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Effect of Enzyme Induction on Nephrotoxicity of Halothane-Related Compounds

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Nephrotoxicity following administration of methoxyflurane has been shown to be directly related to anesthetic metabolism to inorganic fluoride. Enzyme induction should increase metabolic rate and the amount of inorganic fluoride that is released. *In vivo* studies in Fischer 344 rats show that enzyme induction with phenobarbital or phenytoin increases defluorination following methoxyflurane anesthesia but not after enflurane or isoflurane. *In vitro*, methoxyflurane defluorinase activity was increased far more than that of any of the other anesthetics. These data suggest that treatment with enzyme inducing drugs increases the risk of nephrotoxicity only if methoxyflurane is the anesthetic agent.

For purposes of this discussion, the potent, fluorinated, inhalational anesthetics are considered halothane-related compounds. They differ from halothane in that they are aliphatic ethers which when biotransformed liberate inorganic fluoride. Halothane is a fluorinated ethane which, except under conditions of extreme hypoxia, does not breakdown to inorganic fluoride (*1*). Since post-anesthetic nephrotoxicity is directly related to inorganic fluoride concentration in the serum, halothane should not be considered a nephrotoxin.

Nephrotoxicity associated with anesthetic administration was first reported by Crandell et al. (*2*) in a study of patients anesthetized with methoxyflurane ($CHCl_2—CF_2—O—CH_3$). Thirteen of 41 patients had polyuria with negative fluid balance, increased serum sodium and blood urea nitrogen concentrations and fixed urinary osmolality, close to that of serum. Polyuria was resistant to fluid deprivation and vasopressin administration. These findings were later confirmed in both man and animals (*3, 4*). Besides methoxyflurane, only enflurane ($CHClF—CF_2—O—CF_2H$) administration has resulted in decreased urinary concentrating ability. This review will discuss the relationship of anesthetic metabolism and enzyme induction to inorganic fluoride nephrotoxicity.

Evidence Relating Anesthetic Metabolism to Nephrotoxicity

Until the reports of Van Dyke et al. (*5, 6*) describing the biotransformation of diethyl ether, chloroform, and halothane, it was thought that inhalational anesthetics were pharmacologically active but metabolically inert chemicals. It is now known that all of the volatile inhalational anesthetics are metabolized. The relationship between anesthetic metabolism and nephrotoxicity was first suggested when increased concentrations of inorganic fluoride, a metabolite of methoxyflurane were noted in the serum and urine of a patient with renal dysfunction following methoxyflurane anesthesia (*7*).

Mazze and associates (*8, 9*) subsequently related serum inorganic fluoride concentration, methoxyflurane exposure in MAC-hours and the degree of nephrotoxicity. (MAC is defined as the minimum alveolar concentration of anesthetic required to prevent movement in response to skin incision in 50% of patients. MAC-hours is the product of anesthetic concentration expressed in units of MAC and time expressed in hours.) A clear dose-response relationship was seen. The earliest signs

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of nephrotoxicity occurred at inorganic fluoride levels of 50 μM with clinical nephrotoxicity evident at concentrations of 80–175 μM.

There have been additional studies of urinary concentrating ability following anesthesia with other fluorinated agents. Metabolism of isoflurane (CF_3—CHCl—O—CF_2H) has been demonstrated, but this occurs to a very small extent (*10*). In man, mean peak serum inorganic fluoride level following operations lasting an average of 4.1 hr was 4.4 μM; a concentrating defect was not observed. Enflurane anesthesia averaging 2.7 MAC-hr resulted in a mean peak inorganic fluoride level of 22 μM (*11*). There was no difference in concentrating ability in this study among patients anesthetized with enflurane and with halothane, the control anesthetic agent. However volunteers anesthetized with enflurane for 9.6 MAC-hr had mean peak inorganic fluoride levels of 34 μM. They had a 26% decrease in preanesthetic concentrating ability during the first 24 hr following anesthesia (*12*). This impairment was transient and probably would be clinically insignificant in patients with normal preanesthetic renal function.

Figure 1 correlates peak serum inorganic fluoride level following halothane, enflurane, and methoxyflurane anesthesia with decreased urinary osmolality as measured by the response to vasopressin administration; data are from the studies referred to above. The positive correlation between impaired urinary concentrating ability and serum inorganic fluoride level following administration of different anesthetics is strong evidence that anesthetic nephrotoxicity in man is due to metabolic liberation of inorganic fluoride.

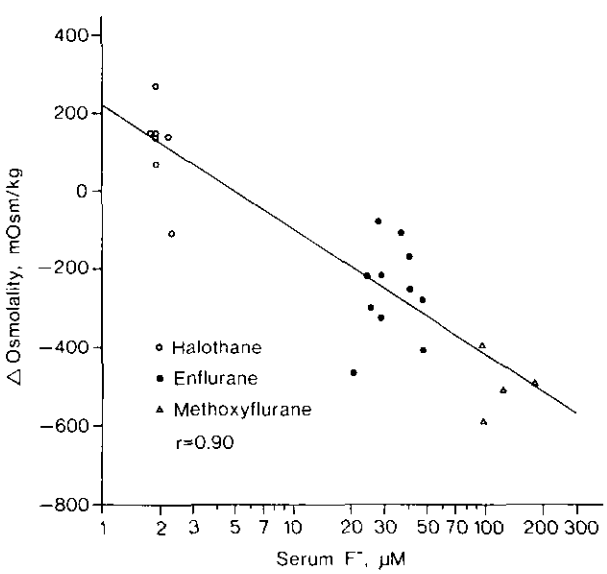

FIGURE 1. Individual changes in urinary osmolality following enflurane, halothane and methoxyflurane anesthesia plotted against peak serum inorganic fluoride levels. As peak serum inorganic fluoride level increased, the ability to concentrate urine decreased (r = 0.90). From Mazze et al. (*12*), reproduced with permission of *Anesthesiology*.

Studies employing Fischer 344 rats have provided further evidence relating anesthetic metabolism to nephrotoxicity. Mazze et al. (*4*) reported vasopressin-resistant polyuric renal insufficiency following either anesthesia with methoxyflurane or injection of inorganic fluoride. Cousins et al. (*13*) showed that phenobarbital treatment of Fischer 344 rats prior to methoxyflurane anesthesia resulted in higher serum inorganic fluoride levels and more severe nephrotoxicity than in control animals (Fig. 2). Conversely, administration of

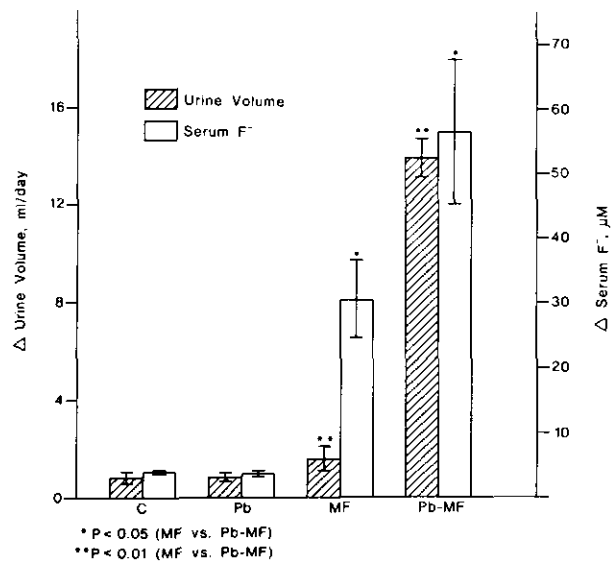

FIGURE 2. Changes in serum inorganic fluoride (F^-) concentration and urine volume for the first 2 days after anesthesia (day 15): (C) control, group I; (Pb) phenobarbital, 25 mg/kg, b.i.d., days 11 to 14, group II; (MF) phenobarbital preceding methoxyflurane, group IV; (Δ) days 7 to 10 minus days 16 to 17, mean ± S.E. From Cousins et al. (*13*), reproduced with permission of *Journal of Pharmacology and Experimental Therapeutics*.

SKF 525A, an inhibitor of drug metabolizing enzymes resulted in lower serum inorganic fluoride concentrations and less nephrotoxicity (Fig. 3). They also showed that inorganic fluoride was the principal nephrotoxic metabolite rather than oxalic acid, the organic end product of methoxyflurane metabolism. Oxalic acid administration resulted in nephrotoxicity only when injected in quantities 10-fold greater than the amount excreted following a nephrotoxic exposure to methoxyflurane; at that, oxalic acid induced nephrotoxicity was of an oliguric rather than a polyuric nature (Fig. 4). Additionally, Barr et al. (*14*) reported that 6 hr of enflurane exposure resulted in vasopressin resistant

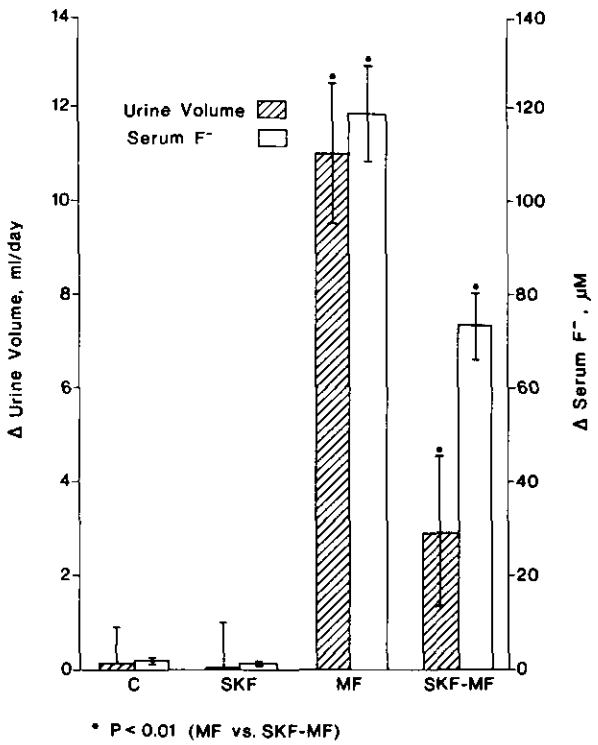

FIGURE 3. Changes in serum inorganic fluoride (F^-) concentration and urine volume for the first 2 days after anesthesia (day 11): (C) control, group I, SKF 525-A, 50 mg/kg, group II; (MF) methoxyflurane, group IV; (Δ) days 7 to 10 minus days 12 to 13, mean ± S.E. From Cousins et al. (*13*), reproduced with permission of the *Journal of Pharmacology and Experimental Therapeutics*.

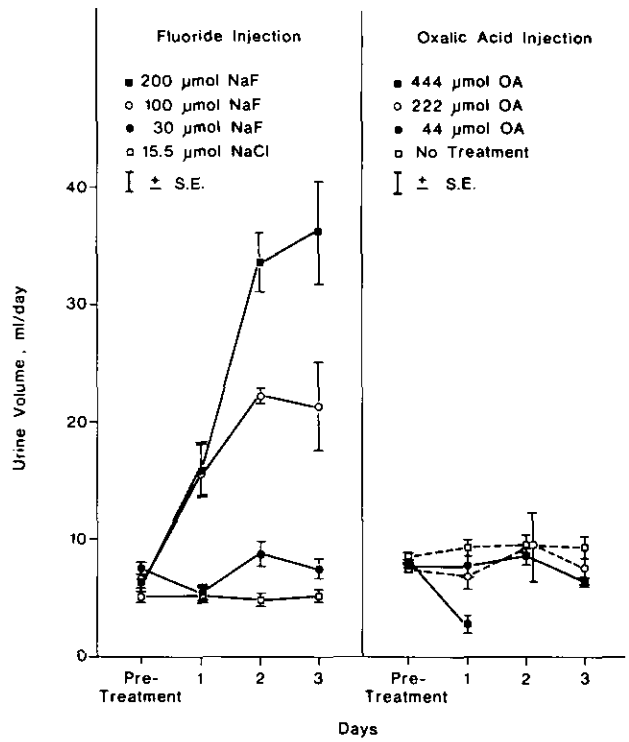

FIGURE 4. Daily urine volume (mean ± S.E.) before and after treatment with oxalic acid (OA) and sodium fluoride (NaF). Dose-related polyuria occurred after NaF injection but not after OA. Treatment with 44 μmole of oxalic acid resulted in oliguria. From Cousins et al. (*13*), reproduced with permission of the *Journal of Pharmacology and Experimental Therapeutics*.

polyuria with serum inorganic fluoride concentration of 57 μM; a level similar to that seen during methoxyflurane induced polyuria. Since the only common metabolite of methoxyflurane and enflurane is inorganic fluoride, these data strongly support the concept that inorganic fluoride, present as a result of anesthetic biodegradation, is nephrotoxic.

Enzyme Induction as a Factor in Anesthetic Nephropathy

It is established that volatile anesthetics are metabolized by the same microsomal enzyme system which catalyzes the biodegradation of other drugs. Cytochrome P450, the terminal oxidase in the system, is induced by many compounds to which surgical patients are exposed. If the activity of enzymes which defluorinate volatile anesthetics is increased, the risk of anesthetic nephropathy should also be increased. In fact, Cousins et al. (*14*) demonstrated that inorganic fluoride levels were higher and nephrotoxicity more severe in enzyme induced rats than in control rats simultaneously exposed to methoxyflurane. Therefore, it is important to determine if defluorination of other volatile anesthetics might be increased thereby increasing their nephrotoxic potential.

Mazze et al. (*15*) compared the effects of phenobarbital treatment on the metabolism and nephrotoxicity of methoxyflurane and isoflurane *in vivo* and *in vitro* in Fischer 344 rats. Phenobarbital treatment prior to methoxyflurane exposure resulted in higher serum inorganic fluoride concentration and greater nephrotoxicity. The same treatment preceding isoflurane anesthesia did not increase either serum inorganic fluoride concentration or urinary fluoride excretion. Thus, enzyme induction does not significantly alter isoflurane metabolism *in vivo* and does not increase its already very low nephrotoxic potential. In contrast to this were the results of *in vitro* studies. Specific activities of both methoxyflurane (MOF) and isoflurane (ISO) defluorinating enzymes were greater in microsomes prepared from the livers of phenobarbital treated animals than in those prepared from untreated animals (Table 1). Phenobarbital treatment increased methoxyflurane defluori-

Table 1. Anesthetic defluorination *in vitro*.

	Defluorination, μmole/F⁻30 min/mg protein			
	ISO	ENF	SEVO	MOE
Noninduced				
$\bar{X}^a$	0.51	1.31	1.82	2.00
S.E.	0.09	0.23	0.30	0.11
Induced				
$\bar{X}^a$	1.51	1.63	3.22	18.89
S.E.	0.25	0.15	0.46	2.40
I/N				
$\bar{X}^a$	3.60[b]	1.35	1.90[b]	9.75[b]
S.E.	0.80	0.15	0.25	1.50

[a]Mean ± S.E., $n = 9$.
[b]$p < 0.1$ $I/N \neq 1$.

nation by 9.75-fold and isoflurane by 3.6-fold. In light of this finding, the inability to demonstrate an increase in isoflurane metabolism *in vivo* can not be fully explained. It was suggested that the low solubility of isoflurane in fat (oil/gas = 98), relative to methoxyflurane (oil/gas = 930), led to its rapid pulmonary excretion; thus, substrate (isoflurane) availability rather than enzyme availability was rate limiting *in vivo*, masking the effect of enzyme induction.

In vivo studies with enflurane (ENF) supported this concept, as phenobarbital treatment neither increased metabolism of the relatively insoluble anesthetic (oil/gas = 98) nor the nephrotoxic response to its administration. *In vitro* findings were surprising, in that enflurane defluorination was not increased by phenobarbital treatment (Table 1). A study of the effects of phenobarbital treatment on the metabolism and nephrotoxicity of sevoflurane [$(CF_3)_2$—CH—O—CH_2F; oil/gas = 55] however, suggested that substrate availability was not the rate-limiting factor governing its *in vivo* defluorination (*16*). Phenobarbital-treated rats had a 100% increase in urinary fluoride excretion as compared to nonphenobarbital treated controls. *In vitro* microsomes prepared from phenobarbital treated rats defluorinated sevoflurane (SEVO) 1.9 times more than microsomes prepared from control rats. Sevoflurane is the least soluble of the volatile inhalational anesthetics; if substrate availability were rate limiting, its metabolism *in vivo* should not have been increased by phenobarbital treatment.

Effect of Other Inducing Agents

Most research on the induction of enzymes which defluorinate the volatile anesthetics has been performed using phenobarbital as the inducing agent. The rationale for this method of procedure has been that all volatile anesthetics belong to the same class of substrates and, presumably, are metabolized by the same enzyme (*17*). If this were correct, induction of defluorination of different anesthetics by phenobarbital should have been uniform. Data in Table 1 indicate that this is not the case. Therefore, we have begun to examine the effects of other inducing agents on the defluorination of the volatile anesthetics. Preliminary data suggest that 3-methylcholanthrene has no effect on methoxyflurane metabolism and nephrotoxicity, whereas phenytoin was found to have nearly the same effect as phenobarbital, both *in vivo* and *in vitro* (*18*).

Effect of Enzyme-Inducing Agents on Anesthetic Nephrotoxicity in Humans

There are no data in humans relating drug treatment and anesthetic defluorination. However, studies of anesthetic metabolism have demonstrated large variations in the amount of defluorination among individuals which could be due to enzyme induction (*3*, *8*–*12*). In one study, Cousins et al. (*11*) reported a patient with a peak serum inorganic fluoride concentration of 106 μM following enflurane anesthesia, considerably greater than the mean volume of 22 μM measured in the remaining patients. This patient was on a multiple drug treatment program and it was felt that his enzymes may have been induced. These suggestions of enzyme induction in surgical patients clearly indicate the need for further clinical investigations. At the present time, however, we can only speculate as to the effects of treatment with enzyme inducing drugs on the metabolism and nephropathy of the fluorinated anesthetics. Based on animal data, we would predict the following: methoxyflurane should be more nephrotoxic in enzyme-induced patients; it is unlikely that enflurane metabolism can be significantly induced so that its nephrotoxicity should be unrelated to drug treatment history; isoflurane and sevoflurane metabolism may be increased by enzyme induction, however, inorganic fluoride levels still should be well below the nephrotoxic threshold of 35–50 μM; and halothane defluorination ordinarily should not be increased by enzyme induction, so that it will not be nephrotoxic in clinical practice.

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REFERENCES

1. Van Dyke, R. A., and Gandolfi, A. J. Anaerobic release of fluoride from halothane relationship to the binding of halothane metabolites to hepatic cellular constituents. Drug. Metab. Disp. 4: 40 (1976).
2. Crandell, W. B., Pappas, S. G., and Macdonald, A. Nephrotoxicity associated with methoxyflurane anesthesia. Anesthesiology 27: 591 (1966).
3. Mazze, R. I., Shue, G. L., and Jackson, S. H. Renal dysfunction associated with methoxyflurane anesthesia. A randomized prospective clinical evaluation. J. Amer. Med. Assoc. 216: 278 (1971).
4. Mazze, R. I., Cousins, M. J., and Kosek, J. C. Strain differences in metabolism and susceptibility to the nephrotoxic effects of methoxyflurane in rats. J. Pharmacol. Exptl. Therap. 184: 481 (1973).
5. Van Dyke, R. A., Chenoweth, M. B., and Van Poznak, A. Metabolism of volatile anesthetics. I. Conversion *in vivo* of several anesthetics of $^{14}CO_2$ and chloride. Biochem. Pharmacol. 13: 1239 (1974).
6. Van Dyke, R. A., and Chenoweth, M. B. Metabolism of volatile anesthetics. II. *In vitro* metabolism of methoxyflurane and halothane in rat liver slices and cell fractions. Biochem. Pharmacol. 14: 603 (1965).
7. Taves, D. R., et al. Toxicity following methoxyflurane anesthesia. II. Fluoride concentration in nephrotoxicity. J. Amer. Med. Assoc. 214: 91 (1970).
8. Mazze, R. I., Trudell, J. R., and Cousins, M. J. Methoxyflurane metabolism and renal dysfunction: Clinical correlation in man. Anesthesiology 35: 247 (1971).
9. Cousins, M. J., and Mazze, R. I. Methoxyflurane nephrotoxicity: A study of dose-response in man. J. Amer. Med. Assoc. 225: 1611 (1973).
10. Mazze, R. I., Cousins, M. J., and Barr, G. A. Renal effects and metabolism of isoflurane in man. Anesthesiology 40: 536 (1974).
11. Cousins, M. J., et al. Metabolism and renal effects of enflurane in man. Anesthesiology 44: 44 (1976).
12. Mazze, R. I., Calverley, R. K., and Smith, N. T. Inorganic fluoride nephrotoxicity. Prolonged enflurance and halothane anesthesia in volunteers. Anesthesiology 46: 265 (1977).
13. Cousins, M. J., et al. The etiology of methoxyflurane nephrotoxicity. J. Pharmacol. Exptl. Therap. 190: 530 (1974).
14. Barr, G. A., et al. A comparison of the renal effects and metabolism of enflurane and methoxyflurane in Fischer 344 rats. J. Pharmacol. Exptl. Therap. 188: 257 (1974).
15. Mazze, R. I., Hitt, B. A., and Cousins, M. J. Effects of enzyme induction with phenobarbital on the *in vivo* and *in vitro* defluorination of isoflurane and methoxyflurane. J. Pharmacol. Exptl. Therap. 190: 523 (1974).
16. Cook, T. L., et al. A comparison of renal effects and metabolism of sevoflurane and methoxyflurane in enzyme-induced rats. Anesth. Analag. 54: 829 (1975).
17. Takahashi, S., Shigematsu, A., and Furukawa, T. Interaction of volatile anesthetics with rat hepatic microsomal cytochrome P450. Anesthesiology 41: 375 (1974).
18. Caughey, G., et al. Dephenylhydantoin (DPH) induction of methoxyflurane (MOF) metabolism in rats. Federation Proc. 36: 3838 (1977) (Abstract).