

Title: COMPARATIVE STUDY OF HALOGENATED HYDROCARBON TOXICITY AND FREE RADICAL FORMATION IN RAT LIVER

Authors: W.H. Massion, M.D., J.L. Poyer, Ph.D., P. Downs and L. Fagraeus, M.D., Ph.D.

Affiliation: Department of Anesthesiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190 and Biomembrane Research Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

Introduction. A number of halogenated hydrocarbon anesthetic agents, in particular halothane have been linked to the occurrence of hepatotoxicity in the postoperative period. Other halocarbon anesthetic agents such as enflurane and isoflurane appear to have a lower potential for hepatotoxic sequelae in clinical use. However, a recent study (1) has claimed that they also produce centrilobular necrosis in a fasting rat model. Previous work (2) has demonstrated the formation of free radicals during the metabolism of halothane by a spin-trapping method using electron spin resonance (ESR) techniques. The present study was designed to quantitate and compare the incidence of free radical formation among various halogenated hydrocarbon anesthetic agents and to correlate these findings with the occurrence of clinical hepatotoxicity reported in the literature.

Methods. Male Sprague-Dawley rats, weighing between 250 and 300 gm, were fed phenyl-t-butyl-nitrone (PBN), a spin-trapping compound which reacts with free radicals to form a more stable adduct detectable by the ESR spectrometer. PBN was administered by stomach tube at a dose of 1 ml of a 0.1 M solution in 0.02 M phosphate buffer at pH 7.4 and homogenized with 1 ml of corn oil. Immediately following PBN feeding, halogenated anesthetic agents were administered by inhalation at a dosage of 0.5% in air for 2 hours, using a 16L plexiglass chamber. The animals were then sacrificed, the livers removed, rapidly weighed and homogenized in chloroform-methanol (2:1). The total lipids were extracted and the CHCl_3 layer evaporated in vacuo to a volume of approximately 3 ml. The concentrated extract was analyzed immediately in a Varian E-9 ESR spectrometer. The amount of halogenated hydrocarbon-derived free radicals was calculated according to the method of Floyd et al (3) employing the nitroxide free radical standard, 4-hydroxy-2,2,6,6-tetramethylpiperidinoxy. Four volatile anesthetic agents were studied: halothane, enflurane, isoflurane and trichloroethylene. Free radical formation was expressed in picamoles of PBN radical adducts and compared to carbon tetrachloride as a standard for reproducible liver damage (Fig. 1).

Results. The mean free radical count for 20 rats after halothane was 0.38 ± 0.15 pmole per gram of liver. The corresponding value for enflurane was 0.02 pmole/g. Trichloroethylene anesthesia resulted in liberation of 0.78 ± 0.45 pmole/g free radicals, which is about double the amount seen after halothane. Livers from rats exposed to isoflurane were free of PBN radical adducts. CCl_4 administered by inhalation yielded the highest amount of free radicals, 1.59 ± 1.25 pmole/g. The major portion of the total trapped radicals in the liver homogenate was found in the extract from the microsomal fraction. No ESR signals could be demonstrated in the liver extracts of rats which were given PBN but no anesthetic agents, or anesthetics but no PBN.

Discussion. These results indicate that PBN-radical adduct measurement of free radical formation in rat livers by ESR techniques may provide a quantitative method for predicting hepatotoxicity of halogenated hydrocarbon anesthetic agents. Both trichloroethylene and halothane consistently showed liberation of free radicals which could be potentially damaging to microsomes and other subcellular elements. Enflurane was virtually free and isoflurane completely free of radical adduct formation in the liver after a 2-hour exposure. These results seem to parallel clinical experience of hepatotoxic sequelae and are also in agreement with histologic studies of hepatic centrilobular necrosis in rats exposed to halocarbon anesthetics at reduced oxygen tensions. Free radical determination by ESR technique does not require preconditioning by enzyme induction or hypoxia which is used widely to exacerbate histological evidence of liver damage. It therefore would appear to be a superior technique in screening newly synthesized anesthetic agents as to their potential for liver damage. In addition, it may also serve to establish a relative index of safety in grading anesthetic agents for surgical procedures involving the biliary and portal systems as well as hypoxic and ischemic conditions. ESR studies in these areas are currently underway in our laboratories.

References.

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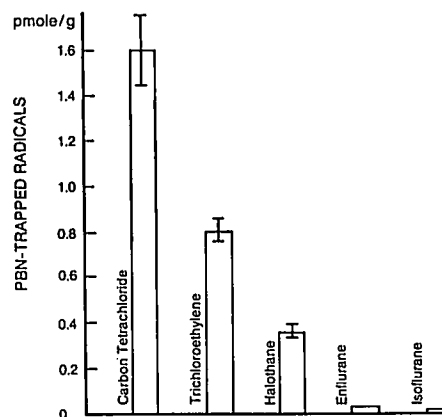


Fig. 1. Free radical count (mean + SEM) in picamoles/g of rat liver. n = 20 for each agent.