Hepatic fibrosis

Thiazolidinediones and hepatic fibrosis: don't wait too long

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Lack of effect of delayed thiazolidinedione treatment may imply that these drugs are of limited efficacy in hepatic fibrosis, and that treatment of chronic liver disease might be successful only if the therapy were started very early

Liver tissue scarring, or fibrosis, is considered a common pathway leading to the alterations typical of cirrhosis, and delaying its progression likely results in longer patient survival and reduced need for liver transplantation. The past 15 years have witnessed a tremendous advance in our understanding of the cellular and molecular mechanisms responsible for the fibrogenic response in the liver (reviewed by Bataller and Brenner¹). Damage to hepatocytes, or alterations in the biliary tree, activate mesenchymal cells that acquire a myofibroblastic phenotype, actively proliferate, produce extracellular matrix components, and secrete soluble mediators that contribute to maintenance of a chronic "wound healing" response, resulting in progressive scarring and alteration of the microvascular architecture. Activation of hepatic stellate cells (HSC) is considered a major source of myofibroblast-like cells during liver injury, together with the contribution of other myofibroblasts, such as those originating from the portal tracts.² In spite of the clinical need for antifibrotic therapies, and a detailed knowledge of the processes of fibrogenesis in cellular and animal models, to date no drugs have been approved for the treatment of hepatic fibrosis.

A number of studies have recently suggested that antidiabetic thiazolidinediones (TZDs) or "glitazones" (from the prototype troglitazone, withdrawn from the market, and currently comprised of pioglitazone and rosiglitazone) may represent a possible novel pharmacological treatment for liver fibrosis. TZDs are employed for the treatment of insulin resistance in patients with type 2 diabetes and are selective ligands for the nuclear transcription factor peroxisome proliferator activated receptor (PPAR) γ .³ PPARs, which include PPAR α , PPAR β (or δ), and PPAR γ belong to the nuclear receptor superfamily, the members of which modulate gene expression after binding to specific

ligands.⁴ Like other PPARs, on ligand binding, PPAR γ heterodimerises with the retinoid X receptor and binds to target DNA sequences. Ligand mediated conformational changes also lead to recruitment of multiple coactivators, explaining the variability in the sets of genes regulated by different ligands. PPAR γ may also repress gene transcription, negatively interfering with other transcription factors, such as nuclear factor κ B, independent of binding to target DNA sequences.⁵ This factor is expressed at high levels in adipose tissue, but different cell types show low level expression of PPAR γ . The insulin sensitising mechanism of TZDs is multifactorial and still incompletely understood but increased fatty acid storage in adipose tissue, as well as augmented secretion of adiponectin, an adipokine which also has anti-inflammatory and antifibrotic properties, are likely to play a major role.³

A connection between PPAR γ and liver fibrosis was established in 2000, when three groups independently showed that this transcription factor is expressed in quiescent HSC (that is, isolated from normal liver), and that its abundance and/or transcriptional activity decrease along the activation process that accompanies the acquisition of fibrogenic properties.⁶⁻⁸ More importantly, exposure of HSC to PPAR γ ligands, including different glitazones, was able to revert most features of the activated phenotype of HSC. In these cells, PPAR γ activation reduces expression of interstitial collagens and other matrix proteins, downregulates the ability to proliferate and migrate in response to PDGF, blocks the secretion of proinflammatory chemokines such as monocyte chemoattractant protein 1, and induces apoptosis.⁶⁻¹⁰ Moreover, exposure to tumour necrosis factor α (TNF- α) or PDGF reduces PPAR γ expression or transcriptional activity while PPAR γ ligands counteract these effects.⁶⁻⁸ In some systems the actions of

TZDs have been shown to be independent of PPAR γ . However, ectopic expression of PPAR γ in activated HSC recapitulates the effects of TZD treatment, indicating that these drugs modulate the biology of fibrogenic cells due to their ability to ligate PPAR γ .¹¹ Interestingly, expression of PPAR γ by quiescent HSC is part of a more complex "adipogenic" phenotype that includes expression of C/EBP β and SREBP-1c, the abundance of which is also decreased along the activation process, and the ability to store lipids, in line with the former name of "fat storing cells".¹² All of these findings strongly argue for TZDs as a means of maintaining a quiescent phenotype in HSC and reverting most of the features of the activated state via PPAR γ .

Accumulating evidence on the role of TZDs as a possible antifibrogenic tool in vitro has been rapidly followed by studies aiming to establish if these drugs are active under conditions of liver injury and repair in vivo. Using three different models of fibrosis in the rat, Galli and colleagues¹⁰ demonstrated that daily intragastric administration of rosiglitazone or pioglitazone, started at the same time as injury, leads to a marked reduction in fibrotic tissue accumulation and fibrogenic cell proliferation. These effects persisted for as long as eight weeks in animals treated with carbon tetrachloride (CCl₄), and for four weeks in those undergoing bile duct ligation. In addition, the antifibrogenic action of pioglitazone was also shown in a dietary model of steatohepatitis where hepatic fibrosis and formation of preneoplastic foci were inhibited after supplementing the diet with pioglitazone.¹³ The mechanisms underlying reduced fibrogenesis were in keeping with the actions of TZDs on isolated HSC, and included reduced expression of transforming growth factor β 1 (TGF- β 1), procollagen I, fibronectin, and tissue inhibitor of metalloproteinases (TIMPs), and increased DNA binding activity of nuclear extracts to peroxisome proliferator response element. None the less, HSC independent factors certainly contribute to the antifibrogenic action of these drugs. In an acute model of CCl₄ intoxication, pioglitazone was shown to reduce serum levels of transaminases and TNF- α , indicating interference of TZDs with the necroinflammatory responses.¹⁴ These data are in line with those observed during chronic CCl₄ or dimethylnitrosamine intoxication¹⁰ and with the observation that TZDs modulate cytokine expression by inflammatory cells.³ Finally, a TZD has recently been shown to limit the extent of the ductular reaction observed in bile duct

ligated rats, an event that may reduce the subsequent proliferation of fibrogenic myofibroblasts.¹⁵ These data provide compelling *in vivo* evidence supporting an antifibrogenic role of TZDs.

In this issue of *Gut*, a study from Isabelle Leclercq and colleagues¹⁶ challenges, at least partially, our conviction of a bright future for TZDs as antifibrogenic drugs (*see page 1020*). Unlike all previous studies employing TZDs, treatment with pioglitazone was introduced at various time points after induction of liver damage that was chronically generated by CCl₄ intoxication, administration of a choline deficient diet, or by bile duct ligation. In all three models, the TZD was added at an early and a later time point, and after addition of the drug the fibrogenic insult was maintained. Several parameters were carefully evaluated, and included expression of TGF- β , procollagen I, and TIMP-1, evaluation of the number of fibrogenic α smooth muscle actin positive cells, and hepatic levels of hydroxy proline, the “gold standard” for determination of collagen deposition. In rats treated for five weeks with CCl₄, pioglitazone was effective in reducing these parameters of fibrogenesis if added for the last three weeks of intoxication. Conversely, when TZD treatment was started after five weeks and continued for an additional four weeks of CCl₄ intoxication, collagen accumulation, myofibroblast proliferation, and expression of profibrogenic cytokines were not different when treated and untreated animals were compared. Surprisingly, even at the five week time point, where an antifibrogenic effect was detectable, pioglitazone lacked any action on inflammatory cell infiltration and expression of proinflammatory cytokines. Lack of a therapeutic effect of late time TZD addition was also confirmed in a dietary model of steatohepatitis where pioglitazone was effective on fibrosis if added for the last four weeks in a six week protocol but not if administered for the last six of a total of 12 weeks. Interestingly, reduction in liver lipid content, an index of steatosis, was significantly achieved with both treatment protocols, ruling out a general refractoriness to the therapeutic effect of TZDs in this context. If some beneficial effects could be obtained in these two models when pioglitazone was added early and the damaging protocol interrupted shortly thereafter, no therapeutic effects whatsoever could be detected in the bile duct ligation model. In this case, even addition after five days over a total two week protocol was ineffective on levels of hydroxy proline or α smooth muscle actin positive cells.

There are clearly several issues that arise from the data provided by Leclercq

and colleagues,¹⁶ and most of them will need additional experimental work to be addressed. Lack of effect of delayed TZD treatment was independent of the type of model used because it failed during CCl₄ intoxication as well as in diet induced steatohepatitis, while in cholestatic fibrosis pioglitazone was ineffective even when added shortly after surgery, suggesting that this latter condition may be particularly resistant to the effects of the drug. While the efficacy of TZD treatment at the time of surgery in bile duct ligated animals was demonstrated in two independent studies,^{10, 15} analysis of procollagen expression at late time points (four weeks) did not differ comparing treated and untreated animals,¹⁵ confirming that the early phases of intervention are critical for the antifibrogenic response. The different identity of fibrogenic cells in the three models is another possible reason for the reduced action of TZDs in bile duct ligated animals.² Matrix producing cells are thought to derive predominantly from HSC or from portal myofibroblasts in steatohepatitis and cholestasis, respectively, while in chronic CCl₄ intoxication the origin of septal myofibroblasts is probably diverse. Thus different sensitivity to TZDs by these different cell lineages could be hypothesised and, if confirmed, would also suggest that the responsiveness of various types of human fibrosis may be different.

A critical point is obviously represented by the mechanisms responsible for the lack of therapeutic effects of TZDs in established liver fibrosis. A first possibility was ruled out by the authors themselves who demonstrated that pioglitazone was effective throughout the study protocol, as determined by its ability to increase expression of a PPAR γ regulated gene in adipose tissue even at the latest time point.¹⁶ In addition, the antisteatotic and antifibrogenic effects of TZDs could be dissociated in the dietary model, indicating that resistance to the effects of the drug occurs specifically for inhibition of fibrogenesis. In principle, it is possible that as long as fibrosis proceeds, the drug becomes unable to efficiently target fibrogenic cells. In this case, experiments employing drug targeting protocols could be helpful.¹⁷ Another possibility is that the effects of TZDs are counterbalanced by other profibrogenic pathways that become upregulated. It has been demonstrated that HSC isolated from fibrotic animals treated with TZDs have a greater DNA binding activity of PPAR γ to its cognate responsive elements.¹⁰ Carrying out these determinations in animals undergoing delayed administration of TZDs

would determine whether PPAR γ is effectively activated in the cells responsible for fibrogenesis. An additional possibility is that in advanced fibrosis, levels of PPAR γ become critically low to allow the system to respond to TZDs. This hypothesis is supported by data, provided by different groups, showing that levels of PPAR γ decrease during activation. Moreover, when liver myofibroblasts were isolated using the outgrowth method, no PPAR γ expression could be detected, and non-TZD ligands were shown to have effects independent of this factor.¹⁸ While this is a reasonable hypothesis, it should be considered that fully activated human myofibroblastic HSC *in vitro* retain the ability to respond to TZDs even in the presence of a marked decrease in PPAR γ levels.^{6, 7}

The most relevant issue is if, and how, these data influence our interest in TZDs as possible therapeutic agents in humans. Animal models are generally used as a “proof of concept” in the road leading to development of new therapeutic strategies. In most studies, treatment is started at the time of initial damage, as in all studies testing TZDs and liver fibrosis reported so far, or is administered after interruption of the toxin. Obviously, these protocols poorly mimic the condition we face in the clinic where we have to treat a patient with established fibrosis and ongoing damage, a situation much more similar to the protocol employed by Leclercq *et al.* A few recent studies have indeed reported successful treatment of established experimental fibrosis, for example interfering with TGF- β signalling or overexpressing haeme oxygenase 1 in animals undergoing CCl₄ intoxication or bile duct ligation,^{19–21} indicating that in these models fibrosis may be modulated despite persistent injury. Therefore, trying to extrapolate the available data on TZDs from animal models to the human situation, it might be anticipated that TZD treatment is of limited efficacy in biliary fibrosis, and that treatment of inflammatory and metabolic forms of chronic liver disease would be successful only if the treatment is started very early. A theoretical applicability of these considerations could be the prevention of fibrosis recurrence in high risk patients after liver transplantation, a condition where TZDs could be administered in the presence of a normal liver, thus reproducing the setting of animal studies where efficacy was shown. On the other hand, the limitations of rodent models of fibrosis should not be overlooked. In particular, all of the available models poorly reflect clinically observed conditions of chronic liver disease, and fibrosis develops (and resolves) over a time frame that is

considerably shorter than that in humans, even taking into account the life span of rodents. Moreover, drug dosage is generally several fold higher than that used in clinical practice, and duration of treatment more limited.

A final question is whether clinical trials employing TZDs as antifibrogenic drugs in humans should be discouraged. Although the data reported in this issue of *Gut* indicate that these drugs are unlikely to be a magic bullet in fibrogenesis, several aspects suggest that exploring their usefulness in patients with liver disease may be worthwhile. TZDs have been used in the treatment of type 2 diabetes for several years, and their safety profile is acceptable, unlike experimental treatments that are based on gene therapy or with anticipated heavy secondary effects. In patients with non-alcoholic steatohepatitis, treatment with TZDs was effective in reducing steatosis, inflammation, and fibrosis, although these studies lacked a placebo control group.^{22, 23} Although in non-alcoholic steatohepatitis the primary action of TZDs is to counterbalance insulin resistance, steatosis associated with the metabolic syndrome is detrimental for progression of fibrosis in most chronic liver diseases.²⁴ Thus the possibility that amelioration of insulin resistance, together with reduction of proinflammatory genes and modulation of expression of adipokines is sufficient to exert an antifibrogenic action in humans deserves consideration. Support for this contention derives from the fact that the effects of TZDs have not been studied in models of fibrosis associated with the metabolic syndrome and that the biology of adipokines differs considerably across species. These considerations lead to a final, and important reflection on the status of research in the fibrosis field. While a riches of experimental data on molecular mechanisms and possible treatment

strategies have been accumulating in the past 10 years, we are still unarmed in front of patients' diseases. Even in the presence of some conflicting data from preclinical studies, it is time to try and test some of the compounds that translational research has provided for clinical investigators, particularly when the likelihood of being harmful is very low.

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