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Editorial Views

Methoxyflurane Metabolism

THE DEMONSTRATION that methoxyflurane nephrotoxicity is related to its biotransformation has emphasized the need for understanding anesthetic metabolism. This issue of ANESTHESIOLOGY contains reports by Yoshimura, Holaday and Fiserova-Bergerova and by Adler, Brown and Thompson that address this subject. Although administration of methoxyflurane has decreased in recent years, the current reports are of interest because the principles of drug biotransformation that apply to methoxyflurane metabolism apply, as well, to biotransformation of other inhalation agents.

One of the mechanisms, if not the primary one, by which organ toxicity occurs following anesthesia relates to the formation of reactive metabolites. Methoxyflurane nephrotoxicity is due to formation of large amounts of inorganic fluoride and perhaps other reactive metabolites. At the cellular level, toxicity may occur as a result of covalent, *i.e.*, irreversible, binding of metabolic intermediates to cellular constituents. In previous studies, Van Dyke and Gandolfi[1] demonstrated covalent binding of radioactively labeled halothane metabolites to hepatic microsomal phospholipids, primarily phosphatidylethanolamine and lecithin, important constituents of cell membranes. Cohen *et al.*[2] identified in human urine cysteine and ethanolamide conjugates of halothane that could only have been formed as a result of production of highly reactive intermediates. Irreversibly bound metabolites also have been found in the liver following halothane administration.[3] Not only could covalent binding of intermediates result in cellular necrosis but, if intermediates were to bind to DNA, aberrant cell lines, *e.g.*, neoplasms, might evolve.

In this issue, Yoshimura *et al.* report metabolic balance studies in patients anesthetized with methoxyflurane, and suggest that as much as 30 per cent of the absorbed dose is covalently bound to tissue. Their evidence for covalent binding was the inability to account for all of the injected dose of methoxyflurane. Sixty-three per cent of absorbed methoxyflurane was traced; 19 per cent was exhaled, unchanged; 29 per cent was found in the urine as nonvolatile organic fluoride compounds; 15 per cent was accounted for as inorganic fluoride, either excreted in urine or deposited in bone. The remaining 37 per cent of methoxyflurane was not recovered and was presumed by the authors to be bound to tissue. This presumption cannot go unchallenged. Perhaps the most difficult task in performing clinical metabolic balance studies is obtaining complete urine collections. An approach to demonstrating that experimental rigor has been achieved is to show that excretion of a marker, such as endogenous creatinine, is constant, *i.e.*, does not vary by more than ±10 per cent from day to day. Unfortunately, such evidence is not presented. The authors defend their results by citing previous studies in which

similar techniques were used to account for 85 per cent of absorbed enflurane and 95 per cent of isoflurane.[4,5] However, in those studies excretion of nonvolatile urinary metabolites accounted for only 2.4 and 0.17 per cent of the absorbed anesthetics, respectively, so that large errors in urine collection would not have significantly affected the results. In the current study of methoxyflurane metabolism, approximately 44 per cent of absorbed methoxyflurane was accounted for as urinary excretory products. Thus, significant errors in urine collection would be of major importance. Additionally, measurements of excretion of urinary metabolites by patients who have renal insufficiency probably are not reliable. Based on the very low inorganic fluoride clearances reported by Yoshimura *et al.*, several of their patients either had compromised renal function or had incomplete urine collections. In summary, although it is highly likely that methoxyflurane biotransformation proceeds through the formation of reactive intermediates, which may covalently bind to tissue constituents, the extent to which this occurs is still open to question.

Adler *et al.*, in their experiments with hepatic microsomes, employed classic *in-vitro* techniques to study kinetics of methoxyflurane metabolism. These preparations may be used to measure the ability of the NADPH-dependent mixed-function oxidase system to biotransform drugs. The authors particularly address the question of whether high concentrations of drug inhibit metabolism. That substrate inhibition might occur was originally suggested by Sawyer *et al.*,[6] who reported proportionally greater *in-vivo* extraction of halothane at low concentrations by the livers of miniature swine exposed to either anesthetic or subanesthetic concentrations of halothane. Adler *et al.* show that substrate inhibition does not occur. Rather, their data fit either of two model curves; an asymptotic curve that is classically seen with enzyme-catalyzed reactions and an unbounded exponential curve that would pertain only if the biotransformation of methoxyflurane were not totally dependent upon an enzyme catalyst. *In-vitro* enzyme kinetic data from our laboratory suggest that methoxyflurane defluorination is an enzyme-catalyzed reaction.

In classic enzyme kinetics the rate of metabolism (v) can be predicted from the substrate concentration (S) by the equation:

$$v = \frac{V_{max}\, S}{K_m + S}$$

where V_{max} is the maximal rate of metabolism and is dependent upon the amount of enzyme. K_m is the enzyme–substrate dissociation constant and determines the fraction of V_{max} that pertains at a given substrate concentration. Adler *et al.* have not reported their value for K_m; however, this value may be determined by plotting the reciprocal of rate $\left(\frac{1}{V}\right)$ against the reciprocal of substrate concentration $\left(\frac{1}{S}\right)$.

In our experiments we have obtained results similar to those of Adler *et al.* and have determined the K_m of methoxyflurane to be approximately 33 μM. The significance of these data lies in the fact that blood concentrations of methoxyflurane at MAC are approximately 1000 μM, or 30 times K_m. If K_m *in vitro* can be applied to *in vivo* metabolism, then, from the equation above, it can be calculated that the rate of methoxyflurane metabolism at MAC is $\frac{30}{31} V_{max}$. More important, rates of metabolism in excess of 75 per cent of V_{max} should occur at methoxyflurane concentrations as low as 100 μM (0.1 MAC) and will remain greater than 50 per cent of V_{max} until methoxyflurane concentration decreases to K_m, *i.e.*, 33 μM. Given the great lipid solubility of methoxyflurane, K_m may be exceeded for many hours, even days, following anesthesia. Thus, the great majority of methoxyflurane metabolism occurs in the period following anesthesia, as can be inferred from the data of Yoshimura *et al.*

Another significant finding in the report of Adler *et al.* is that there are qualitative, as well as quantitative, changes in methoxyflurane metabolism resulting from phenobarbital treatment. The ratio of total fluoride to free fluoride was decreased when the induced preparations were compared with the non-

induced preparations. These data are interesting in light of the report of Haugen *et al.*,[7] who have demonstrated four electrophoretically distinct cytochrome P-450 proteins. Phenobarbital treatment induces only one of these proteins. It is possible that the protein induced by phenobarbital treatment catalyzes the pathway primarily leading to free fluoride formation. The effects of any of the hundreds of other enzyme-inducing agents on the quantitative and qualitative aspects of anesthetic biotransformation are unknown.

These reports reflect a growing interest in anesthetic biotransformation and the increasing experimental sophistication needed to obtain useful information from these studies. It is clear that an important factor in anesthetic toxicity relates to metabolism and, it seems only reasonable, that the more that is known about anesthetic biotransformation the safer will be our anesthetic practice. We have come a long way from the era when inhalation anesthetics were thought to be classic examples of pharmacologically active but metabolically inert compounds.

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Circulation

HYPERTENSION AND MENTAL STATUS The authors examined the course of modest (mean arterial pressure 110 torr) hypertension in 19 patients. Five received no therapy. Fourteen patients were instructed in "a technique based on Buddhist meditation exercises designed to elicit a relaxation response." They were told to repeat the technique twice a day for 10–15 minutes. After six months, no change was observed in the control patients. In the experimental group, an average decrease of 12 torr in mean arterial pressure had occurred. The plasma level of dopamine-beta-hydroxylase (an indicator of sympathetic nervous system activity) was significantly decreased at the end of six months in the experimental group but showed no change in control patients. No change in either plasma volume or peripheral venous renin level was found in either group. There was a significant increase in furosemide-stimulated renin activity in the treated group. Since adrenergic activity may influence renin secretion, the authors hypothesize that this decrease resulted from diminished sympathetic activity. The authors conclude that for certain patients, a psychotherapeutic modality of therapy may be efficacious in treating hypertension. Furthermore, the decreased blood pressure appears to be associated with reduced peripheral adrenergic activity. (*Stone RA, DeLeo J: Psychotherapeutic control of hypertension. New Engl J Med 294:80–84, 1976.*)