accepted for publication January 17, 1973. Supported in part by VA Hospital, Palo Alto, California, USPHS Grants GM-18514-D1A1 and GM-00862, and Ohio Medical Products, division of Airco, Inc., Murray Hill, New Jersey.
¶ Trademark of Ohio Medical Products, division of Airco, Inc.

Methods

Thirty 10-month-old, inbred male Fischer 344 rats weighing an average of 369 g were divided at random into five groups of six rats each. Group I received no treatment. Group II received 0.25 per cent methoxyflurane for 1.5 hours. Group III received 0.5 per cent methoxyflurane for 3 hours. Group IV received 2 per cent isoflurane for 4 hours. Group V received 2 per cent isoflurane for 10 hours. Otherwise, all animals were treated identically. Food containing 1.1 μM/g inorganic fluoride and tap water containing one part per million inorganic fluoride (52.6 μM/l) were allowed *ad lib.* Artificial light was present from 8 AM to 6 PM each day, and room temperature was maintained at 22 to 24 C.

The rats were placed in individual metabolic cages. Four days were allowed for the animals to adapt to their cages; then four 24-hour urine samples were collected. On day 8, anesthesia was administered as defined above. Each group of rats was placed in a closed plastic chamber. Anesthetic vapor was administered through an inlet port in one side of the chamber and allowed to escape through a vent in the opposite side. Methoxyflurane was administered with a Pentec vaporizer. Isoflurane was administered with a Fluotec vaporizer that had been calibrated for isoflurane with a Varian 1440 gas chromatograph. Oxygen at 6 l/min was employed as the carrier gas. Anesthetic concentrations in the chambers were checked at 30-minute intervals by gas chromatography, and vaporizer adjustments were made when necessary to keep chamber concentrations constant. Rectal temperature was continuously monitored with a Yellow Springs telethermometer and maintained between 37 and 39 C with the aid of a water mattress on the floor of the

Table 1. Serum and Urinary Values, Mean ± SE

	Period	Serum				Urine				
		Sodium† (mEq/l)	Osmolality (mOsm/kg)	Urea Nitrogen (mg/100 ml)	Inorganic Fluoride (μg/l)	Sodium† (mEq/l)	Potassium† (mEq/l)	Osmolality (mOsm/kg)	Urea Nitrogen (mg/100 ml)	Inorganic Fluoride (μg/l)
Group I, control	Premeasthetic*	137.7 ±0.5	292.2 ±0.9	18.1 ±0.8	2.2 ±0.2	150 ±3	371 ±5	2,435 ±48	3,869 ±102	108 ±8
	Post anesthetic*	137.5 ±0.5	292.1 ±0.7	17.2 ±0.7	3.0 ±0.3	160 ±7	374 ±19	2,508 ±70	3,608 ±108	253 ±19
Group II, 0.25 per cent methoxyflurane, 1.5 hours	Premeasthetic	138.2 ±0.5	290.7 ±2.5	17.9 ±0.6	2.8 ±0.5	155 ±4	276 ±9	2,521 ±78	3,883 ±132	237 ±13
	Post anesthetic	138.7 ±0.8	291.4 ±0.9	18.6 ±0.7	56.0‡ ±2.6	122‡ ±2	291‡ ±12	2,153‡ ±58	3,208 ±116	6,824‡ ±389
Group III, 0.5 per cent methoxyflurane, 3.0 hours	Premeasthetic	139.5 ±0.8	293.7 ±3.0	20.8 ±1.5	3.3 ±0.8	167 ±6	404 ±9	2,584 ±86	4,188 ±104	243 ±16
	Post anesthetic	139.0 ±0.8	297.1 ±2.6	19.9 ±0.9	70.1‡ ±2.2	83‡ ±8	184‡ ±17	1,547‡ ±141	1,910‡ ±141	6,155‡ ±512
Group IV, 2.0 per cent isoflurane, 1.0 hours	Premeasthetic	139.5 ±0.8	290.5 ±2.5	18.5 ±0.7	2.1 ±0.2	162 ±5	399 ±7	2,509 ±20	4,128 ±101	242 ±6
	Post anesthetic	138.3 ±0.9	298.5 ±1.6	18.2 ±0.7	6.5† ±1.2	151‡ ±4	374 ±12	2,552 ±10	4,033 ±208	2,525‡ ±520
Group V, 2.0 per cent isoflurane, 10.0 hours	Premeasthetic	138.6 ±0.4	297.5 ±3.5	18.5 ±0.4	2.3 ±0.2	157 ±6	372 ±12	2,448 ±84	3,820 ±82	219 ±8
	Post anesthetic	139.5 ±0.4	293.6 ±1.2	15.6 ±0.7	7.3‡ ±0.6	120 ±27	383 ±10	2,262 ±93	3,273 ±220	3,178‡ ±142

* Rats in this group were not anesthetized. Collection periods parallel those for anesthetized rats.
† $P < 0.05$, significant premeasthetic–postanesthetic difference compared with control group.
‡ $P < 0.01$, significant premeasthetic–postanesthetic difference compared with control group.

TABLE 2. Urinary Volumes, Solute Excretion, and Results of Miscellaneous Tests, Mean ± SE

Group	Period	Urine Volume (ml/21 hours)	Solute Excretion				Miscellaneous			
			Sodium Excretion (mEq/21 hours)	Potassium Excretion (mEq/21 hours)	Osmolal Excretion (mOsm/21 hours)	Inorganic Fluoride Excretion (mM/21 hours)	Urea Clearance (ml/min)	Osmolal Clearance (ml/min)	Body Weight (g)	Hematocrit (Per Cent)
Group I, control	Preanesthetic*	7.4 ±0.3	1.16 ±0.05	2.75 ±0.12	18.0 ±0.7	1.5 ±0.1	1.10 ±0.02	0.041 ±0.001	369 ±9	48.7 ±2.8
	Postanesthetic*	6.4 ±0.4	1.05 ±0.06	2.39 ±0.20	16.0 ±1.2	1.6 ±0.2	0.95 ±0.06	0.038 ±0.003	362 ±9	47.0 ±1.9
Group II, 0.25 per cent methoxyflurane, 1.5 hours	Preanesthetic	6.5 ±0.3	1.01 ±0.06	2.43 ±0.10	16.3 ±0.6	1.5 ±0.1	0.99 ±0.01	0.038 ±0.002	376 ±10	48.3 ±1.3
	Postanesthetic	8.5‡ ±0.4	1.03 ±0.06	2.44 ±0.05	18.2‡ ±1.0	68.3‡ ±5.5	1.02 ±0.06	0.042 ±0.003	367‡ ±9	48.8 ±1.2
Group II, 0.5 per cent methoxyflurane, 3.0 hours	Preanesthetic	6.1 ±0.2	±1.02 ±0.05	2.46 ±0.07	15.7 ±0.4	1.5 ±0.1	0.88 ±0.06	0.037 ±0.001	357 ±9	49.0 ±1.3
	Postanesthetic	14.4‡ ±1.5	1.12 ±0.12	2.62‡ ±0.09	21.3‡ ±1.2	71.1‡ ±6.2	0.93 ±0.08	0.050‡ ±0.003	344 ±9	47.8 ±1.2
Group IV, 2.0 per cent isoflurane, 4 hours	Preanesthetic	7.1 ±0.4	1.14 ±0.05	2.70 ±0.12	18.2 ±1.0	1.7 ±0.1	1.11 ±0.05	0.042 ±0.002	348 ±10	49.3 +1.0
	Postanesthetic	5.7 ±0.3	0.80‡ ±0.05	2.11 ±0.08	14.5 ±0.8	14.3‡ ±3.1	0.87 ±0.04	0.034 ±0.002	338 ±10	45.8 ±1.7
Group V, 2.0 per cent isoflurane, 10 hours	Preanesthetic	7.0 ±0.3	1.10 ±0.07	2.60 ±0.14	17.1 ±0.9	1.5 ±0.1	1.01 ±0.06	0.040 ±0.002	307 ±17	48.5 ±2.6
	Postanesthetic	5.4 ±0.6	0.69 ±0.12	1.65‡ ±0.19	12.2 ±1.2	19.3‡ ±3.8	0.83 ±0.14	0.029 ±0.003	281 ±10	44.7 ±1.5

*Rats in this group were not anesthetized. Collection periods parallel those for anesthetized rats.
† $P < 0.05$, significant preanesthetic–postanesthetic difference compared with control group.
‡ $P < 0.01$, significant preanesthetic–postanesthetic difference compared with control group.

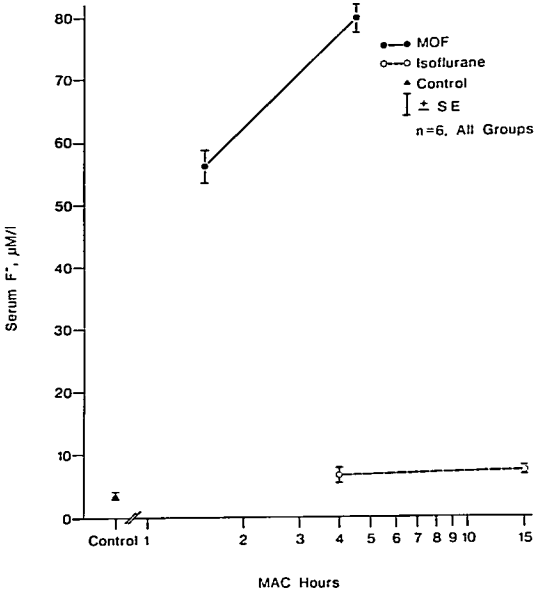

FIG. 1. Serum inorganic fluoride (F^-) concentrations at the times of peak response, days 1 and 2 postanesthesia. Values are plotted at the anesthetic dose, in MAC hours, measured for each group: methoxyflurane (MOF) at 1.5 and 4.5 MAC hours: isoflurane at 4 and 15 MAC hours. Values for the unanesthetized control group, for the same period, are shown for comparison. Both methoxyflurane- and isoflurane-treated rats had significant increases in serum inorganic fluoride concentration; however, the changes were much greater in the methoxyflurane-treated groups.

chamber. During the last 30 minutes of anesthesia, a 0.3 ml blood specimen was obtained from the tail of each animal; anesthetic concentration was determined using a direct-injection gas chromatographic method,[6] and *p*H, P_{O_2} and P_{CO_2} were measured with an Instrumentation Laboratories #113 Blood Gas Analyzer. At the termination of anesthesia, rats received 100 per cent oxygen until awake, then were returned to their individual metabolic cages, where food and water were allowed *ad lib.* Twenty-four-hour urine collections were then resumed.

One ml of blood was obtained from the tail of each rat four days prior to anesthesia and on the first and second days after anesthesia. One rat from each group was sacrificed on the first and second postanesthetic days and the remainder were sacrificed on the fourth postanesthetic day. At the time of sacrifice a blood sample was obtained and kidney tissue was removed and prepared for study with light and electron microscopy.[5] Seventy-five microliters of blood from each sample were anticoagulated for hematocrit determination, and the remainder allowed to clot. After centrifugation, the serum was decanted and frozen at −10 C. Urinary volume was measured at the end of each 24-hour collection and a sample was frozen for subsequent analysis.

Serum and urine specimens were analyzed for: sodium and potassium concentrations with an Instrumentation Laboratories #143 Flame Photometer; urea nitrogen concentration with a Technicon AutoAnalyzer; fluoride activity with an Orion ion-specific fluoride electrode and #801 Ionalyzer; osmolality with a Fiske Model G-82 Osmometer. Twenty-four-hour sodium, potassium, inorganic fluoride and osmolal excretions were calculated, as were urea nitrogen and fluoride clearances. Hematocrit was determined by micromethod. Animals were weighed daily.

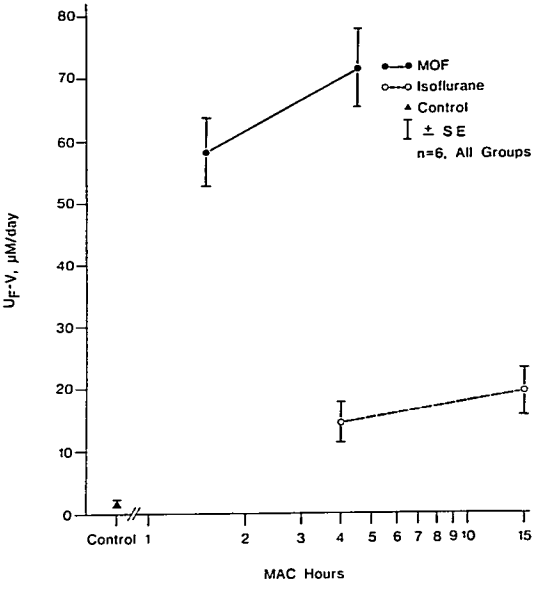

FIG. 2. Twenty-four inorganic fluoride excretion (U_F-V) at the times of peak response, days 1 and 2 postanesthesia. Values for the unanesthetized control group, for the same period, are shown for comparison. Both methoxyflurane- and isoflurane-treated rats had significant increases in inorganic fluoride excretion; however the changes were much greater in the methoxyflurane-treated groups.

ANALYSIS OF DATA

The mean preanesthetic value of each variable for each rat was calculated and means were determined for each group. The effect of anesthetic treatment was determined by subtracting the preanesthetic mean from the mean of determinations on the first two postanesthetic days, and by comparing differences in treated groups with differences in control animals. The first two postanesthetic days were selected for analysis because the greatest changes in serum fluoride occurred during this period. An expression for anesthetic dosage was derived as follows: Blood anesthetic concentration was converted to MAC, assuming values ranging from 10 to 13 mg/100 ml in the blood were equivalent to one MAC for both isoflurane and methoxyflurane. The resulting MAC value, 1.0 or 1.5, was multiplied by duration of anesthesia, yielding a value the dimension of which was *MAC hours*. Student's t test was used for statistical analysis.

Results

Prior to anesthesia all variables for all groups were within normal limits and there was no significant difference between groups except those which might be attributed to chance alone (tables 1 and 2). There was no significant change in the variables measured in control animals throughout the study. Following methoxyflurane anesthesia there were dose-related increases in 24-hour urine volume and reciprocal decreases in urinary osmolality; serum inorganic fluoride level and 24-hour urinary fluoride excretion also showed dose-related increases (figs. 1 and 2, tables 1 and 2). Isoflurane anesthesia resulted in no change in urinary volume or osmolality. There was an increase in mean serum inorganic fluoride concentration; however, the mean peak values were less than 8 μM/l in both isoflurane-treated groups, with no single value greater than 13.9 μM/l. Urinary fluoride excretion was increased in both isoflurane-treated groups, but was still

TABLE 3. Blood Gases and Anesthetic Doses, Mean ± SE

	pH	P_{O_2} (torr)	P_{CO_2} (torr)	Anesthetic Concentration (mg/100 ml)	Equivalent MAC	×	Duration of Anesthesia (Hours)	=	Dose (MAC Hours)
Group II, 0.25 per cent methoxyflurane	7.55 ±0.01	87* ±4	31 ±1	11.9 ±0.3	1.0	×	1.5	=	1.5
Group III, 0.5 per cent methoxyflurane	7.49 ±0.01	89* ±1	31 ±2	20.4 ±0.4	1.5	×	3.0	=	4.5
Group IV, 2.0 per cent isoflurane	7.53 ±0.01	374 ±6	34 ±1	13.0 ±0.4	1.0	×	4.0	=	4.0
Group V, 2.0 per cent, isoflurane	7.51 ±0.01	254 ±15	32 ±1	18.9 ±0.3	1.5	×	10.0	=	15.0

* Sampled outside the anesthetic chamber.

only a third that seen following low-dose methoxyflurane anesthesia, or less (figs. 1 and 2, table 2). Changes in other variables following anesthesia with either methoxyflurane or isoflurane followed no discernible pattern and occurred with no greater frequency than would be due to chance alone.

One rat exposed to isoflurane for 10 hours had gross hematuria and only 2.5 ml of urine during the 24 hours following anesthesia. This was not accompanied by an increase in BUN or any abnormality in other variables.

There was no evidence of intra-anesthetic acidosis, hypoxia, or hypercarbia. Blood *p*H, P_{O_2}, P_{CO_2} anesthetic concentration, the equivalent MAC, and anesthetic dose are shown in table 3.

PATHOLOGY

No renal abnormality was demonstrable with light or electron microscopy in rats anesthetized with isoflurane in either dose. The rat anesthetized with isoflurane which had gross hematuria had hemorrhagic cystitis but no other urinary-tract abnormalities. Renal histologic changes following methoxyflurane were essentially the same as those reported earlier[2, 5]; dose-related, mitochondrial swelling of proximal convoluted tubule cells was visible with electron microscopy, while proximal convoluted tubule dilatation was visible with light microscopy at the higher methoxyflurane dose only.

Discussion

Isoflurane and methoxyflurane are both fluorinated methylethyl ethers. Since metabolism of methoxyflurane to inorganic fluoride is responsible for its dose-related nephrotoxicity,[1-5] it is important to determine whether isoflurane is metabolized to inorganic fluoride and, if so, whether this metabolism results in nephrotoxicity. Initial animal and human studies of isoflurane have failed to reveal any significant metabolism or organ toxicity.[7-9] In studies of miniature swine receiving a subanesthetic concentration of isoflurane, Halsey *et al.*[7] found negligible hepatic extraction; their results imply that catabolism of isoflurane did not occur in the liver of this species. Serum and urinary inorganic fluoride concentrations were not measured. Byles *et al.*[8] observed no abnormality in urine, blood, or kidney sections of rhesus monkeys exposed to 1.5 to 2.5 per cent isoflurane for 4 to 16 hours. That renal abnormalities were also absent following administration of enflurane, halothane, and methoxyflurane suggests that the rhesus monkey is not a suitable animal species for renal toxicity studies of anesthetic agents.[4] In the only human studies in which renal status has been evaluated, Dobkin *et al.*[9] found no increase in blood urea nitrogen or creatinine concentration following 204 clinical isoflurane anesthetics lasting an average of 178 minutes.

In the present study, isoflurane was administered to Fischer 344 rats to assess its metabolism to inorganic fluoride and its effects on renal function. In previous reports we have shown that the Fischer 344 rat strain develops nephrotoxicity from metabolism of methoxyflurane to inorganic fluoride or from parenteral administration of sodium fluoride.[3-5] To as-

sure that the sensitivity of the strain had not changed, two groups of Fischer 344 rats receiving methoxyflurane acted as internal standards for this experiment. These rats developed essentially the same renal functional and pathologic lesions as Fischer 344 rats previously studied,[2-5] indicating the strain was still suitable for studies of nephrotoxicity of inorganic fluoride. In comparison, there were no renal functional or pathologic lesions in Fischer 344 rats receiving isoflurane. Halothane anesthesia has also been shown not to cause renal abnormalities in this rat strain.[3] Metabolism of isoflurane to inorganic fluoride did occur, but even after ten hours of anesthesia at a blood concentration equivalent to 1.5 MAC (15 MAC hours), inorganic fluoride levels remained well below those associated with nephrotoxicity. In other experiments, Fischer 344 rats pretreated with 50 mg/kg/day of phenobarbital prior to isoflurane anesthesia had no renal functional or pathologic changes. This dose of phenobarbital induced the metabolism and exacerbated the nephrotoxicity of methoxyflurane in this rat strain (Cousins and Mazze, unpublished data).

The only genitourinary abnormality following isoflurane was hemorrhagic cystitis in one rat anesthetized for ten hours. In a previous study,[3] this complication occurred in four of six Fischer 344 rats anesthetized with 0.75 per cent methoxyflurane for six hours. Hemorrhagic cystitis has not been reported in humans anesthetized with isoflurane or methoxyflurane. This may represent organ-specific sensitivity of the bladder mucosa of Fischer 344 rats to inorganic fluoride; however, it is possible that a similar lesion could occur in man.

In summary, we have demonstrated that isoflurane, although minimally defluorinated, does not cause adverse renal effects in an animal model sensitive to the nephrotoxic effects of inorganic fluoride. If this pattern of response can be extrapolated to humans, it is likely that the extent of isoflurane metabolism will be slight and its renal effects will not be clinically significant.

Addendum

Additional studies were carried out in 16 Fischer 344 rats exposed to 2.0 per cent isoflurane for ten hours to better define the changes in serum inorganic fluoride in the 24 hours following anesthesia. There was an increase in serum inorganic fluoride level immediately after anesthesia, with the peak concentration, 19.5 ± 0.8 μM/l, occurring 12 hours postanesthesia. Twenty-four and 48 hours postanesthesia, serum inorganic fluoride levels were similar to those reported in the present study. The occurrence of peak serum inorganic fluoride levels 12 hours after isoflurane administration, compared with 24–48 hours following methoxyflurane anesthesia,[2] is probably related to the latter's greater solubility in fat and higher blood–gas partition coefficient.

The authors thank B. Hitt, Ph.D. for helpful discussion and support. Messrs G. Hernandez, L. Mosconi, M. Rendes, J. Scoggins and Mrs. S. Scoggins provided skillful technical assistance.

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