INVESTIGATION OF THE IMMUNOLOGICAL BASIS OF HALOTHANE-INDUCED HEPATOTOXICITY


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INTRODUCTION

It is well established that halothane (CF₃CHClBr), an inhalation anesthetic, causes both a mild and a severe form of hepatotoxicity in patients.¹ The milder form of hepatotoxicity is characterized by minor elevations in serum transaminase levels and has been reported in about 20% of patients anesthesized with halothane.²,³ The severe form of hepatotoxicity, however, is often fatal and is much rarer.⁴,⁵ Most of the patients with the severe disease have high serum transaminase values and massive hepatic necrosis. The necrosis is often centrilobular,⁶ although other histologic lesions have been reported.⁷,⁸

Reactive Metabolites of Halothane

The metabolism of halothane has been extensively studied in order to determine whether a metabolite or metabolites may be involved in these toxicities. It is now clearly recognized that liver microsomal cytochrome P-450 metabolizes halothane into two reactive products. Under anaerobic conditions, CF₃CHClBr is reduced by cytochrome P-450 to produce the reactive radical intermediate, 1-chloro-2,2,2-trifluoroethyl radical (CF₃CHCl·).⁹-¹² This product can react with liver microsomal protein, lipid, and presumably other unidentified target substances in the liver, to form adducts or can abstract a hydrogen atom to produce 1-chloro-2,2,2-trifluoroethane (CF₃CH₂Cl). The radical can also be reduced further by cytochrome P-450 to form 1-chloro-2,2,2-trifluoroethyl carbanion (CF₃CHCl⁻¹), which can eliminate fluoride to produce 1-chloro-2,2-difluoroethylene (CF₂CHCl). In contrast, liver microsomes in air catalyze the oxidation of halothane to a trifluoroacetyl halide (CF₃COX) intermediate that either acylates tissue molecules to form trifluoroacetylated (TFA, CF₃CO-) adducts or reacts with water to form trifluoroacetic acid (CF₃COOH).⁹,¹³-¹⁷
Mild Form of Hepatotoxicity in Animals

The mild form of hepatotoxicity appears to have been produced in animals. Most of the studies with rats indicate that the toxicity is due to the reductive radical metabolite. For example, it was found that substitution of a deuterium atom in place of a hydrogen atom in halothane (CF₂(CD₃)Br) slowed the rate of the oxidative pathway of metabolism without decreasing the extent of hepatotoxicity. Other studies have shown that treatment of rats with halothane under moderate hypoxia (14% O₂), enhanced both the rate of the reductive pathway of metabolism and the extent of the hepatotoxicity. Similarly, females rats were found to metabolize halothane more slowly by the reductive pathway than males and to be less susceptible than males to the hepatotoxic effect of halothane. In addition, treatment of rats with cimetidine selectively inhibited the reductive pathway of halothane metabolism and provided partial protection against its hepatotoxic effect. In contrast to these results, recent studies with a guinea-pig model of halothane-associated hepatotoxicity suggested that either the oxidative or reductive metabolites may produce the hepatotoxicity.

Evidence for an Immune Basis of the Fulminant Form of Hepatotoxicity

Progress in understanding the basis of the fulminant form of hepatotoxicity has been considerably slower than that of the mild form of toxicity, mainly because no animal model has been developed to study this disease. Nevertheless, recent findings have indicated that this rare toxicity may have an immune basis.

It had been suggested several years ago that the fulminant form of halothane-induced hepatotoxicity might have an immune basis, because most of the patients with this toxicity had received halothane on previous occasions and because its clinical features, such as eosinophilia, fever, rash, and serum liver-kidney microsomal autoantibodies were similar to those found in idiosyncratic drug sensitization reactions. This hypothesis has been strengthened by several lines of evidence. For example, a cell migration test revealed that leucocytes from 8 of 12 patients with unexplained fulminant hepatic failure after halothane were sensitized to a cell-subfraction of liver homogenate from halothane-treated rabbits. Similar evidence for cellular sensitization to an antigen formed during halothane anesthesia was obtained by a direct lymphocyte cytotoxicity assay. Furthermore, specific circulating antibodies were found only in the sera of patients with fulminant hepatic failure after several episodes of anesthesia. These antibodies were shown by indirect immunofluorescence to react with the cell surface of hepatocytes from halothane-treated rabbits. Moreover, they rendered the hepatocytes susceptible to antibody-dependent cell-mediated cytotoxicity (ADCC). These results suggested that halothane-induced fulminant hepatotoxicity may be initiated by a reactive metabolite that alters the surface structure of hepatocytes and in susceptible individuals, induces an immune response, which in turn leads to fulminant hepatotoxicity.

Recent investigations have suggested that it is the oxidative metabolite, CF₃COX, and not the reductive radical metabolite, CF₃CHCl., of halothane that alters the surface of the rabbit hepatocytes so that they are recognized by the antibodies from the halothane hepatitis patients. For example, only hepatocytes from rabbits administered halothane at oxygen tensions that promoted its oxidative and not its reductive metabolism were susceptible to ADCC induced by the human antibodies. Subsequently, sensitive peroxidase enzyme-linked immunosorbent and immunofluorescence antibody staining methods for identifying