Title: FREE RADICAL FORMATION IN VIVO AND HEPATOTOXICITY DUE TO ANESTHESIA WITH HALOTHANE

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Introduction: In an animal model in which halothane biotransformation is similar to that in man, halothane was consistently hepatotoxic and reductive metabolism of the anesthetic was associated with hepatotoxicity. Indirect evidence suggests that a free radical is responsible for this toxicity: the amounts of volatile reductive halothane metabolites, including 2-chloro-1,1,1-trifluoroethane, which is derived from a free radical, are increased by factors promoting hepatotoxicity, and under conditions of enzyme induction halothane initiates lipid peroxidation in rats. The aim of the present study was to determine if free radical formation in vivo, during anesthesia with halothane, enflurane or isoflurane, is associated with hepatotoxicity. Carbon tetrachloride (CCl₄) was included in the study as a model free radical hepatotoxin.

Methods and Results: Male Fischer 344 rats were fasted for 18hr before experiments. Where indicated, mixed function oxidase induction was obtained by addition of sodium phenobarbital (PB) (1g/l) to drinking water for seven days, followed by one day of tap water prior to experiments. Free radicals were trapped in vivo, during anesthesia, as stable adducts using the spin trap 2,2,6,6-tetramethylpiperidinyl-1-oxide (PBN). These adducts were extracted from the liver and studied by electron spin resonance spectrometry (ESR). PBN (125mg/kg) was administered i.p. injection in coconut oil at a concentration of 50mg/ml. CCl₄ was administered i.p. at a dose of 0.5ml/kg as a 15% v/v solution in coconut oil. Halothane 1%, enflurane 2.1% or isoflurane 1.5% were administered for 2 hours in 14% oxygen-balance nitrogen using a glass mask designed for anesthetic administration to rats. Free radical formation: In rats pretreated with PB and anesthetised with halothane for 2 hours under conditions of 14% oxygen (n=3), free radical was trapped in the liver (Figure). In rats similarly treated with enflurane (n=2) or isoflurane (n=2), no free radical was trapped. Control experiments also yielded no free radical under the following conditions: PB induced rats receiving PB and placed in 14% oxygen for 2 hours (n=2); rats given i.p. PBN and allowed to breathe room air (n=2), and rats anesthetised with 1% halothane in air for 2 hours and given i.p. coconut oil (n=2). In rats treated with CCl₄ (n=4), free radical was trapped from the liver and gave a strong ESR signal, identical to that of a chemically prepared trichloromethyl radical-PBN adduct. Hepatotoxicity: For each anesthetic agent studied, four rats received the anesthetic under conditions of enzyme induction and 14% oxygen while in 4 PB treated rats placed in 14% oxygen for 2 hours served as controls. Twenty-four hours after anesthesia, rats anesthetised with halothane under conditions of enzyme induction and hypoxia had significant (p < 0.05) increases in serum alanine aminotransferase (ALT) (ALT increase = 450 ± 100 U/L) and all livers showed hepatocyte degeneration and necrosis in the region of the central veins. Under the same conditions of anesthesia, enflurane and isoflurane were not followed by increased serum ALT or liver damage.

Discussion: Free radicals were trapped in vivo after administration of CCl₄ or halothane, which were both hepatotoxic under the conditions of the present study. No free radical was trapped after enflurane or isoflurane, which were not hepatotoxic under identical conditions. This indicates that the anesthetic state per se is not responsible for free radical formation. The radical trapped by PBN after CCl₄ treatment was identified as a metabolite of CCl₄, demonstrating that the techniques used in this study are capable of trapping free radical intermediates formed during metabolism of halogenated compounds. No lipid derived radicals were trapped by PBN after CCl₄, though this compound is a potent initiator of lipid peroxidation. This evidence supports the hypothesis that the free radical trapped by PBN was derived from halothane rather than from lipid.

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References:

(a)
receiver gain = 1.0*10^4
10 G

(b)
receiver gain = 5.0*10^4

ESR spectra of liver extracts from phenobarbital-induced rats anesthetised in 14% oxygen-balance nitrogen and treated with PBN. (a) Halothane; (b) Enflurane.