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Immunological Basis of Anesthetic-induced Hepatotoxicity

PRESENT EVIDENCE INDICATES halothane-induced hepatotoxicity can be considered as two entities in clinical practice. One, a mild form of toxicity, is seen shortly after anesthesia and can be reproduced in animals regardless of the mechanism of toxicity (chemotoxic or ischemia-hypoxia) or animal species. In the other form of hepatotoxicity, a delayed, severe, and often lethal clinical toxicity is observed. This form may be due to an allergic response producing a fulminant type of hepatotoxicity. The rarity of the disease, its association with repeated administrations, and the difficulty in reproducing it in animals suggests an immune mediated mechanism.[1]

The idea that a hypersensitivity response may result in halothane-associated liver injury is not new. Studies have been initiated at various times through the years only to be blurred by the lack of a "complete picture." The inability to produce in animals a form of the disease involving an immune mechanism and/or the inability to demonstrate conclusively the role of the immune system in liver injury have been two reasons why a more definitive cause-effect relationship has not been established.[2–5]

How, then, have current studies improved the understanding of the role of the immune system in halothane-induced liver injury? In part, the application of newer, more sensitive technology that clearly demonstrates production of antibodies and the detection of liver antigens in these cases provides great insight. The importance of recent findings, such as those investigated by Christ *et al.* and reported in this issue of ANESTHESIOLOGY,[6] suggests that hepatotoxicity following all volatile halogenated anesthetics may be linked by a common mechanism. Unquestionably, the incidence of hepatic injury following enflurane and isoflurane anesthesia is less than that reported following halothane, but it must be accepted that these volatile anesthetics can produce rare liver injury, albeit at a much lower incidence. The report by Christ *et al.* (National Institute of Health) as to the potential for a metabolic basis of hepatotoxicity following enflurane anesthesia *via* production of covalently bound liver antigens recognized by antibodies generated from patients with halothane hepatitis is significant. As clearly stated in their studies, the principal reason that isoflurane did not produce detectable liver antigens might be solely related to the level of biotransformation in their particular animal preparation. But, in fact, isoflurane, enflurane, and halothane all have the potential for producing acetylating intermediates that can alter liver proteins rendering them immunogenic. For example, such reactive metabolic intermediates of halothane could include, among others, either a trifluoroacetyl halide from the oxidative pathway, free radicals, or carbene intermediates from reductive pathways.[7]

How does this work differ from earlier studies? Basically, very little. However, many of the earlier techniques used were of insufficient sensitivity, and variation in results from laboratory to laboratory were often contradictory.[2–4] Many researchers tried to "clear the picture" by studies in antigenically sensitized animals; negative results frequently led to termination of studies.

At the time of many early investigations, little was known about the bioactivation of halothane along with

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its subsequent covalent binding of intermediates, and liver injury could not be reliably reproduced in animals. The realization of a hypersensitivity-mediated mechanism came indirectly with the demonstration of bioactivation of halothane. Finding that metabolites could bind covalently to liver tissue[8] proved important, because these substances could potentially act as haptens and evoke an immune response. Although this information was suggested by early investigations, recent work has demonstrated clearly the importance of bioactivation and covalent binding to liver protein to produce immunogens.[9]*

Early studies in both rabbits and guinea pigs demonstrated that multiple exposures to halothane in hyperoxic atmospheres evoke an antibody that cross-reacts with trifluoroacetylated albumin.[5,10] These data imply that, during halothane anesthesia, animals oxidatively biotransform halothane to a trifluoroacetyl halide moiety, which then acetylates endogenous liver protein. Endogenous liver protein is, in effect, changed from self to non-self, thus becoming immunogenic. Antibodies are elicited against this non-self protein creating an immune response. Importantly, patients with halothane hepatitis have been shown to generate a similar antibody response that reacts with trifluoroacetylated albumin.[11] The chronology of this antibody response has been reported with antibody titers remaining at high levels for over a month post-exposure. In one patient who succumbed to liver failure postoperatively, very high titers of antibody dropped dramatically prior to death. Controls included laboratory personnel, anesthesiologists, and patients anesthesized with halothane. None of these groups demonstrated ill effect from halothane and all had negative or marginal antibody titers. Others have also shown similar results in their patient studies, indicating that the response is fairly specific for patients with halothane hepatitis and not evident in patients developing liver complications from other drugs, toxins, or viruses.[12,13] Recent evidence states, however, that there is a 12% incidence of false-positives from the antibody ELISA test, thereby masking the specific antibody-antigen response.[14]

Thus, the anti-TFA antibody test has the potential as a diagnostic marker specific for halothane hepatitis or other halogenated anesthetic-induced liver injury. One must use caution with this assay as an absolute indicator, because there is the possibility of false positives or a negative result in patients whose antibody is rapidly cleared.

The actual identification of the halothane-induced liver antigens is underway. This was never attempted in past work. Due to the sensitivity of the presently available immune techniques, improved methods for isolating and separating protein, and development of gel-transfer assays (*i.e.*, western assays), all research groups have been able to identify specific liver proteins (identified by molecular weight) that would cross-react with antibodies generated either in animals or from patients with halothane hepatitis. We have been able to identify five liver proteins induced in rabbits and guinea pigs exposed multiple times to inhalation of halothane. Liver proteins derived from liver homogenates were most prevalent after three exposures and persisted for 3–5 days post-exposure.[15] Satoh *et al.* following an intraperitoneal injection of halothane in rats detected proteins in microsomal fractions that were fairly similar to those noted in both rabbits and guinea pigs.[16] Similarly, Kenna *et al.* have also evaluated liver samples from patients and found a similar array of liver antigens.[17] Initial studies from the NIH stated that one of the proteins labeled by the trifluoroacetyl moiety was the hepatic cytochrome P-450.[16] However, subsequent studies have determined that this is not the dominant protein acetylated. The current emphasis of the NIH group is to identify the endogenous proteins that are conjugated with the halothane metabolite to form immunogens.

The identification of potential antigens has generated many questions. Is the uniqueness of the halothane-induced liver injury the result of specific proteins being labeled in specific patients? Is this the reason the latent, progressive form of hepatic injury is rare or sporadic? If the specific acetylated proteins could be identified, these proteins could then be molecularly cloned. After production of adequate quantities of the protein, these proteins could be chemically acetylated to mimic the antigen and used for pre-sensitization of animals prior to anesthetic exposure to determine if the innoculated animals are more or less susceptible to halothane-induced liver injury.

Recent efforts in determining a role for the immune system in halothane-induced liver injury have concentrated primarily on the humoral response. One must also consider the cellular immune response as well. Current efforts are underway to determine if the cellular immune response can produce liver injury. However, recent evidence has only noted a weak cellular immune response that lacks the intensity and specificity of the humoral reaction.†

Finally, the role of the immune system in the hepatotoxicity of the volatile anesthetics must be addressed. Al-

* Roth TP, Hubbard AK, Gandolfi AJ: Localization of halothane-induced antigen in situ by specific anti halothane metabolite antibodies. Toxicologist 8:11, 1988

† Hubbard AK, Roth TP, Gandolfi AJ: Elicitation of a cell mediated immune response to a reactive intermediate of halothane. Toxicologist 8:12, 1988

though numerous groups have expounded the virtues of their animal preparations for the acute phase of injury, these are not reflective of the clinical situation. In contrast, demonstration by Christ *et al.* that pretreating animals (albeit by ip injections) with enflurane to produce labeled liver protein cross-reactive with antibodies from halothane hepatitis patients has clinical relevance. Their data suggest that the other halogenated anesthetics have much the same potential as halothane for acetylating liver protein as halothane, which explains why a common pattern of liver injury, albeit at a much lower frequency, may be associated with any halogenated anesthetic. Both isoflurane and enflurane can form either an acid halide or reactive ester intermediate, with potential for acetylating proteins.[18] The principle difference in the rate of incidence of various anesthetic-induced liver injury could be related to the rate of formation of these acetylating species. As is well documented, halothane is biotransformed far more than enflurane which, in turn, is biotransformed more than isoflurane.[19] Therefore, the prediction is that the incidence of liver injury relative to this immunopathological mechanism would be in the same order.[20]

The report in this issue of ANESTHESIOLOGY represents the first unifying hypothesis for a common mechanism between the halogenated volatile anesthetics for production of the liver injury, and suggests that changing halogenated anesthetics for patients requiring multiple anesthesias will not necessarily reduce the risk for a susceptible individual from anesthetic-induced liver injury. Because the antibodies generated by one anesthetic apparently cross-react with antigens generated by a different one, changing anesthetics will perhaps reduce the incidence but not necessarily negate an occurrence. Patient risk would most likely be reduced if halothane exposure preceded exposure to enflurane or isoflurane, because the extent of their metabolism, 2.4% and 0.2%, respectively, is lower than that for halothane (20%).[19]

Halogenated anesthetics produce a unique immunological response. We have attempted to replicate the halothane-induced antibody response by exposing animals to other non-anesthetic halocarbons. These chemicals (trifluoroethanol and 1,1,2-trichloroethane) will be processed by the liver to reactive intermediates that should covalently bind to the lysine residues on liver proteins. Interestingly, even after multiple and extensive exposures, neither the rabbit nor the guinea pig produced any humoral antibodies against these chemicals conjugated to protein (unpublished observations). These data suggest that the anesthetic-induced immune response is relatively unique to the fluorocarbon acetylated intermediates that are produced from the volatile halogenated anesthetics.

We believe that this current research opens a new area for consideration of the mechanism of anesthetic-induced liver injury. More importantly, proof of an immune-mediated mechanism would best explain the clinical situation of delayed injury (7–10 days) seen after multiple exposures. In none of the existing animal preparations can the liver injury seen clinically be exactly duplicated. Of importance, however, is the fact that any fluorohalocarbon volatile anesthetic has a potential for acetylating a liver protein with the possibility of evoking an immune response. Much effort is needed now to clarify if this immune response is responsible for a toxicological event. Establishing a cause-and-effect relationship between host response and anesthetic damage is the future direction of research efforts.

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