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Mechanism of Halothane-induced Hepatotoxicity: Another Step on a Long Path

UNEXPECTED AND UNEXPLAINED hepatitis following general anesthesia with halothane has been the subject of an intensive research effort for a number of years. The discovery that halothane is metabolized opened the door to a simple, but logical, suggestion that one or more of the products of this metabolism is responsible for the hepatotoxicity. This idea was further nurtured by the fact that the experimental model for halothane-induced hepatotoxicity required pretreatment with phenobarbital (to induce cytochrome P-450 with subsequent increase in halothane metabolism), accompanied by hypoxia during halothane administration.[1-4] Halothane is uniquely metabolized, since it undergoes a simple oxidation when ample oxygen is present, but a more complex reductive metabolism occurs when tissue (hepatic) P_{O_2} decreases below 6 mmHg.[5] The hypoxia used in the *in vivo* rat model is sufficient to shift metabolism of halothane to the reductive pathway, increasing the number of metabolites and raising the possibility that one of the metabolites may be cytotoxic. In fact, phenobarbital treatment not only increases cytochrome P-450, but induces the isozymic form of cytochrome P-450 which catalyzes the reduction of halothane, thereby increasing the quantity of each metabolite.[6] If these were all the facts available, this would implicate the metabolism of halothane as the factor principally responsible for halothane-induced hepatotoxicity. However, there are other observations available which do not fit such a neat and simple mechanism suggesting that metabolism may not have a primary role in hepatotoxicity. The list of observations is long, but some of the more important facts are: 1) enflurane and isoflurane (as well as a number of other anesthetics and drugs) administered with hypoxia to rats pretreated with phenobarbital, also produce hepatotoxicity, in spite of their very low metabolism[7,8]; 2) neither enflurane nor isoflurane are reductively metabolized[9-11]; 3) fasting enhances the hepatotoxicity produced by the volatile anesthetics, but has no effect on metabolism[7]; 4) the metabolites produced by the reductive metabolism of halothane have been isolated and identified, but do not produce hepatotoxicity by themselves; 5) while other species, such as the mouse, have the appropriate isozymic form of cytochrome P-450 which can be induced by phenobarbital treatment and can metabolize halothane as effectively as the rat, they have not shown a similar tendency to develop hepatic injury[12]; 6) the guinea pig, which does not metabolize halothane well without phenobarbital induction, develops hepatotoxicity (the same type as the rat) without phenobarital pretreatment and/or hypoxia[13]; 7) higher doses with brief exposures to halothane are associated with a more pronounced hepatic damage than lower concentrations with longer exposures[14]; lengthy exposure increases the amount of halothane metabolized, while higher doses of halothane decrease the cytochrome P-450 activity; and[15,16] 8) triiodothyronine (T3) pretreatment of rats exposed to halothane without hypoxia or barbiturate pretreatment

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leads to hepatic necrosis despite the fact that T3 treatment reduces cytochrome P-450 content, consequently reducing the metabolism of halothane.[17]

On the other hand, many experimental observations which were interpreted only from a metabolic theory standpoint can be successfully explained by the hypoxic hypothesis, which simply implies that hepatic damage is due to a decrease in the hepatic oxygen supply/demand ratio (decrease in oxygen supply by respiratory and/or circulatory, systemic and regional, depression, and/or an increase in demand by enzyme induction).[18-20] In this case, the role of halothane metabolism *per se* is negligible or non-existent. For example, hypothermia decreases liver damage in phenobarbital pretreated hypoxic rats.[21] * This protective effect may be related to a decrease in cytochrome P-450 activity with a subsequent decrease in halothane biotransformation, but it can be also attributed to a decrease in oxygen demand by the liver, resulting in an improved hepatic oxygen supply/demand ratio. The association of halothane-induced hepatic necrosis with cytochrome P-450 concentrations and the depth of halothane anesthesia[21] fits the metabolic theory, but does not contradict the hypoxic hypothesis: the concentration of cytochrome P-450 affects the hepatic oxygen demand, while halothane concentrations affect the hypoxic oxygen supply, altering the hepatic oxygen supply/demand relationship. Metyrapone and cimetidine may protect rats from halothane-induced liver damage[22] † by either a decrease in cytochrome P-450 activity, which reduces the metabolism of halothane, or by a reduction in the overall hepatic metabolism and oxygen demand, which benefits the hepatic oxygen supply/demand relationship. The demonstrated importance of genetic profile in halothane-induced hepatic damage in humans,[23,24] rats,[25] and guinea pigs[26] can be explained by the metabolic or immunologic theories, but does not contradict the hypoxic hypothesis either: guinea pigs, some particular rat strains, and, possibly, some humans might have a higher hepatic oxygen demand and/or might develop a more severe reduction in hepatic oxygen supply (due to a "hypersensitivity" of the hepatic vasculature to halothane) than other animals, resulting in a lower oxygen supply/demand ratio. Halothane can probably cause different entities of hepatic injury, explaining some of the contradictions.[27] These issues have been extensively reviewed recently.[20,28]

Thus, it becomes relatively clear that halothane-induced hepatic necrosis, in many instances, is associated with hepatic oxygen deprivation, increased concentrations of cytochrome P-450, and halothane metabolism. But these are only associations, and the question of a cause-effect relationship is still unanswered. Strictly speaking, only experiments with a complete elimination of one of the associated factors can solve the mystery. There has been an attempt to produce hepatic oxygen deprivation by blood loss equal to that caused by decreased hepatic blood flow observed during halothane anesthesia.[19] If the degree of hepatic oxygen deprivation developed by blood loss were similar to that achieved during halothane anesthesia and produced the same degree of hepatic necrosis as that produced by halothane, then this would negate the metabolic theory associated with halothane anesthesia. Alternatively, a marked disparity in hepatic necrosis would support a mechanism other than hypoxia as being a major contributor. However, the desirable degree of hepatic oxygen deprivation could not be achieved in these experiments; all animals with such a degree of hepatic oxygen deprivation died, and there was not sufficient time for hepatic necrosis to develop.[19]

The study by Schieble *et al.* published in this issue[29] is a different, and certainly more successful, attempt to establish the role of halothane *per se versus* hepatic oxygen deprivation. They used monolayer cultures of rat hepatocytes, which obviously bypasses any concerns of hepatic blood (oxygen) supply, as well as certain hormonal effects. This technique involves the isolation and harvesting of rat hepatocytes, which are then allowed to attach to glass plates coated with collagen. While this appears simple, it is actually very difficult, requiring highly specialized equipment and training. Properly maintained, the cells will remain viable for days and retain hepatocyte characteristics. This procedure, therefore, offers considerable advantages over either cells maintained in suspension in buffers which limits the life of the cells to a matter of a few hours, or cells in culture where cells transform and lose the important hepatocyte characteristics in the process of division. On the basis of the results from this technique, Schieble *et al.* conclude that the mechanism of halothane hepatotoxicity is multifactorial and that hypoxia, phenobarbital pretreatment, and halothane each contribute to the hepatocyte cytotoxicity.[29] Isoflurane, unlike halothane, does not contribute additionally to the hypoxia-phenobarbital-related effect. It seems that this study by Schieble *et al.*[29] is a tombstone on the grave of the hypoxic theory of halothane-induced hepatotoxicity in phenobarbital-pretreated hypoxic rats. But, is there a grave? Is this a triumph for the metabolic theory? Not yet. The significant role of hepatic oxygen deprivation has again

* Ford D, Coyle DE: Effect of body temperature on the halothane-hypoxia-induction model of halothane (abstract). ANESTHESIOLOGY 59:A222, 1983

† Wood M, Uetrecht J, Phythyon JM, Wood AJJ: Cimetidine protects against halothane-induced hepatotoxicity (abstract). ANESTHESIOLOGY 57:A221, 1982

been demonstrated; halothane is not cytotoxic by itself, but requires hypoxia (as well as phenobarbital pretreatment) to produce this toxicity.[29]

Schieble *et al.* concluded that "reductive halothane metabolism is a factor in the pathogenesis of injury."[29] While this may be true, the question is, how large of a factor is metabolism? Strictly speaking, the investigators did not study halothane metabolism, nor the effects of metabolites. It may not be appropriate to implicate metabolism without some qualifications because, as pointed out above, there are still many questions raised in the *in vivo* studies that have gone unanswered. More importantly, control of cellular metabolism may play a key role in the cytotoxicity. In this regard, the hepatocyte has an abundance of receptors on its outer membrane which largely controls the events within the cell, whether the cell is *in situ* or *in vitro* in monolayers. For example, hepatic adrenergic receptors have been directly identified and characterized in plasma membranes isolated from rat hepatocytes.[30] Three types of adrenergic receptors are present on liver plasma membranes; α_1, α_2, and β_2, the majority being the α_1, activation of which is associated with mobilization of intracellular Ca^{++}.[30] Do the volatile anesthetics affect the activity of these adrenergic receptors? The answer is, very likely. Exposure to hypoxia results in an increase in intracellular free Ca^{++},[31] and exposure to halothane produces an increase in phosphorylase activity,[32] suggesting that intracellular free Ca^{++} has been increased.[33] While it is uncertain whether hypoxia and the volatile anesthetics are acting on the intracellular Ca^{++} reserves through a direct stimulation of the α-adrenergic receptors, the effect of hypoxia and halothane is very likely additive and associated with Ca^{++} release from intracellular stores. This mechanism should be recognized as being important for any mechanism of toxicity. A variety of chemicals that initiate toxic events leading to liver cell death exhibit marked alterations in intracellular Ca^{++} homeostasis with excessive accumulation of Ca^{++}.[34,35] The only important factors may be the duration and degree to which calcium homeostasis is disrupted. Thus, in light of this "calciogenic" hypothesis (involving an increase in free intracellular Ca^{++}), the difference in toxicity between halothane and isoflurane might result from different ability of anesthetics to disrupt the intracellular calcium homeostasis, and/or may be simply dose-related; only one dose of anesthetics (1.5 MAC) was studied,[29] while end-points for toxicity might be different from end-points for movement response. These comments are intended as a plea for an open interpretation of the data related to the toxicity of the volatile anesthetics. If, as many agree, the mechanism is multifactorial, then the complete pharmacodynamics of these agents should be considered before any definite conclusions are reached. A method for studying hepatotoxicity in a definitive manner has been offered by Schieble *et al.*[29] We should now take advantage of this opportunity.

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Should We All Have a Sympathectomy at Birth? Or at Least Preoperatively?

THE SYMPATHETIC NERVOUS SYSTEM appears useful to wild animals in helping to mobilize energy stores and in facilitating escape from threatening situations. But, as the article by Stone *et al.*[1] in this issue of ANESTHESIOLOGY suggests, such reactions may not be beneficial in anesthetized humans inasmuch as myocardial oxygen requirement may increase beyond supply. Do the adverse effects of stress now outweigh the benefits an intact sympathetic nervous system conveys? Should we ideally all be sympathectomized at birth, or at least preoperatively? Before answering this not so tongue-in-cheek question, we should first consider the details of this study by Stone *et al.*[1] which has stimulated this question.

Stone *et al.* gave one of a variety of beta-adrenergic blocking drugs or a placebo as preoperative medication to a group of mildly hypertensive patients and, knowing to which group the patients were assigned, the investigators then looked for ischemic episodes. They observed a significantly greater incidence of brief ischemic episodes during induction and emergence in the untreated patients compared with patients receiving a beta-adrenergic blocking drug as premedication. Al-

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