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COMMENTARY

BIOTRANSFORMATION AND HEPATOTOXICITY OF HALOTHANE*

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The introduction of halothane (1,1,1-trifluoro-2-bromo, 2-chloroethane) into clinical practice in 1957 represented a significant advance in anesthesia pharmacology since the drug possesses characteristics of rapid induction and emergence, potency, and nonflammability, with minimal nausea and vomiting. By 1960 it was, and has remained, the most popular potent anesthetic employed in the Western world.

After a brief period of widespread clinical use, anecdotal case reports of unexplained post-anesthetic jaundice began to appear [1–5]. The essentials of this syndrome were rarity, pathologic and clinical features indistinguishable from viral hepatitis, unpredictability, and non-statistical inference that there was a higher incidence after a second administration. Similarly, there was belief that middle age and obesity were predisposing factors to the development of the complication. These reports triggered several independent clinical investigations of the problem [6–8] and a large nationwide retrospective survey [9]. No scientific conclusions could be drawn from these reports except that unexplained jaundice following halothane anesthesia was a rare occurrence (approximately 1:30,000 administrations) and that the overall safety record of the anesthetic was excellent. Laboratory animal experiments during this era failed to reveal a direct hepatotoxic action of halothane at clinically inspired concentrations [10–15]. Primarily because of lack of an appropriate animal model of direct toxicity, hepatologists searched for other possible mechanisms of halothane hepatotoxicity and implicated an allergic or hypersensitivity reaction primarily by exclusion [16]. Documentation of this mechanism was attempted [17–19], but the concept that allergy arises from the parent anesthetic molecule is highly suspect in the light of recent human and animal experiments [20–24].

At the time halothane was introduced, and during the early part of the 'halothane hepatotoxicity' rancor, it was categorically believed and taught that inhalation anesthetics (except trichloroethylene [25]) were totally resistant to enzymatic breakdown. However, in the early 1960s it was conclusively determined that chloroform was biotransformed [26, 27]. Because chloroform was already obsolete as an anesthetic at the time, these experiments had more academic than clinical pharmacologic impact. In 1964, Van Dyke *et al.* [28] described halothane biotransformation *in vitro*. This group subsequently observed that this metabolism was NADPH-O_2 dependent, occurred primarily in the hepatic microsomal fraction and was inducible by phenobarbital [29, 30]. Thus, it appeared that halothane biotransformation occurred via the classic mixed function oxygenase pathway. Confirmation of the biotransformation of halothane in man was rapidly forthcoming [31]. As much as 20 per cent of halothane absorbed by man could be accounted for as non-volatile urinary metabolites recovered over a 13-day period [32]. The primary urinary metabolites of halothane were initially identified by Stier [33] as trifluoroacetic acid and bromide ion, neither of which could be strongly implicated with hepatotoxicity, although parenthetically the plasma bromide level frequently reached borderline soporific concentrations (>2.0 mEq/l) [34, 35]. Administration of trifluoroacetic acid, free or conjugated with albumin, failed to produce liver damage [36], although it did result in liver enlargement and alteration of glycolytic and gluconeogenic enzymes [37]. Production of the reactive trifluoroethanol was postulated but never proven [38].

It was determined that, for a given dose of an inhalation anesthetic, biotransformation was more extensive in the obese subject [39]. In addition, studies in a small series of twins showed that non-volatile urinary metabolite difference was less in identical than in fraternal twins [40]. These findings suggest that genetic factors may significantly alter halothane biotransformation.

By 1972, three mechanisms for halothane biotransformation were postulated: (1) oxidative dehalogenation (NADPH-O_2 dependent) of Br^- and Cl^- [29]; (2) abstractive dehydrogenation with production of the radical, CF_3CClBr, followed by oxidation, dehalogenation and hydrolysis to trifluoroacetic acid and Br^- [41]; and (3) oxidative dehalogenation to the intermediate trifluoroacetaldehyde with subsequent hydration and oxidation to trifluoroacetic acid [42]. It is of interest to note that enzymatic defluorination of the trifluoro bond of halothane was considered impossible.

Evidence that the biotransformation of halothane could be a vector in hepatic necrosis began to accumulate from animal data. Cohen [43] demonstrated that fluorine-containing non-volatile metabolites of halothane were bound covalently to liver macromolecules of the mouse and persisted for almost 2 weeks. Although halothane anesthesia in rats pretreated with phenobarbital for microsomal induction does not lead to centrolobular necrosis in the presence of an oxygen environment greater than 20 per cent [44, 45], such covalent binding increases over 400 per cent in the induced animals [46]. However, in none of these studies did the covalent bonding correlate with necrosis, since no necrosis was produced.

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Another postulate as to the mechanism of halothane-induced hepatotoxicity was that halothane, or more likely one of its metabolites, initiated lipoperoxidation similar to the hypothesis of Recknagel and Ghoshal [47] for carbon tetrachloride. Using this approach, Brown [48] found early rises in diene conjugates in phenobarbital-pretreated, halothane (1% in 99% O_2)-anesthetized rats. However, no malondialdehyde or lipofuscin pigments, late products of lipoperoxidation usually associated with necrosis, were observed [49], strongly undermining a role of the lipoperoxidation hypothesis with halothane. After repeated administrations of [^{14}C]halothane in the rat, no enhanced metabolite excretion was observed, indicating that in this species the anesthetic is not an inducing agent [50]. The reason for this latter work was to study the implication that multiple administrations produced a higher incidence of hepatic necrosis in man.

Halothane in an air environment produces a type I spectral difference binding to hepatic microsomes [51] when examined by the method of Schenkman *et al.* [52]. Uehleke *et al.* [53] were the first to point out that this spectral binding characteristic of reduced microsomes and halothane is enhanced and modified in an anaerobic environment. In the absence of oxygen, the combination of halothane and hepatic microsomes produces an absorption maximum at 473 nm and a trough at 408 nm. In addition, these investigators found enhanced covalent binding after anaerobic incubation of [^{14}C]halothane with phenobarbital-induced rabbit hepatic microsomal protein and NADPH. These experiments were felt to give some substantiation to the concept of Stier [41] that initiation of halothane biotransformation was dehydrogenation with the formation of the $CF_3CClBr\cdot$ radical or anion. Actually the $CF_3CClBr\cdot$ radical may be less reactive than the debrominated $CF_3CClH\cdot$ radical, which is a possible dehalogenated intermediate postulated by Van Dyke and Chenoweth [29]. Synthesis of the unstable reduced and debrominated compound, CF_3CH_2Cl, was performed and when injected (in anhydrous propylene glycol) into portal veins of rats produced extensive hepatic necrosis.*

Van Dyke's group has investigated several interesting points pivotal to biotransformation of halothane in the presence of reduced O_2 concentrations. An initial publication indicated that within the first 2 hr after administration the covalent binding of radioactive halothane to microsomes is greater in the phospholipid component than in protein [54]. It was found that this binding, when carried out in an hypoxic environment *in vitro* could be linearly correlated with lipid peroxidation products [55]. Using both ^{36}Cl-halothane and [^{14}C]halothane, it was determined that the covalent binding of halothane metabolites to microsomal phospholipid retained the chlorine atom. However, neither the binding nor the lipid peroxidation was of sufficient intensity to destroy cytochrome P-450. In summary, these studies, coupled with those of Uehleke's group, strongly implied that halothane biotransformation in an anaerobic or hypoxic environment apparently produced some degree of reactive intermediates, particularly if metabolism were enhanced by phenobarbital induction.

Cohen *et al.* [56] published a truly sophisticated investigation of human halothane biotransformation. They utilized heart transplant donors (legally 'dead') and gave three donors 1 mCi and five donors 25 μCi [^{14}C]halothane intravenously. Urinary non-volatile metabolites were identified, after separation, by NMR and mass spectroscopy techniques. Three major organic metabolites were found: trifluoroacetic acid, *N*-trifluoroacetyl-2-aminoethanol, and *N*-acetyl-5-(2-bromo-2-chloro-1,1-difluroethyl)-L-cysteine. The ability of human liver to defluorinate the extremely stable CF_3 bond was revealing and completely unexpected. The presence of the cysteine and ethanolamine conjugates is of concern to human toxicology, since the finding of these substances implies the presence of reactive intermediates. In all likelihood these conjugated metabolites indicate urinary excretion of degraded hepatic lipid and protein macromolecules containing covalently bound halothane metabolites.

Recently, two animal models mimicking the human lesion of 'halothane hepatitis' (centrolobular necrosis, elevated SGPT and SGOT, etc.) have been produced. In rats pretreated with polychlorobiphenyls, potent inducers of a wide variety of biotransformation pathways which qualitatively and quantitatively alter cytochrome P-450 [57], a 2-hr exposure to 1% halothane in 99% oxygen causes classic centrolobular necrosis in rats [58]. No evidence of lipid peroxidation is seen with this model. The weakness of this model is that polychlorobiphenyls themselves produce modest liver morphologic changes (sudanophilic vacuole accumulation, although no centrolobular necrosis). Therefore, this phenomenon could be the net result of two additive liver-damaging substances rather than due to formation of a specific reactive metabolite of halothane.

Widger *et al.* [59] found that rats anesthetized with halothane under hypoxic conditions (7% oxygen) demonstrated the following: (1) an elevated plasma fluoride (enhanced trifluoro bond cleavage in hypoxic conditions) producing reactive difluoro compounds; (2) a 3-fold increase in the binding of [^{14}C]halothane metabolites as compared to halothane anesthesia with 20% oxygen; and (3) a microsomal lipid/protein binding ratio of 3.24 compared to 0.76 in halothane–oxygen anesthetized animals. They postulated that hypoxic atmospheres promote biotransformation of halothane by reductive pathways and that the metabolites produced are potentially more hepatotoxic than those produced by conventional oxidative pathways. However, they did not elucidate this reductive pathway or its metabolites. Two other investigations hinging on this concept are important. Adler *et al.* [60] demonstrated that phenobarbital pretreatment may alter not only the quantitative biotransformation of inhalation anesthetics but also change the qualitative nature of this metabolism. These findings at reduced oxygen tensions are extremely relevant to the clinical cases of 'halothane hepatitis' because halothane anesthesia in man significantly decreases hepatic blood flow and in many cases causes hepatic artery perfusion to drop to zero [61].

From the preceding discussion it is possible to

* B. R. Brown, Jr. and R. Baker, unpublished observations.

speculate a mechanism of halothane-induced liver damage. Key points in the model are that: (1) bioactivation of halothane occurs in the endoplasmic reticulum; (2) this bioactivation is inducible by pretreatment with phenobarbital; (3) bioactivation involves a reductive pathway; and (4) reactive intermediates are produced which interact with lipids and protein. In our laboratory, we can now consistently reproduce halothane-induced centrolobular liver necrosis in rats [62]. In this animal model, phenobarbital-pretreated rats are anesthetized with clinical concentrations of halothane (1%) in 14% oxygen for 2 hr. Within 24 hr, extensive centrolobular necrosis and sharply elevated SGPT levels are observed. None of these changes are apparent in induced animals exposed to hypoxia alone (14% O_2) or in non-phenobarbital-pretreated halothane-anesthetized rats. Elucidation of the mechanism and identification of non-volatile halothane metabolites produced in this model are currently being pursued.

In summary, the useful and safe halogenated inhalation anesthetic, halothane, apparently possesses a rare, unpredictable complication of hepatic necrosis. Evidence is strong from animal experiments that this could possibly be due to qualitatively and/or quantitatively altered biotransformation to reactive intermediates, particularly via a reductive or oxygen-deficient pathway. Lipid peroxidation and extensive covalent binding of halothane metabolites may occur but may be effects, rather than causes. It is conceivable, but certainly not proven, that these new substances could induce a hypersensitivity phenomenon accelerating hepatic cellular destruction. Although only the surface of this problem is scratched, future investigations into the precise mechanisms and variation of halothane biotransformation should be performed. The widespread use of this otherwise safe anesthetic in clinical practice dictates such studies.

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