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Deuterated Methoxyflurane Anesthesia and Renal Function in Fischer 344 Rats

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Inorganic fluoride (F^-) production and renal function were assessed in six groups of Fischer 344 rats administered either methoxyflurane (MOF) or deuterated methoxyflurane (d_4-MOF). One untreated and one phenobarbital (PB)-treated group were exposed for two hours to either air, 0.5 per cent (v/v) MOF, or 0.5 per cent (v/v) d_4-MOF. Serum and urinary F^- and serum urea nitrogen and creatinine were measured. Urine volume and urinary F^- excretion were only slightly greater among MOF than among d_4-MOF exposed animals. Pretreatment with PB, however, greatly enhanced F^- production in MOF-exposed animals leading to marked renal impairment but only slightly enhanced F^- production in d_4-MOF animals leading to mild renal impairment. Thus, only in PB-pretreated animals could a biologically significant difference in nephrotoxicity be demonstrated for MOF and d_4-MOF. (Key words: Anesthetics, volatile: methoxyflurane; deuterated methoxyflurane. Toxicity, renal.)

SHORTLY AFTER ITS INTRODUCTION in 1960, methoxyflurane ($CHCl_2CF_2$-O-CH_3, Penthrane; MOF) gained wide use as a volatile inhalational anesthetic. It was soon reported, however, that exposure to MOF resulted in vasopressin-resistant polyuric renal insufficiency in humans.[1] The same syndrome could be produced in Fischer 344 rats, a strain which provided an animal model for the human condition.[2] Nephrotoxicity is the result of high serum levels of inorganic fluoride (F^-) released during biotransformation of MOF by the hepatic mixed function oxidase system.[3,4] Administration of other fluorinated anesthetics does not result in such high F^- levels because they are less prone to enzymatic attack and are not as lipid soluble. As a direct result of its toxicity, MOF is now little used in clinical practice. The substitution of deuterium for hydrogen in MOF, however, offers a possible method for reducing MOF metabolism and, thus, its toxicity. In an *in vitro* study, Hitt *et al.*[5] demonstrated that completely deuterated MOF (d_4-MOF) released less F^- than ordinary MOF. Furthermore, McCarty *et al.*[6] showed that rats anesthetized with d_4-MOF excreted slightly less F^- in urine than rats anesthetized with ordinary MOF. The presumed explanation for these findings is the fact that it is more difficult to break a carbon-deuterium bond than a carbon-hydrogen bond, *i.e.*, the so-called primary hydrogen isotope effect. In the present study we have assessed serum and urinary F^- levels and the renal effects which follow d_4-MOF or MOF administration to Fischer 344 rats. We also have assessed the role of enzyme induction by phenobarbital (PB) in the nephrotoxicity of both drugs.

Materials and Methods

ANIMALS

Sixty-four, 11-month-old male Fischer 344 rats§ weighing 350–400 g were used. They were housed six to a cage on ground corn cob bedding¶ for two weeks prior to the study. Temperature (21 ± 1° C) and light (6 A.M. to 7 P.M.) were controlled and rats were fed standard chow** and tap water *ad libitum* throughout the experiment. Replicate experiments were performed on two separate occasions.

On day 1, rats were transferred to individual metabolic cages. After a two-day adaptation period, 24-h urine collections were made for four consecutive days to establish normal urinary volumes and urinary levels of urea nitrogen (UN), F^-, and creatinine (Cr). A 2-ml tail blood sample was taken during this time for determination of basal serum F^- levels. On day six, half the animals were chosen randomly and PB, 1 mg/ml, was added to their drinking water. During the following week, three 24-h urine collections and a 2-ml tail blood sample were taken from all animals.

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¶ Tab Litter®, Paxton Processing Co, Paxton, Illinois 60957.

** Wayne Lab-Blox, Allied Mills, Inc, Chicago, Illinois 60951.

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Anesthetic Exposure

On day 15, PB and non-PB treated animals were divided randomly into three groups. One group of PB and non-PB treated rats were exposed for 2 h to 1) air; 2) 0.5 per cent (v/v) MOF; or 3) 0.5 per cent (v/v) d_4-MOF. Groups were designated: Air, MOF, d_4-MOF, Air and PB, MOF and PB, and d_4-MOF and PB. Exposures were performed in three identical, 150-l Plexiglas® chambers. Soda lime was spread on the chamber floor to absorb carbon dioxide. Rectal temperature was monitored with Yellow Springs® telethermometers and was maintained between 37° and 39° C with the aid of a heated water mattress under the chamber.

Oxygen concentration was monitored continuously using an Instrumentation Laboratory, model 402, polarographic oxygen analyzer and was maintained at 21 per cent by adding medical grade oxygen, as necessary. Because of the limited supply of d_4-MOF which has the same vaporization characteristics as MOF, anesthetics were vaporized by dripping them slowly onto gauze pads which were moved around the chamber. No anesthetic concentration gradients were detected within the chamber. Anesthetic concentration was determined every 3–5 min using a Hewlett-Packard® model 5830A gas chromatograph. When the correct concentration was achieved, the chamber was sealed and static conditions maintained. From time to time, maintenance amounts of anesthetic and oxygen were added in a manner similar to that used for charging the chamber. Air-exposed animals were subjected to the same conditions. At the end of exposure, the chambers were flushed with compressed air before rats were removed and returned to their metabolic cages. Twenty-four hour urine collections were made on days 1–4 and blood samples were taken on days 1, 2, and 4 after anesthesia. Rats were weighed daily.

Assays

Serum and urinary osmolarity were measured using an Advanced Cryomatic® osmometer. Urea nitrogen and Cr were determined with a Technicon® AAII dual channel AutoAnalyzer. Serum and urinary F^- levels were determined by the method of Fry and Taves[7] using an Orion® Model 801 ionanalyzer and ion-specific fluoride electrode. Urinary sodium (Na^+) and potassium (K^+) were measured with an Instrumentation Laboratory flame photometer. Urinary Na^+, K^+, and F^- excretions, and serum UN and Cr clearances were calculated.

Anesthetics

MOF was purchased from Abbott Laboratories,†† d_4-MOF was prepared by reacting 1,1-dichloro-2,2-difluoroethylene‡‡ with CD_3OD§§ containing 2 M $NaOCD_3$. All washings were done using 99.9 per cent D_2O.§§ d_4-MOF was purified as described by Miller *et al.*[8] and Larsen.¶¶ Isotopic purity was ascertained by both mass spectrometry and proton nuclear magnetic resonance. Analysis of d_4-MOF showed a CD_3/CH_3 ratio of 99 and a CCl_2D/CCl_2H ratio of 98; purity was greater than 99 per cent as determined by gas-liquid chromatography.

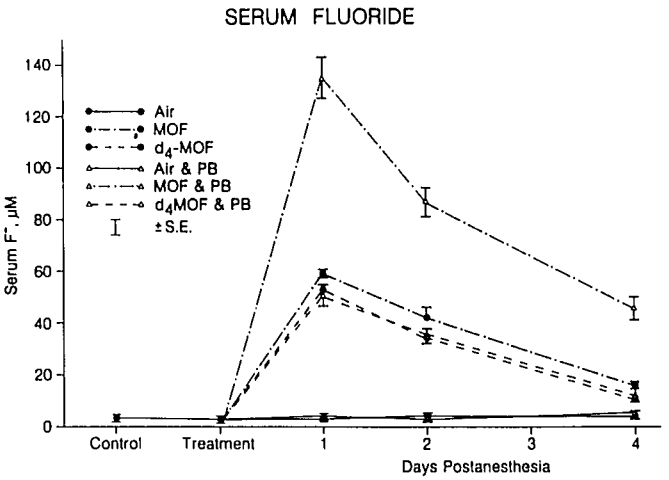

FIG. 1. Serum F^- prior to and following exposure to air, 0.5 per cent MOF, or 0.5 per cent d_4-MOF. MOF and PB group had greater serum F^- levels than other groups on days 1 ($P < 0.01$) 2, and 4 ($P < 0.05$) postanesthesia.

Statistical Analysis

Paired and unpaired Student's *t* tests, analysis of variance, and Newman-Kuels post hoc test were used as appropriate, for statistical comparisons; $P < 0.05$ was considered significant.

Results

Phenobarbital treatment greatly enhanced the defluorination of ordinary MOF. Peak serum F^- (135 ± 8 μM; fig. 1) and urine volume (36 ± 2 ml; fig. 2) on day 1 after anesthesia were greater ($P < 0.01$) for the MOF and PB group than for all other groups. In addition to polyuria, evidence of renal dysfunction in MOF and PB rats included increases in serum UN and Cr levels ($P < 0.01$) and decreased clearance of these substances (figs. 3 and 4). Abnormal renal function values returned to near preanesthetic levels by day 4 after anesthesia. Urinary F^- excretion (U_F-V) in the MOF and PB group was lower than in the MOF group ($P < 0.05$), no doubt

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¶¶ Larsen ER: US Patent No. 3, 104,202, Dow Chemical Corporation, 1963.

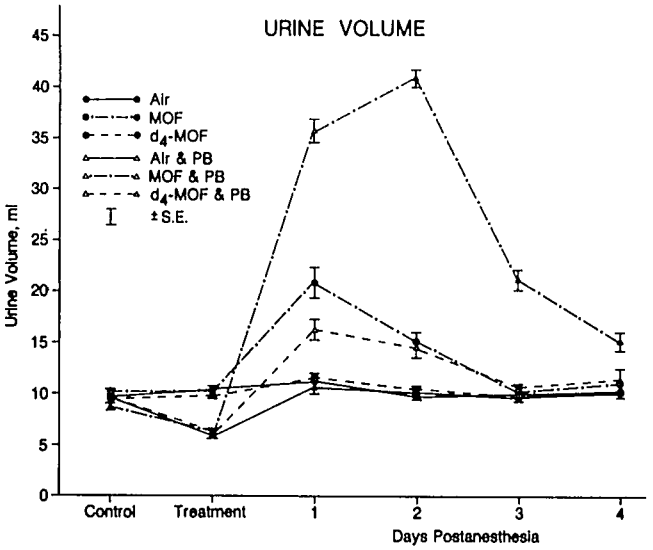

FIG. 2. 24-h urine volume prior to and following exposure to air, 0.5 per cent MOF, or 0.5 per cent d_4-MOF. MOF and PB group had greater urine volumes than other groups on days 1–3 postanesthesia ($P < 0.01$).

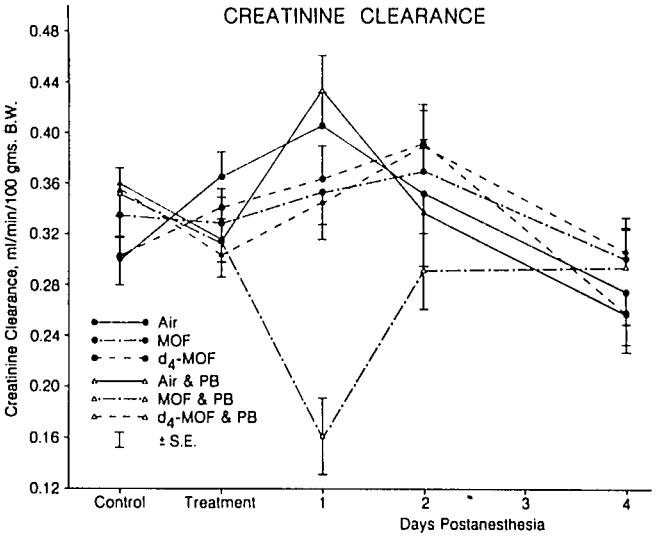

FIG. 4. Serum Cr clearance prior to and following exposure to air, 0.5 per cent MOF, or 0.5 per cent d_4-MOF. There was no difference among the groups other than for the MOF and PB group which had lower Cr clearances on day 1 postanesthesia ($P < 0.01$).

because kidney function was impaired in the former animals (fig. 5). As renal function recovered in MOF and PB rats, additional F^- was excreted.

In contrast to the striking differences in urine volume, serum F^-, UN, and Cr clearances between the MOF and PB and the MOF groups, there were no differences between the d_4-MOF and PB and the d_4-MOF groups (figs. 1, 3, and 4) except for a modest increase in urine volume on day 1 postanesthesia (fig. 2).

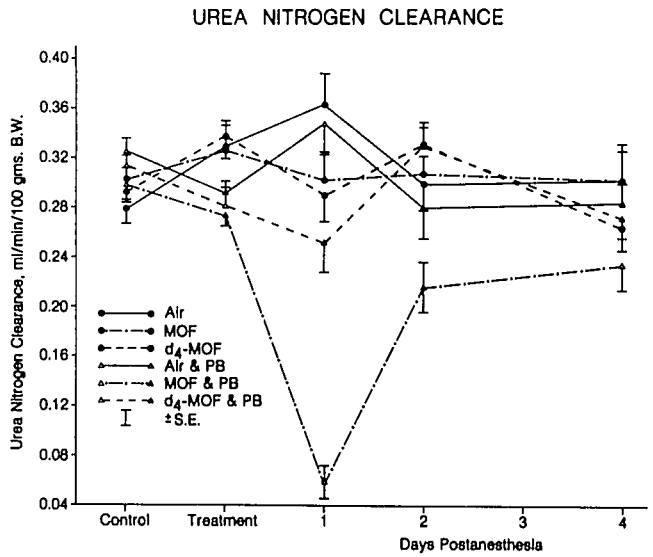

FIG. 3. Serum UN clearance prior to and following exposure to air, 0.5 per cent MOF, or 0.5 per cent d_4-MOF. There was no difference among the groups other than for the MOF and PB group which had lower UN clearances on days 1 and 2 postanesthesia ($P < 0.01$).

Among non-PB treated animals (MOF and d_4-MOF groups), urine volume (22 ± 3 *vs.* 13.2 ± 1 ml) and U_F-V (65 ± 5 *vs.* 44 ± 3 μmol/day) on day 1 after anesthesia were greater ($P < 0.05$) for MOF than for d_4-MOF (figs. 2 and 5) treated groups. U_F-V during the 4-day period after anesthesia was a modest 22 per cent greater in MOF than in d_4-MOF treated animals. Serum F^-, however, was not increased in the MOF group compared with the d_4-MOF group.

Discussion

In the present study, deuteration of MOF protected against increased defluorination and renal dysfunction associated with MOF anesthesia in PB-treated, *i.e.*, enzyme-induced, Fischer 344 rats. The exact extent of the reduction in defluorination cannot be quantified because the amount of F^- sequestered into bone or excreted by other than the urinary route was not determined. The approximate twofold difference in serum F^- levels between MOF and PB, and d_4-MOF and PB groups throughout the experiment, however, suggests that the reduction in defluorination was considerable. In contrast, there was no difference between the d_4-MOF and MOF groups in serum F^- levels.

The results of the present study are consistent with those obtained in *a prior in vitro* assay by Hitt *et al.*[5] In that experiment, d_4-MOF was defluorinated less than MOF by microsomes prepared from livers of PB-treated rats. Values derived at zero order kinetics were 3.8 and 19.6 nmol F^-/15 min per mg protein, for d_4-MOF and MOF exposed microsomes, respectively. The calculated

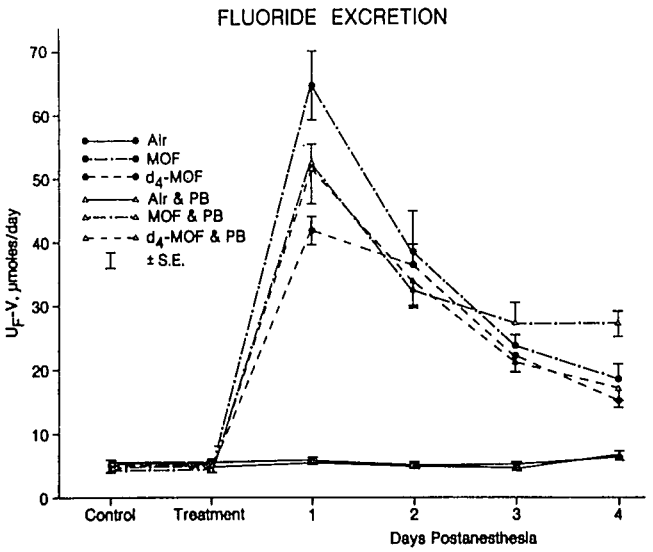

FIG. 5. Urinary F^- excretion prior to and following exposure to air, 0.5 per cent, MOF, or 0.5 per cent d_4-MOF. MOF group had greater F^- excretion than other groups on day 1 postanesthesia ($P < 0.01$).

kH/kD ratio, 5.24, indicates a primary hydrogen isotope effect. Using microsomes prepared from non-induced rat livers, however, the kH/kD ratio was only 1.19. Additionally, the present *in vivo* study extends the findings of McCarty *et al.*[6] who measured U_F-V in non-enzyme induced Fischer 344 rats exposed to 0.5 per cent (v/v) d_4-MOF and MOF for two hours. They observed that urinary F^- excretion over a 48-h period was decreased about 29 per cent in d_4-MOF exposed animals compared with MOF exposed animals. Since MOF administration did not result in polyuria in their study, it was impossible to assess the potential protective effect of MOF deuteration on renal function.

The data from the present and previous studies indicate that although defluorination of d_4-MOF is less than MOF, especially after enzyme induction, the clinical advantages of d_4-MOF would be small. Serum F^- levels and renal dysfunction following only two hours of d_4-MOF anesthesia were still unacceptable. Thus, it is unlikely that d_4-MOF will ever be commercially available, although it will remain a useful probe for understanding mechanisms of anesthetic biotransformation.

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