

ADVANCES IN HEPATOLOGY

Current Developments in the Treatment of Hepatitis and Hepatobiliary Disease

Section Editor: Eugene R. Schiff, MD

Reversibility of Liver Fibrosis



David A. Brenner, MD
Vice Chancellor for Health Sciences
Dean of the School of Medicine
University of California, San Diego
San Diego, California

G&H What causes liver fibrosis?

DB Liver fibrosis is the final result of most types of chronic liver injury. Most habits and events that injure the liver will lead to a fibrotic liver. The main causes of liver fibrosis in the United States are hepatitis C virus (HCV), which I would say is the liver disease of the 20th century in the United States, and nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, which I would say are the liver diseases of the 21st century. Besides these conditions, other causes of liver fibrosis include hepatitis B virus infection, various autoimmune diseases such as primary biliary cirrhosis and primary sclerosing cholangitis, and liver injury caused by alcohol abuse (alcohol-induced hepatitis, liver fibrosis, and cirrhosis). In each case, the liver is injured in some way, the injury leads to inflammation, and the inflammation leads to fibrosis.

G&H Is fibrosis a process whereby scarring occurs as the immune system tries to right the wrong done to it?

DB That is the teleologic way of thinking about the process. The same mediators that cause inflammation are also causing fibrosis in the way that the same inflammatory macrophage that is recruited to the injured liver releases tumor necrosis factor α but also releases transforming growth factor beta (TGF- β), which is the major fibrogenic cytokine in the liver.

G&H Is complete regression of liver injury achievable?

DB There is clear evidence, based on findings from repeat biopsy, that mild to moderate fibrosis is reversible. For example, livers of patients who are successfully treated for HCV infection and test HCV RNA–negative will, upon repeat biopsy, show no evidence of the fibrosis seen in the index biopsy. However, although fibrosis regresses, it is not fully reversible in patients with cirrhosis. The question is, what is the point of no return that stymies reversal of fibrosis? The cause might be structural. Extensive crosslinks develop in collagen, the fibrotic bands consisting mainly of fibrillar collagen, as the collagen bands mature. Some of these crosslinks are irreversible so that the normal collagenases that the body makes cannot then degrade the collagen; the collagenases cannot break up these crosslinks. The point of no return may be when extensive crosslinking has occurred. At this time, the fibroscar is very stable, and the body has no way to remove the causes of the effect.

When this scenario ultimately results in the need for liver transplantation, it is not so much because of the fibrosis but because of the sequelae of fibrosis. Fibrosis may cause portal hypertension, leading to variceal bleeding, ascites, and portal systemic encephalopathy. With liver failure come coagulopathy and failure of the liver to clear toxins from the body. Fibrosis itself is not the threatening condition per se because a person can have some fibrosis and be just fine, but when the fibrosis progresses to the point of causing liver

decompensation, or if, over time, enough inflammation and fibrosis occur, then hepatocellular carcinoma (HCC) results. It is very rare that HCC develops in the normal liver; it is almost exclusively seen in the setting of fibrosis.

G&H How is the quality or extent of liver fibrosis measured?

DB The gold standard is liver biopsy. A biopsy is performed, and a decision is then made about whether or not to treat the liver injury with pharmacotherapy. Then, a repeat biopsy is done. Although this procedure is considered the “gold standard,” it is imprecise because, if different parts of the liver are sampled, different results might be obtained. The liver might be exactly the same between biopsy evaluations, but the particular part sampled in 1 biopsy might have more fibrotic tissue and the part sampled in another might have less. Another challenge regarding liver biopsy is that the sample must be long enough to capture enough portal tracts to make a proper analysis. In addition, liver biopsies involve high cost and also some risk—although a very small risk—of mortality.

Given these caveats, clinicians say, “There must be better ways to do this.” Researchers have been looking at both imaging and serum biomarkers for insights into evaluating the presence of liver fibrosis and whether fibrosis, when present, is reversing. No modality has yet replaced liver biopsy, but there are resources—everything from a FibroScan (Echosens/Sandhill Scientific), which is a method of measuring the elasticity of the liver (the more fibrotic, the more rigid the liver is) to looking in the serum for markers of the collagens or other fibrotic particles. Data show that these modalities work well, but they have not been so robust that clinicians have stopped performing biopsies and replaced their criteria for success.

G&H What are the measurable signs of liver fibrosis?

DB The biopsy is analyzed by several different specialized stains. The 2 that are most useful for detection of fibrosis are the Sirius red stain, which only stains the collagen, and the Masson trichrome stain, which stains the cells 1 color and the collagen—that is, the fibroscar—blue. Some pathologists prefer 1 stain to the other. The Sirius red stain is easy to quantitate using a scanner, so it is sometimes preferred.

G&H Are there therapeutic strategies or agents that encourage regression of liver fibrosis?

DB Yes, but none are approved. Very many drugs are undergoing trials now, and results from several drugs are showing mixed or not enough success to make them standards of

care. Recently, however, a series of observations of molecules that seem to be linked to fibrosis have been disseminated. Researchers have had concerns that if an agent that is basically responsible for collagen production is used, it will affect normal collagen, such as the collagen in tendons and ligaments. If this were so, the outcome would be terrible because a person would dissolve into jelly. To avoid this, researchers try to identify pathologic pathways that are not normally present. For example, there is a crosslink of collagen that requires the hydroxylation of lysine. There are many different types of enzymes that are lysyl oxidases, but lysyl oxidase-like 2 (LOXL2) is only found in fibrotic organs, not normal muscles, tendons, and bone. Therefore, researchers say, “Maybe if we target LOXL2, the agent used will not affect normal physiology and just affect the pathophysiology.”

LOXL2 inhibitors are currently under study in clinical trials. Another promising therapy is inhibition of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is responsible for generating reactive oxygen species, but some of the varieties of NADPH oxidase are only found in injured tissues, not in normal tissues. These include *NOX1*, which is induced in liver injuries, and *NOX4*, for which there is now a small inhibitor that is in clinical trials.

G&H What have your studies on myofibroblasts found?

DB Other organs undergoing fibrosis contain myofibroblasts, but these cells are not normally seen in the liver. Myofibroblasts respond to injury before development of the fibroscar and come from cells that are nonfibrotic. For example, fibroblasts can become myofibroblasts or, in the liver, hepatic stellate cells can become myofibroblasts. Therefore, normal cells can be activated to become myofibroblasts. They start making large amounts of extracellular matrix proteins, resulting in the formation of a fibroscar. One area of study that my research team and others have an interest in is how removing the fibrotic stimulus allows the liver to revert toward normalcy. When the liver is examined again, myofibroblasts are gone. The question is, what happened to them? My colleagues and I showed that they can undergo 2 possible outcomes. One is that they can die through apoptosis or senescence. The other is that they are actually able to revert to an inactivated phenotype that looks like the original quiescent cell, although gene profiling and other techniques will show that they are actually different. Our findings lead us to believe that there are 4 types of hepatic stellate cells: the original quiescent hepatic stellate cell found in a normal liver; the activated hepatic stellate cell, which is called a myofibroblast; the inactivated myofibroblast, which is similar to the quiescent cell but has a different gene expression; and the senescent stellate

cell, which is dying and no longer replicating but releases novel cytokines that the other stellate cells do not. The new observation discovered last year was that the myofibroblast does not have to die to reverse the fibrosis. The quiescent phenotype can be targeted.

G&H What modalities in development seem most promising for the treatment of liver fibrosis?

DB One modality, as mentioned, would be the LOXL2 inhibitor. Another modality would be *NOX* inhibitors. A third modality being tested are inhibitors of lysophosphatidic acid, which is released in the injured lung and liver and seems to activate the resident cells to become myofibroblasts. There are also inhibitors of TGF- β . Systemic inhibition would result in immunogenicity and carcinogenicity. If the local areas of excessive TGF- β or the integrands they are interacting with can be identified, there might be a way to locally block TGF- β , and there are specific molecules that block the interactions between TGF- β and the integrands that activate them that might be more selective. The general theme is to identify pathologic pathways that stimulate fibrosis but are not required for a normal physiology.

G&H Do some persons have a greater ability to achieve regression of liver fibrosis than others?

DB Yes. If a group of cirrhotic patients with HCV infection are treated with effective antivirals, the livers in some will regress toward normal but will not in others. When these patients are examined, something other than the viral load or genotype seems to be at play and seems to be related to the host response.

Predicting which patients will respond to therapy and achieve regression of liver fibrosis is currently a topic of research, but it is still an unknown. Although the concept that a person may exert a genetic response that turns off TGF- β and results in successful therapy makes sense, it has not been demonstrated. Environmental factors that might be relevant to fibrogenesis are also of interest as well as the role of the gut microbiome.

G&H Ideally speaking, how might liver fibrosis regression be systematically achieved?

DB There are several core mediators of fibrosis; blocking only one may not be sufficient. A combination that includes an angiotensin receptor blocker, a LOXL2 inhibitor, and a *NOX* inhibitor might target several pathways. I think that the goal should be to affect the ability of myofibroblasts to activate and perpetuate. If the progression of an endogenous cell to a myofibroblast to a fibroscar is blocked, then a patient's natural collagenases would be able to degrade the residual fibroscar.

Dr Brenner has no conflicts of interest to disclose.

Suggested Reading

Kisseleva T, Brenner DA. Inactivation of myofibroblasts during regression of liver fibrosis. *Cell Cycle*. 2013;12(3):381-382.

Kisseleva T, Cong M, Paik Y, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. *Proc Natl Acad Sci U S A*. 2012;109(24):9448-9453.

Kisseleva T, Brenner DA. Anti-fibrogenic strategies and the regression of fibrosis. *Best Pract Res Clin Gastroenterol*. 2011;25(2):305-317.

Liu X, Xu J, Brenner DA, Kisseleva T. Reversibility of liver fibrosis and inactivation of fibrogenic myofibroblasts. *Curr Pathobiol Rep*. 2013;1(3):209-214.

Paik YH, Brenner DA. NADPH oxidase mediated oxidative stress in hepatic fibrogenesis. *Korean J Hepatol*. 2011;17(4):251-257.