Title : BIOACTIVATION AND HEPATOTOXICITY OF $^{2}H/^{3}H$ HALOTHANE

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Introduction. The development of our hypoxic animal model for halothane induced hepatotoxicity was predicated on the hypothesis that reductive biotransformation of halothane was prerequiste for lesion development. Because scission of the C-H bond may occur during this reductive biotransformation of halothane, scission of this bond may also be a critical factor in halothane induced liver injury. To test this hypothesis, the hepatotoxic effects of deuterium substituted halothane were determined in the "hypoxic model of halothane induced hepatotoxicity." The rationale for this study was that the increased stability of the C-D bond as compared to the C-H bond should result in a decreased hepatotoxicity of deuterium substituted halothane, if scission of the C-H bond is involved. Since the carbon skelton of halothane is known to become covalently bound to lipids and protein, a comparison of the binding of ^{14}C-halothane and ^{3}H-halothane should also indicate the improtance of C-H bond scission in the bioactivation of halothane to a binding species.

Methods. The deuterated halothane (d-halothane) was prepared by a base catalyzed exchange reaction using D_2O and halothane. Analysis by gas chromatography-mass spectrometry showed it to be >99% pure. Tritiated halothane (^{3}H-halothane) was prepared by a similar procedure using $^{3}H_2O$ and had a final specific activity of 0.6 mCi/mmole. For the hepatotoxicity studies phenobarbital pretreated, male Sprague-Dawley rats were anesthetized for 2 hrs in a hypoxic environment (F_IO_2 = 0.14) with 1% d-halothane or 1% halothane. At the end of anesthesia blood was collected from certain animals for the determination of serum fluoride (F^-), bromide (Br^-), and trifluorochloroethane (CTE) and difluorochloroethylene (CDE), the volatile, reductive metabolites of halothane. At 24 hrs after exposure the remainder of the animals were sacrificed, and the livers, blood and total urine collected for the assessment of histological changes, SGPT and urinary F^- excretion. For in vitro studies, livers were removed from phenobarbital pretreated animals, homogenized in Tris-KCl buffer and microsomes obtained by differential centrifugation. These microsomes were incubated under atmosphers of N_2 or O_2 with NADPH and 1-^{14}C-halothane or 2-^{3}H-halothane. At the end of incubation, lipids and proteins were isolated, subjected to exhaustive extraction procedures and the bound radioactivity determined.

Results. At the end of anesthesia, no significant differences were observed between d-halothane or halothane exposed animals in the plasma levels of F^- (18.2 ± 4.6 vs 15.2 ± 3.9 µM),CDE (3.0 ± 0.9 vs 3.5 ± 1.4 nmole/g) or CTE (6.0 ± 1.3 vs 8.4 ± 1.9 nmole/gm), respectively. Both d-halothane and halothane exposure (N=36/group) resulted in hepatotoxicity by 24 hr, with no apparent differences in liver morphological alterations, in SGPT (80 ± 30), or 24 hr urinary F^- (4.54 vs 4.76 µmoles). The only significant difference observed between d-halothane and halothane was a 60% decrease in serum Br^- levels, observed at both end of anesthesia and at 24 hr for rats exposed to d-halothane. Incubations of ^{14}C-halothane with microsomes under a N_2 atmosphere resulted in a two fold increase in binding of ^{14}C-halothane equivalents to protein (1090 ± 280pmole/mg) and a four fold increase to lipid (6420 ± 280 pmole/µmole P), over that observed in incubations under an O_2 atmosphere. This supports previous work demonstrating enhanced binding of halothane under a N_2 atmosphere.[1,2] Only negligible quantities of ^{3}H-halothane bound to protein (50 ± 40 pmoles/mg) or lipid (11 ± 8 pmoles/µmole P) during aerobic microsomal incubation. However, under a N_2 atmosphere high levels of ^{3}H-halothane were bound to protein (390 ± 120 pmole/mg) and lipid (4920 ± 1220 pmole/µmole P), indicating a specific requirement of reductive metabolism for the ^{3}H to be retained. Under a N_2 atmosphere, the ratio of $^{3}H/^{14}C$ halothane equivalents bound to protein and lipid was 0.36 and 0.75, respectively.

Discussion. The results suggest that scission of the C-H bond is not a determining factor in the hepatotoxicity of halothane, since no apparent differences in liver injury were observed between halothane and d-halothane. Also, no significant changes between halothane and d-halothane were found in the levels of those metabolites produced during the reductive biotransformation of halothane (F^- and the volatile metabolites, CDE and CTE). However, deuterium substitution markedly reduced the oxidative biotransformation of halothane, as evidenced by the 60% decrease in debromination. The covalent binding data also indicate that scission of the C-H bond does not occur during reductive bioactivation of halothane, since the ^{3}H was retained under reductive conditions. The differences in stoichiometry of the binding of ^{3}H-halothane and ^{14}C-halothane are interesting and may indicate that different reactive intermediates exist for binding to lipid or protein and/or for O_2 or N_2 atmospheres, or reflect isotope effects or isotope exchange reactions. However, binding of ^{3}H-halothane equivalents may be a useful marker for reductive biotransformation of halothane and its associated toxicity.

References.

1. Van Dyke RA and Gandolfi AJ: Drug Metabolism and Disposition 4:40-44, 1976.
2. Sipes IG and Brown BR, Jr.: Anesthesiology 45: 622-628, 1976.