

Metabolism of Isoflurane in Fischer 344 Rats and Man

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The metabolism of isoflurane was studied in Fischer 344 rats and man. In both species, ionic fluoride and non-ionic fluoride were detected in urine, with ionic fluoride the principal metabolite in rats and non-ionic fluoride the predominant end-product in man. Thin-layer chromatography indicated that the non-ionic fluoride metabolite was trifluoroacetic acid (TFA). (Key words: Anesthetics, volatile: isoflurane; Biotransformation: isoflurane.)

ISOFLURANE (2,2,2-trifluoro-1-chloroethyl difluoromethyl ether, Forane,**), a new fluorinated inhalation anesthetic agent, has been reported not to be metabolized.¹ Metabolism of fluorinated anesthetics is important, since metabolism of methoxyflurane to ionic^{††} fluoride has been shown to result in dose-related, polyuric renal insufficiency in man² and Fischer 344 rats.^{3,4} Our recent study of the renal effects of isoflurane in Fischer 344 rats indicated that its administration was not associated with renal dysfunction, but metabolism to ionic fluoride was observed.⁵ The present study has

examined the excretion of nonvolatile urinary metabolites of isoflurane in Fischer 344 rats and man.

Methods and Materials

ANESTHESIA ADMINISTRATION

Eight 9-month-old inbred male Fischer 344 rats, bedded for 30 days on ground corncob, were placed in individual metabolic cages. They were allowed four days to adapt to their environment, following which 24-hour urine collections commenced. Food and water were allowed *ad lib.* throughout the experiment. Artificial light was present from 8 AM to 6 PM each day, and room temperature was maintained between 21 and 23 C.

On day 9, all rats were placed in a plastic chamber with a volume of approximately 100 liters. Anesthesia was administered with a Fluotec vaporizer calibrated for isoflurane at a concentration of 2 per cent for 4 hours, employing oxygen, 6 l/min, as the carrier gas. Anesthetic concentration in the chamber was determined at 30-minute intervals by gas chromatography. Rectal temperature, continuously monitored with Yellow Springs telethermometers, was maintained between 37 and 39 C with the aid of a water mattress under the chamber. During the last 30 minutes of anesthesia a 0.3-ml blood specimen was obtained from the tail of each animal; isoflurane concentration was determined by direct injection into a Varian 1440 gas chromatograph,⁶ and pH, P_{O₂} and P_{CO₂} were measured with an Instrumentation Laboratories #113 blood-gas analyzer. After anesthesia, rats received 100 per cent O₂ for an additional 10-15 minutes, by which time they were awake. They then were returned to their individual metabolic cages and 24-

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†† We have used the terms "non-ionic" and "ionic" fluoride rather than "organic" and "inorganic" fluoride. The former are more precise, since fluoride could be present in inorganic complexes which would not be detected with the ion-specific electrode prior to treatment with a chelating agent or combustion.

FIG. 1. Mean fluoride excretion following isoflurane anesthesia in eight Fischer 344 rats. Ionic fluoride was the major fluoride species detected.

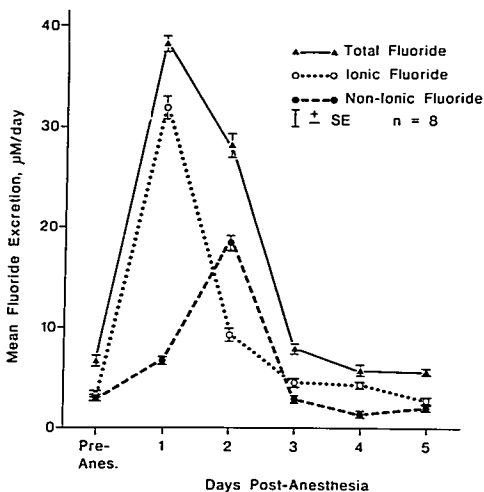
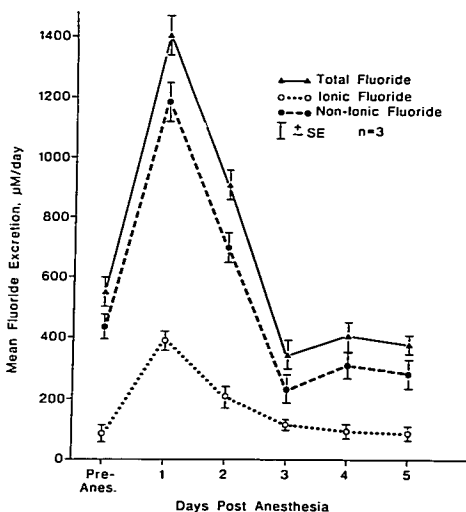


FIG. 2. Mean fluoride excretion following isoflurane anesthesia in three surgical patients. Non-ionic fluoride was the major fluoride species excreted.



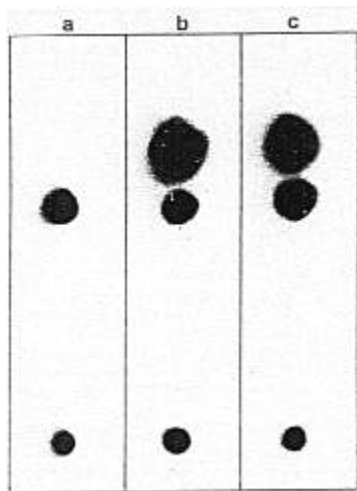


FIG. 3. Sketch of TLC plate spotted with three samples of rat urine. *a*, preanesthetic urine; *b*, preanesthetic urine to which TFA had been added to a concentration of 30 mg/ml; *c*, urine collected during the second postanesthetic day, the period of peak non-ionic fluoride excretion. R_f 0.60 spots were seen in all samples. R_f 0.72 spots were seen in both sample *b* and sample *c*, strongly suggesting the presence of TFA in sample *c*.

hour urine collections were continued for the next five days.

FLUORIDE ACTIVITY

Ionic fluoride activity of each urine sample was determined by the method of Taves, using an Orion ion-specific fluoride electrode.⁷ Total fluoride was determined by evaporating 0.25 ml of urine in the boat of a quartz combustion flask and combusting the residue at 850 C in oxygen.⁸ The residue was absorbed in 50 ml of 2.5 M sodium acetate buffer, pH 4.8, and fluoride activity again determined. Non-ionic fluoride was calculated as the difference between total and ionic fluoride. Ionic fluoride activity was also determined in each urine sample after EDTA was added to a concentration of 0.05 M.

THIN-LAYER CHROMATOGRAPHY (TLC)

TLC was performed on three urine samples from each rat: *a*, preanesthetic urine; *b*, preanesthetic urine to which trifluoroacetic acid (TFA) had been added to a concentration of 30 mg/ml; *c*, urine collected during the period of peak non-ionic fluoride excretion, the second postanesthetic day in rats and the first postanesthetic day in patients. A 1-ml amount of each sample was extracted three times with an equal volume of chloroform:methanol (1:1). Ten μ l of the aqueous phase were spotted on Analtech, 250 μ , silica gel, thin-layer plates and chromatographed in ascending fashion in ethanol:chloroform: NH_4OH (5:2:1). After drying, plates were sprayed with 0.5 per cent bromthymol blue in 80 per cent methanol which had been treated with 1.0 N NaOH until the solution just turned blue. All spots were scraped from the plate and combusted in the quartz flask. Residues were absorbed in 1.0 ml of acetate buffer and fluoride activity measured.

PATIENT STUDIES

Three adult, male surgical patients, ASA Class I, received isoflurane at a concentration of 0.9 per cent (0.8 MAC) for an average of 2.8 hours. Twenty-four-hour urine specimens were collected prior to and for five days following anesthesia and fluoride activity determined. TLC was performed as noted above, except that 6 ml of urine were concentrated to 1 ml by evaporating to dryness and suspending the residue in distilled water.

Results

FLUORIDE EXCRETION IN RAT URINE

Maximum excretion of ionic fluoride and total fluoride occurred in the first 24 hours postanesthesia, while non-ionic fluoride peaked during the second 24 hours postanesthesia (fig. 1). The ionic to non-ionic fluoride ratio was 5.3:1 on the first postanesthetic day and 0.53:1 on the second postanesthetic day. All values returned to preanesthetic levels by the third day after

TABLE 1. μM of Non-ionic Fluoride Resulting from Combustion of TLC Spots, Rat Urine*

Sample	μM Fluoride	
	R _f 0.60	R _f 0.72
Preanesthesia	72 \pm 5.0	—
Preanesthesia plus TFA	96 \pm 10.0	4136 \pm 150
Postanesthesia	74 \pm 6.0	400 \pm 25

* Mean \pm SE.

TABLE 2. μM of Non-ionic Fluoride Resulting from Combustion of TLC Spots, Human Urine*

Sample	μM Fluoride	
	R _f 0.60	R _f 0.72
Preanesthesia	71 \pm 9.0	—
Preanesthesia plus TFA	89 \pm 21.0	4077 \pm 210
Postanesthesia	74 \pm 8.0	1146 \pm 75

* Mean \pm SE.

isoflurane administration except ionic fluoride excretion, which remained slightly elevated for two additional days. Urinary volume did not change significantly throughout the experiment. Treatment of urine samples with EDTA prior to determining ionic fluoride activity did not significantly alter the results of this measurement.

FLUORIDE EXCRETION IN HUMAN URINE

Figure 2 depicts the excretion of the species of fluorinated compounds in human urine following isoflurane anesthesia. Peak ionic and non-ionic fluoride excretion occurred on the first postanesthetic day. The ionic to non-ionic fluoride ratio on that day was 1:6.5, and it was 1:5.0 on the second postanesthetic day. By the third day following isoflurane administration all values had returned to preanesthetic or lower levels. EDTA treatment of urine did not result in significant changes in ionic fluoride activity.

TLC OF RAT URINE

Figure 3 is a sketch of a typical chromatogram; acidic spots on the plate were identified by bromthymol blue dye. A spot with R_f 0.60 was present in every sample. The postanesthetic sample and the preanesthetic sample with added TFA had an additional spot, R_f 0.72. The results of combustion of TLC spots are shown in table 1. The R_f 0.72 spot contained a relatively large amount of non-ionic fluoride, whereas the R_f 0.60 spot contained much smaller amounts.

†† R_f = front ratio. Ratio of distance from origin to solvent front and origin to identified spot.

TLC OF PATIENT URINE

R_f 0.60 and R_f 0.72 spots were also seen in TLC plates prepared from urine of patients anesthetized with isoflurane. Results of combustion of these spots are shown in table 2. Once again, the R_f 0.72 spots contained a large amount of fluoride and the R_f 0.60 spots, only minimal amounts.

Discussion

There is conflicting evidence pertaining to the metabolism of isoflurane. Halsey *et al.*¹ measured hepatic extraction of isoflurane in miniature swine which had received sub-anesthetic doses. Extraction was negligible, from which they concluded that metabolism did not occur. However, measurements of isoflurane metabolites were not made. To the contrary, our previous study with Fischer 344 rats indicated that metabolism of isoflurane to ionic fluoride occurred, but at only one fifth the rate of methoxyflurane metabolism.^{3,5} The present study indicates that metabolism of isoflurane to ionic fluoride also occurs in man at approximately the same rate, relative to methoxyflurane metabolism.⁹ Furthermore, non-ionic fluoride metabolites were detected in both rat and human urines. The discrepancy between our results and those of Halsey *et al.*¹ may have resulted from one or more of the following factors: species differences in the metabolism of isoflurane, differences in isoflurane dosage, the occurrence of metabolism in organs other than the liver, or insufficient sensitivity of the hepatic extraction method.

Co-chromatography with TFA and combustion of the non-ionic residue to ionic fluoride

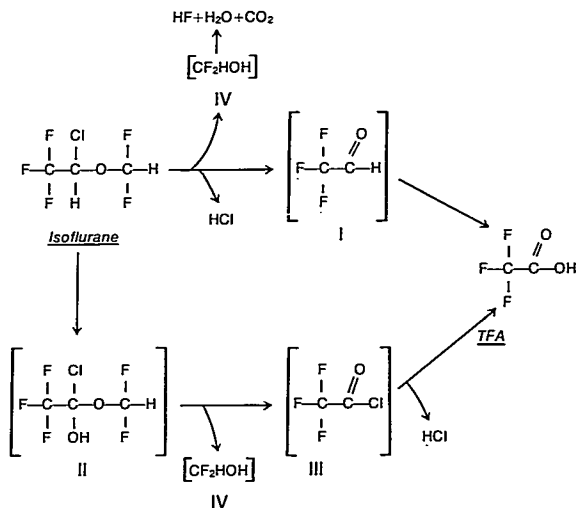


FIG. 4. Possible pathways of isoflurane metabolism to TFA. Compound I, trifluoroacetaldehyde, has not been identified, but may result from O-demethylation and oxidative dechlorination of isoflurane. Compound II, if formed, would decompose to the chloride of TFA (Compound III), which would react with water to form TFA. Compound IV, difluoroethanol, would be formed as result of both pathways and is probably present briefly as an intermediate in the formation of ionic fluoride.

is not unequivocal proof that the non-ionic metabolite of isoflurane is TFA. However, if it is TFA, it probably results from enzymatic O-demethylation and subsequent dechlorination to trifluoroacetaldehyde (TFAL). The latter would then be enzymatically oxidized to TFA (fig. 4). It is possible that trifluoroethanol (TFE) may also be an isoflurane metabolite, through other pathways. TFA has been positively identified as an organic metabolite of halothane¹⁰ and it is thought that TFAL and TFE may also be formed.¹¹ The toxicity of these compounds has been studied in animals in view of the possible association of halothane with postoperative jaundice and hepatic necrosis. TFE and, to a lesser extent, TFAL, were directly hepatotoxic in mice, causing fat accumulation and glycogen depletion in the liver within 24 hours of a large sublethal dose.¹² In the same study, TFA was two to ten times less toxic than TFE or TFAL, with its administration

resulting in increased deposition of glycogen in the liver. Inhibition of energy production, as evidenced by decreased ATP content and ATP/ADP ratio in mouse livers, is further evidence of the direct toxicity of TFA, TFAL, and TFE.¹³ Additionally, Rosenberg and Wahlstrom have shown that TFA and other trifluoro metabolites of halothane can form antigenic determinants, *i.e.*, act as haptens.¹⁴ The relevance of these studies to anesthetic-induced hepatic damage is not known, since TFA administered exogenously does not readily cross the cell membrane, whereas TFA resulting from anesthetic metabolism is formed intracellularly.

Several other aspects of the study are worthy of comment. EDTA added to urine did not increase ionic fluoride activity. If this had occurred it would have been due to chelation of divalent cations, releasing additional ionic fluoride from inorganic complexes. Also, the patterns of fluoride excretion in

man and rats were quite different. Almost all of the fluoride recovered in rat urine on post-anesthetic day 1 was ionic, whereas non-ionic fluoride was the primary fluoride excretory product in man. This could result from species differences in metabolism, more complete biotransformation of non-ionic intermediaries in rats than in man, or to species differences in renal excretion or binding of non-ionic fluoride to protein.

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Respiration

CLOSING VOLUME IN SMOKERS Measurements of conventional lung function and "closing volume" were made in 62 nonsmokers and 46 smokers, using radioactive argon. FEV_1 , MEFR, VC, FRC, airway resistance, and airway conductance were within normal limits in the nonsmokers. The sitting "closing volume" increased progressively with increasing age, and closing volume exceeded FRC at an average of 73 years of age. The supine closing volume surpassed FRC at about 46 years of age. In seven of 46 smokers, closing volume could not be measured because the expired air showed a continuous marked in-

crease in argon concentration, indicative of gross abnormalities in the distribution of ventilation. In 26 of the remaining 39 smokers, closing volume was above the upper limit for nonsmokers, and the critical age at which airway closure in the dependent lung zones was present during normal tidal breathing was lower than in nonsmokers. However, conventional lung function tests were abnormal in only 14 individuals of this group. (McCarthy, D. S., and others: *Measurement of "Closing Volume" as a Simple and Sensitive Test for Early Detection of Small Airway Disease*. *Amer. J. Med.* 52: 747-753, 1972.)