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Title: Non-Radiometric Detection of Covalent Binding of Halothane

Authors: A.Jay Gandolfi, Ph.D., Research Associate, I. Glenn Sipes, Ph.D., Associate Professor
Burnell R. Brown, Jr., M.D., Ph.D., Professor and Head
Affiliations: Department of Anesthesiology, University of Arizona, Tucson, Arizona 85724

Introduction: One of the difficulties confronting the researcher studying the bioactivation and covalent binding of halothane is the necessity for a sensitive technique for the detection of organic metabolites or residues bound to macromolecules. We have used specific radiochemically labeled halothane for our in vitro microsomal bioactivation studies (1) but the large amounts of halothane required for in vivo studies is prohibitively expensive. Since the halothane derived intermediates that bind to macromolecules retain some of their original carbon-fluorine bonds, we have applied our recently published organic fluoride analysis technique (3) to detect any fluorinated residues bound to rat liver tissue samples. This method allows us to assess the amount of fluorinated residues covalently bound to liver tissue in rats exposed to anesthetic levels of halothane in a hypoxic environment, conditions which have been reported to cause hepatic lesions (3).

Methods: Livers from phenobarbital treated rats used in characterizing the conditions required for the production of the halothane-induced liver lesion were used in this study (4). Detection of fluorinated residues covalently bound to rat hepatic tissue were determined as follows: 1) Small sections of the frozen livers from our previous study (4) were homogenized in water. 2) The macromolecules were precipitated with trichloroacetic acid leaving any inorganic fluoride metabolite in the supernatant. 3) The precipitate was resuspended in a new trichloroacetic acid solution, frozen, and lyophilized to remove any trifluoroacetic acid metabolite. 4) The dry residue was fused with sodium metal as previously reported (3). 5) The fluoride ion released by the fusion was quantified with a specific ion electrode. Results were expressed as nmoles of fluoride/10 mg wet weight of liver. Reproducibility and efficiencies for the technique were found to be similar to those previously reported (3). Control rats not receiving halothane were found to have less than 1 nmole fluoride/10 mg wet weight of liver tissue which is also the limit of detection for the fluoride electrode. Liver samples from control rats were treated with sodium fluoride or trifluoroacetic acid (up to 2.6 mM) and analyzed for bound organic fluoride residues. No bound organic fluoride was found indicating the lack of interference by either of these metabolites.

Results: The covalent binding of fluorinated residues of halothane to the hepatic tissues of phenobarbital pretreated rats occurred primarily when the rats were exposed to halothane in a hypoxic atmosphere. Rats exposed to halothane in a 14% oxygen atmosphere bound 16.4±2.2 nmoles fluoride/10 mg liver while rats exposed to halothane in a 50% oxygen atmosphere had 5 nmoles fluoride bound/10 mg liver. When rats were exposed to halothane in a hypoxic atmosphere binding of fluorinated residues was maximal immediately after exposure. The binding of organic fluoride to liver tissue preceeds the development of the liver lesion. In fact, when the halothane induced liver lesion is most pronounced at 24-48 hours post exposure over one half of the bound organic fluoride has been removed from the liver. The binding of the fluorinated residue to liver tissue of halothane exposed rats has been found to be dependent on the concentration of halothane (0.1-1%) and the length of exposure (3-120 min) indicating a true dose-responsive bioactivation of halothane. In addition, rats treated with biotransformation enzyme inhibitors bound less organic fluoride following halothane exposure, and rats treated after halothane exposure with sulfhydryl agents that inhibit lesion development also suppresses the amount of organic fluoride bound to the livers of these rats. Finally, female rats which have not been shown to respond to the halothane-hypoxia liver lesion model also have less fluorinated residue bound to their livers as compared to their male counterparts.

Discussion: In summary, we have presented a non-radiometric technique which can easily detect the fluorinated residues of halothane covalently bound to the liver tissue of rats exposed to halothane under conditions that produce a liver lesion. With this method, we have found that the conditions required for optimizing the lesion development also optimizes the binding of the fluorinated residue. The maximal binding of fluorinated residues to the livers of rats exposed to the halothane-hypoxia liver model preceeds the development of the lesion. The advantages of this procedure are obvious and we are presently applying it to human biological samples to confirm that halothane is also bioactivated in humans. (Supported in part by NIH Grants AM 16715-07 and CA 21820-03).

References:

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