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Steatohepatitis: A Tale of Two ''Hits''?

See article on page 764.

It has long been recognized that hepatic steatosis (fatty liver) occurs frequently in heavy alcohol drinkers and obese individuals.[1,2] It may also follow the ingestion of a wide variety of therapeutic drugs.[3] Steatosis of any etiology can be associated with the development of necroinflammation and fibrosis, so-called steatohepatitis, and even cirrhosis.[4] Furthermore, steatohepatitis caused by alcohol, drugs, or other forms of nonalcoholic steatohepatitis (NASH) share many histological features.[5] The question is whether these disparate causes could lead to steatohepatitis and its potential sequelae of cirrhosis or liver failure by one or more common mechanisms. Any satisfactory unifying mechanism should ideally explain why, in some individuals, steatosis, whatever its etiology, never progresses to steatohepatitis,[6] and also explain the variable incidence and severity of steatohepatitis and fibrosis in fatty liver of different etiologies. In this issue, Berson et al., working in Pessayre's laboratory, provide persuasive evidence that at least one such mechanism linking steatosis to necroinflammation and fibrosis is lipid peroxidation.[7]

A growing body of evidence supports a role for lipid peroxidation in the pathogenesis of alcohol-induced hepatitis and fibrosis.[8,9] Ethanol metabolism results in the formation of reactive oxygen species (ROS) and carbon-centered free radicals capable of initiating peroxidation of the polyunsaturated fatty acid side chains of membrane phospholipids and lipoproteins. Potential sources of free radicals are the ethanol-inducible cytochrome P450 2E1 (CYP2E1), which generates superoxide, hydroxyl, and hydroxyethyl radicals, the mitochondrial respiratory chain (in response to the increased ratio of reduced to oxidized nicotinamide adenine dinucleotide [NADH/NAD]), xanthine and aldehyde oxidases, and peroxisomal β-oxidation of free fatty acids, which generates hydrogen peroxide.[10] CYP2E1-mediated generation of hydroxyethyl radicals in particular correlates closely with the degree of lipid peroxidation and liver damage in animal models of alcoholic liver disease (ALD).[11] A role for lipid peroxidation in NASH has been suggested by recent studies showing its presence in both animal models of nonalcoholic fatty liver and humans with steatosis of different etiologies.[12–14]

A major attraction of lipid peroxidation as an important common pathogenic mechanism of steatohepatitis associated with fatty liver of different etiologies is that it potentially explains most, if not all, of the diverse histological features observed in this condition. Peroxidation of membrane lipids may cause cell necrosis and megamitochondria. The aldehyde products of lipid peroxidation, 4-hydroxynonenal and malondialdehyde (MDA), are capable of activating hepatic stellate cells,[15,16] the principal collagen-producing cells within the liver, cross-linking cytokeratins to form Mallory bodies,[12] and stimulating neutrophil chemotaxis.[17] MDA may also contribute to inflammation by activating NF-κB,[18] a transcription factor regulating the expression of several proinflammatory cytokines and adhesion molecules including tumor necrosis factor α, interleukin 8, intercellular adhesion molecule 1, and E-selectin.[19]

Previous work from Pessayre's group has suggested that the mere presence of oxidizable fat within the liver is enough to trigger lipid peroxidation.[12] However, many patients with steatosis never progress to necroinflammation or fibrosis.[6] This suggests that, in addition to steatosis (the first "hit"), the development of steatohepatitis requires the presence of some other factor(s) (second "hit"). This factor(s) might be expected to be particularly apparent in conditions where steatohepatitis is most commonly associated with steatosis, including alcohol-related liver disease and certain drugs. The results presented by Berson et al. show elegantly that one such second hit is a source of free radicals capable of inducing oxidative stress.

Many drugs are associated with the development of steatosis, including antiviral agents (interferon, zidovudine), aspirin, and other nonsteroidal anti-inflammatory drugs, the antiepileptic sodium valproate, and the tetracycline group of antibiotics. The fat is typically microvesicular in distribution and results predominantly from the inhibition of mitochondrial β-oxidation of fatty acids by a variety of different mechanisms.[3] However, only one class of drugs is commonly associated with classical steatohepatitis, the cationic amphiphilic amines: amiodarone, perhexiline, and the coronary dilator 4,4'-diethylaminoethoxyhexestrol (DEAEH).[3,20,21] Pessayre's group has shown previously that amiodarone[22,23] and perhexiline[24] accumulate in the mitochondria and inhibit not only β-oxidation (causing steatosis, the first hit) but also the transfer of electrons along the respiratory chain. Theoretically, this latter effect would be expected to generate superoxide anions capable of initiating lipid peroxidation,[25] thus providing a potential mechanism for the second hit required for steatohepatitis to develop. In this latest study they first show that, like amiodarone and perhexiline, DEAEH inhibits both β-oxidation and respiration, and then go on to show that all three drugs increase the production of ROS by isolated mitochondria and increase in vivo lipid peroxidation 5–10-fold. These data strongly suggest that it is because these drugs not only cause steatosis but also provide a mechanism for increased oxidative stress that they are capable of inducing steatohepatitis-like lesions.

This concept of steatohepatitis as a double-hit lesion potentially explains its relatively common occurrence in alcohol-related liver disease, where there exist several mechanisms for both steatosis[1] and oxidative stress. This theory may also explain why it has been difficult to develop animal models of alcoholic hepatitis and cirrhosis. In addition to simple ethanol administration, necroinflammation and fibrosis have only been produced by manipulations that have provided an alternative or extra source of oxidative stress. These have included increasing the dietary content of polyunsaturated fat (which induces the activity of CYP2E1[26]) and iron[27] (which favors the production of hydroxyl radicals from hydrogen peroxide) and coadministering carbon tetrachloride vapor.[28] Because most heavy drinkers develop steatosis, the apparent individual susceptibility to advanced ALD seems likely to be explained by interindividual differences in the magnitude of the second hit, oxidative stress. This may be genetically determined, e.g., possession of the more transcriptionally active c2 allele of CYP2E1,[29] or influenced by environmental factors such as dietary intake of anti- or pro-oxidants.

The work of Pessayre's group might also be extrapolated to steatohepatitis of non–drug-related or ethanol-related etiologies. In particular, the data might explain why NASH occurs more commonly in association with some causes of steatosis than others and with greater severity. Some degree of lipid peroxidation can be shown in steatosis of most etiologies.[12] This presumably reflects the mild level of oxidative stress arising from normal physiological processes and is insufficient to cause significant liver injury. By analogy with drug-induced steatohepatitis, for NASH to occur, some additional source of oxidative stress (the second hit) is required that is capable of initiating enough lipid peroxidation to overcome the normal cellular defense mechanisms and produce necroinflammation. Recent studies have suggested several potential sources for this second hit. Increased expression of CYP2E1 has been shown in patients and animal models of NASH.[30,31] In the absence of ethanol, CYP2E1 can generate free radicals from endogenously produced ketones and aldehydes and dietary *N*-nitrosamines. Possible

mediators of its induction in nonalcoholics include ketones and/or fatty acids,[32] both of which may explain the induction of CYP2E1 by a high-fat diet.[26] Interestingly, among patients with obesity-related steatosis, the risk factors for steatohepatitis/fibrosis include rapid weight loss caused by dieting, debilitation or intestinal bypass surgery, surgical stress, alcohol intake, and diabetes, all of which are associated with an increase in the concentration of fatty acids and/or ketones within the liver.[1] In addition to CYP2E1 induction, an increase in the intrahepatic concentration of free fatty acids may provide a further source of oxidative stress via peroxisomal β-oxidation. This pathway becomes important in conditions of substrate overload or when mitochondrial β-oxidation is inhibited.[33] Unlike its mitochondrial counterpart, peroxisomal β-oxidation produces hydrogen peroxide that, in the presence of free iron, is converted to the highly reactive hydroxyl radical. The importance of liver iron in disease pathogenesis has been illustrated by a recent study showing that patients with NASH have an increased frequency of the C282Y mutation in the recently cloned hemochromatosis gene, HFE.[34] Clearly, as for ALD, other genetic determinants of oxidative stress could also play a role in susceptibility to NASH.

This study by Berson et al. of a small group of drugs has provided a basis for understanding at least one of the important mechanisms of steatohepatitis regardless of etiology. Its development requires a double hit, the first producing steatosis, the second a source of oxidative stress capable of initiating significant lipid peroxidation. This concept provides a rationale for both the treatment and prevention of disease progression in steatosis of alcoholic and nonalcoholic causes.

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