Title : CHARACTERIZATION OF HYPOXIC MODEL FOR HALOTHANE HEPATOTOXICITY

Authors : B.R. Brown, Jr., M.D., Ph.D., I.G. Sipes, Ph.D., R. Jee, B.S., and A.J. Gandolfi, Ph.D.

Affiliation: Department of Anesthesiology, University of Arizona, Tucson, Arizona 85724

Introduction. Three years ago we reported a model for halothane-induced hepatotoxicity involving the exposure of phenobarbital induced male rats to 1% halothane in a 14% $O_2$ atmosphere. This model was conceived from earlier findings that halothane could covalently bind to subcellular macromolecules and that the binding could be increased by phenobarbital pretreatment or exposure to hypoxic conditions. Experiments since then have extensively characterized the model and confirmed its applicability to studying halothane-induced hepatotoxicity.

Methods. The basic hypoxic model for halothane-induced hepatotoxicity involves pretreatment of male Sprague-Dawley rats with phenobarbital and their exposure to 1% halothane for 2 hours in an atmosphere of 14% oxygen ($F_IO_2$=0.14). The animals are sacrificed 24 hours later for liver histological examination and determination of SGPT. Four basic parameters of the model were varied; halothane concentration, oxygen concentration, length of exposure, level of induction. Certain rats were also scarificed at various time points following exposure to the halothane-hypoxia model. In addition, similar exposures were performed on animals pre- or post-treated with inhibitors of drug metabolism, inhibitors of protein synthesis, and a variety of sulfhydryl agents. Female rats pretreated with testosterone, other rat strains, and other laboratory animals have also been utilized in the halothane hypoxia model. Studies were also undertaken where rats were reexposed periodically to the halothane hypoxia model.

Results. Histologically, it was found that (1) increased halothane concentration produced a dose response (0.1-1.3%) when $F_IO_2$ = 0.14; (2) $F_IO_2$ greater than the 0.14 decreased liver injury (H at 1%); (3) exposure lengths as short as 30 minutes, produced liver injury and (4) increased hepatic damage occurred with increased hepatic cytochrome P-450 levels (Fig 1). Hepatic lipid accumulation was observed 2 hours after halothane exposure with the greatest degree of cell injury noted at 12 to 48 hours followed by a rapid repair process over the next 48 hours. Protein synthesis inhibitors prolonged lesion occurrence but did not cause progressive liver degeneration. The drug metabolism inhibitors, SKF-525A and metyrapone, decreased halothane induced histology changes when administered prior to halothane exposure. Cysteine, cystamine, and N-acetylcysteine could be given 4 hours after halothane-hypoxia and provide nearly complete inhibition of necrosis. Phenobarbital pretreated female rats did not respond to the halothane hypoxic model. However, pretreatment of female rats with testosterone proprionate and phenobarbital caused these animals to exhibit halothane induced histological changes similar to male animals. Repeated exposure of male rats to the halothane hypoxia model did not illustrate any differences in histology over a single exposure.

Discussion. Most models for halothane associated liver injury are centered on the hypothesis that certain metabolic products or reactive intermediates of halothane initiate the events that lead to liver injury. This hypothesis is supported by the findings presented here and in previous presentations. The two most critical variables necessary to initiate halothane induced liver injury are induction of the hepatic biotransformation enzyme system and a reduced $F_IO_2$, which results in an increased rate of halothane biotransformation by the reductive, hepatotoxic pathway. Additional evidence implicating halothane bioactivation as a causative factor is indicated by the inhibition of the lesion by pretreatment of rats with inhibitors of drug metabolism. At present the hypoxic model does not completely correlate with the reported clinical cases of fulminating liver injury following halothane anesthesia. The lesion that develops in rat liver rapidly repairs and does not progress to liver failure. Also, female rats appear resistant. The latter problem has been partially resolved by pretreatment of female rats with testosterone, a hormone necessary for maximal microsomal cytochrome P-450 mediated reactions in both male and female rats. Some progress has also been made at prolonging the duration of liver injury by inhibition of liver repair processes with inhibitors of protein synthesis. However, it should be stressed that only a small percentage of animals would be expected to develop progressive liver failure and that only a small number have been studied longer than 2 days post exposure. Perhaps the most exciting finding is the inhibition of lesion development by post-anesthetic treatment with sulfhydryl containing compounds. Cystamine, cysteine and N-acetylcysteine produced nearly complete inhibition when administered 4 hours after end of anesthesia and afford partial protection 8 hours post anesthesia.

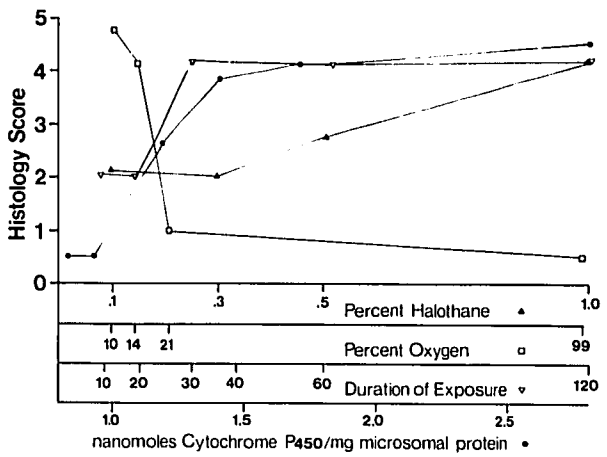


FIGURE 1

Supported in part by NIAMD Grant #AM16715-06.