Therapy for hepatic fibrosis: Revisiting the preclinical models

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Biomedical science has become increasingly public. Disease X — cancer, mental illness, HIV, juvenile diabetes, liver fibrosis or other — draws a group who advocate for research towards a cure. Politicians take up the cause and appropriate large sums. Biologists and physicians rush in with ideas. They study cells in culture. They create new strains of mice to test hypotheses in vivo. Eventually they are able to propose molecular and cellular targets for therapeutic intervention. With proof of concept thus established, the stage is set for human testing and development by the commercial sector. Additional large sums of money are raised from investors willing to take a chance on an untested but potentially major product. The news media announce that treatment for Disease X is just around the corner.

And then, we wait — often many years, sometimes a few decades. What is the problem?

The case of hepatic fibrosis and interferon-gamma

We know that fibrosis in the liver has physiologically important negative effects on liver function. If its progression could be slowed or stopped — or, better, reversed — liver failure could be prevented and much transplantation avoided. The knowledge base on fibrosis is now enormous. Since the mid-1980s, its cellular source(s) are understood, and we know quite a lot about its regulation [1]. Having developed methods for isolation and primary culture of hepatic stellate cells (HSC), we are able to profile the injury responses of HSC in vivo [2,3]. The same models have lent themselves to studies of possible antifibrotic therapies, with the result that we now have a substantial list of possible cellular and molecular targets [4,5]. One of the first agents to be characterized was interferon-gamma (IFg). It blocked fibrogenesis in vivo [6] and potently inhibited HSC activation in culture [7]. Moreover, recombinant IFg (Actimmune® , Genentech) was approved in 1991 for chronic granulomatous disease and thus was demonstrably safe in humans. The potential market for an antifibrotic was very large with respect to liver disease and could be envisioned for other tissues such as lung. IFg was being studied for idiopathic pulmonary fibrosis [8] and for keloids [9] with positive results.

Despite the encouraging prospects, Genentech chose not to pursue new indications for Actimmune®. Quite possibly the company came to this decision after factoring in the length and cost of the required trials (hepatic fibrosis being a slow process) and the expiration of their exclusive marketing rights to Actimmune®. Ultimately the case was taken up by InterMune, a company founded in 1998 for the purpose of developing new clinical uses for recombinant IFg. The disease targets were liver and lung fibrosis. Initial pilot studies in the liver were promising and led to larger randomized controlled trials, including one with 488 subjects with advanced fibrosis or cirrhosis due to chronic hepatitis C. After one year, no difference was found between IFg-treated patients and
controls, with regard to fibrosis reduction or overall survival. There were hints of a subgroup that might have benefited, but otherwise the results were negative [10]. Studies of interstitial pulmonary fibrosis (IPF) were pursued in parallel, with a similar story line. Encouraging pilot data were followed by phase 3 studies, which did not produce a decisive outcome. To settle the issue, the company forged on with a much larger trial involving 826 IPF patients from 81 centers in North America and Europe. Unfortunately, after a median treatment period of 64 weeks, patients receiving Actimmune® showed no improvement in pulmonary function or difference in survival relative to controls [11]. The trial was discontinued, and according to the currently available public information, InterMune has no plans for further studies of this drug in chronic fibrosis.

The problem of the existing preclinical models

The failure of IFg for fibrosis was large and costly but not unique. Many other candidate drugs have fallen by the wayside because of toxicity concerns or lack of efficacy in early human studies. To date fibrosis treatment has been realized only as a secondary benefit of treatment directed at the cause of injury (hepatitis B, hepatitis C, alcohol, iron overload, etc.). We still have nothing for patients with fibrosis of unknown cause or for those who have failed to respond to treatment directed at the causative factor. The same is true for IPF [12]. Drug development is a complex challenge, and the causes of failure are multiple. However, one problem stands out, namely, the reliability of preclinical models for establishing proof of principle. The models are critical, as large sums of money are at risk. Bringing a new chemical entity to regulatory approval requires several hundred million dollars. Establishing a new indication for an existing medication is less costly but still a major investment.

The problem of the experimental models is, in one word, mice — or more precisely, failure to recognize the limitations of the mouse model. Genetically in-bred strains have been central to advancing our understanding of the biology of liver injury. With the methods now available for manipulating gene expression, mice are invaluable for defining the in vivo details of organ-specific pathophysiology and hypothesis testing. Studies in mice have defined, with elegant specificity, numerous new targets for fibrosis therapy. Moreover, mice are generally inexpensive to maintain and study, relative to larger mammals.

Problems arise when the mouse model is used not only for hypothesis building but also for testing the efficacy of a candidate treatment. Testing carries the implicit assumption that injury in the experimental model resembles that in the human liver, to a first approximation. Clearly, this is not the case for mice, as judged by the amount of fibrosis that accompanies an injury. A widely used form of liver injury involves administration of carbon tetrachloride (CCl4), which is hepatotoxic for all mammals including humans [13]. With respect to fibrosis specifically, however, the response of the mouse liver to CCl4 is feeble by comparison with that of larger mammals. It consists, at most, of loosely organized central-central and portal-central collagen, never reaching the stage of cirrhosis. It is also quite labile, as indicated by the fact that it rapidly resolves upon withdrawal of the toxin. The primary injury response of the mouse liver is regeneration. When the intensity or duration of CCl4 administration exceeds regenerative capacity, the mouse succumbs. By contrast, larger mammals mount a fibrotic reaction and survive, albeit at the cost of altered liver structure and compromised function. Even a closely related small rodent, the rat, responds to CCl4 with a fibrotic reaction that is much more robust than that of in-bred mice. Another injury model that has an exact counterpart in human disease is bile duct obstruction. This is a potent fibrogenic stimulus in humans, leading to cirrhosis in just a few weeks. In the mouse, it produces some initial fibrosis, then death. If the animal undergoes selective ligation of the left bile duct only, it survives, but the ligated lobe atrophies while the right regenerates (B. Wang and D.M. Bissell, unpublished observation).

Clearly, in-bred mice are deficient in facets of the injury response that contribute importantly to fibrogenesis in human liver. Whether the same is true for field mice (mus spretus) has not been examined, to my knowledge. It would not be surprising if the out-bred mice mounted an injury response that resembles that of out-bred rats and not in-bred mouse strains. There are differences in this regard among the in-bred strains, with BALB/c generating more fibrosis than does, for example, C57BL/6 [14].

Modeling the initial injury-specific events

As noted above, treatments that eliminate the specific cause of injury (hepatitis viruses, alcohol, etc.) reduce fibrosis and stabilize liver function. When injury-specific interventions are either unknown or ineffective, treatment should be directed at earliest events in the response [15]. The early events are still being defined, but recent studies are revealing subtleties. For example, the inflammatory response clearly comprises cells of widely differing function. Lymphocyte subpopulations express different chemokine receptors and are regulated by the corresponding chemotaxin. Some ligand-receptor pairs elicit profibrogenic effects, while others are antifibrogenic [16]. Proteinases have key roles in the initial response to injury as well as in its resolution. The type(s) of proteinase and their physiological effects likely differ for the two phases, but that remains to be defined along with the roles of counter-regulatory molecules such as tissue inhibitors of metalloproteinases (TIMPs), alpha-2-macroglobulin, and the extracellular matrix (ECM). Mesenchymal-epithelial transition (EMT), involving both hepatocytes [17] and biliary epithelial cells [18,19], may be the basis for the appearance of non-HSC fibrogenic cells in certain types of injury. Regional lobular hypoxemia initiates a series of events mediated by HIF-1, leading to angiogenesis [20]. The latter likely is critical to the formation of pathological fibrosis. For the initial vetting of antifibrotic candidate therapies, the mouse model may be suitable provided it demonstrates faithfully the relevant early event(s) after injury. It need not replicate every facet of the downstream events including fibrosis. Ultimately of course, it will be necessary to show that the intervention has an impact on the end point, fibrosis, but other models — presumably larger mammals — will be required for this.
Mediators of both early injury-specific events and physiological repair

Some biological mediators have multiple roles and are involved in both parts of the injury response as defined in Fig. 1. A prime example is transforming growth factor-beta (TGFβ), which is both upregulated in injury and directly stimulatory to fibrogenic cells [21,22]. However, TGFβ has a number of other roles. It is immunoregulatory: deletion of TGFβ in mice, depending on the isoform targeted, leads to embryonic death or sickly pups with general infiltration of solid tissues by inflammatory cells [23,24]. TGFβ also has strong antimitotic activity, with a postulated role as a tumor stimulatory to fibrogenic cells [21,22]. However, TGFβ has been implicated in both parts of the injury response as defined in the model in Fig. 1. The key insight is that at all stages of active liver disease, fibrogenesis is ongoing and dynamic, with turnover as well as maintenance of the injury micro-environment.

Effective fibrosis treatment may require blocking multiple early events

The focus of attempts to modulate pathological fibrosis for the past 25 years has been activation of HSC, as the final common pathway of the injury response. This is highly logical, because it promises a single remedy ('magic bullet') for a wide range of chronic liver injuries. Unfortunately, as shown by the example of IFγ, efforts to target HSC activation have met with very little success. The causes are speculative, but with factors such as TGFβ playing multiple roles, it is safe to suggest that regulatory events in the HSC micro-environment are extremely complex [15]. Moreover, efficient blockade of HSC activation—if such were possible—would raise the concern of interference with homeostatic repair. For this reason, attention is turning to modulation of the injury-specific events (Fig. 1). The key insight is that at all stages of active liver disease, fibrogenesis is ongoing and dynamic, with turnover as well as maintenance.

If we are now to target injury-specific events, it will be important to bear in mind that these events are not discrete but have multiple secondary effects. Upregulation of a matrix proteinase may result in ECM fragments that have signaling properties, including recruitment of inflammatory cells. Apoptosis of hepatocytes leads to cellular breakdown products that may be ingested by macrophages, eliciting profibrogenic responses [32]. Regional lobular hypoxemia may elicit a host of cellular responses including angiogenesis [20]. Thus, a successful antifibrotic strategy likely will target more than one type of early response in a multidrug approach. As such, fibrosis treatment will evolve in a man-
ner similar to that for other diseases (cancer, HIV, hepatitis C).

**Treatment tailored to the disease entity**

For each disease etiology, the initial injury event(s) likely differ. As already mentioned, "inflammation" encompasses a variety of cell types and biological activities. The inflammatory cells associated with autoimmunity will be the same as those in hepatitis B. Although the details are not well understood, the difference can be inferred from the often dramatic response of autoimmune hepatitis to immunosuppressive therapy (systemic glucocorticoid) and the poor or even deleterious effect of the same treatment in hepatitis B. Oxidative production is important in hepatitis C, steatohepatitis, and alcoholic liver disease. Angiogenesis is prominent in diffuse lobular disease but not in biliary-based injury (until it becomes advanced). The conclusion is that the multi-pronged approach to blocking injury-specific initial events will need to be tailored to the cause of liver disease.

**Summary**

Chronic hepatic fibrosis is a health problem of significant proportions. Preclinical models—mainly, mice—have provided a plethora of possible targets for treatment. To date, however, efforts to translate this progress to treatment of human liver fibrosis have been unproductive. The problem lies in part in excessive reliance on mouse models for demonstrating therapeutic efficacy. While this is understandable from a cost viewpoint, the mouse liver is a poor model for human liver injury. There is also the question of whether direct inhibition of fibrogenesis (at the level of the stellate cell) is safe, in that abrogation of normal homeostatic repair could be risky. Both of these concerns can be addressed by focusing on events that are early and specific to individual types of injury. The mouse models should be judged by the degree to which they reproduce the early events of interest. With the appropriate model, it will be possible to demonstrate that targeting is specific to the injury event, spares normal repair and therefore has a good chance of being safe. Further testing in a larger mammal will be required for demonstrating antifibrotic activity. Candidate treatments that pass these tests, as well as general toxicity screening, will be ready for human studies.

**Disclosure of interest**

The author declares that he has no conflicts of interest concerning this article.

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