The impact of pregnane X receptor activation on liver fibrosis

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Abstract
The PXR (pregnane X receptor) is a nuclear receptor transcription factor that is activated by a range of endobiotics and xenobiotics. The activated PXR modulates the transcription of genes in hepatocytes (the main functional cell of the liver) associated with endobiotic and xenobiotic uptake, metabolism and excretion. However, activation of the PXR also inhibits a deleterious response of the liver to chronic damage – that of fibrosis. The antifibrogenic mode of action is mediated through changes in the expression of genes in hepatic stellate cells and liver macrophages (Kupffers). These results suggest an additional function for the PXR.

The liver’s response to damage
The parenchymal hepatocyte is the main functional cell of the liver. When hepatocytes are irreversibly damaged, an inflammatory response occurs in the tissue through the release of cytokines from liver macrophages (Kupffer cells) and other cells [1]. This reaction mobilizes systems to remove the damaged cells and stimulates the mitosis of existing hepatocytes to restore liver function [2].

Clinicians are most often required to provide treatment for patients with chronic tissue damage. In the case of the liver and many other tissues, the subsequent chronic inflammatory response to damage leads to an aberrant tissue structure driven by the generation of scars or fibrosis. The fibrotic tissue structure prevents effective regeneration and defines cirrhosis, which may eventually result in organ failure and death. Preventing tissue fibrosis may maintain organ viability and function and is considered the only way of treating chronic liver disease.

Non-parenchymal cells and liver damage
In response to hepatocyte damage, nearby endothelial cells lose their fenestrae and release chemokines that recruit circulating leucocytes to the site of injury [1]. Kupffer cells present in the liver sinusoids become activated by the necrosis of hepatocytes and release pro-inflammatory cytokines and reactive oxygen species [1]. HSCs (hepatic stellate cells) (mainly localized in the centrilobular region) transdifferentiate to a myofibroblast-like (α-smooth-muscle actin-expressing) phenotype [1]. In addition, liver myofibroblasts in the periportal regions of the liver lobule proliferate into the liver sinusoid. Trans-differentiated HSCs and periportal fibroblasts constitute a population of ‘fibrogenic myofibroblasts’ within the liver. Fibrogenic myofibroblasts are primarily responsible for the synthesis and accumulation of the extracellular matrix proteins that give rise to fibrotic scarring [1]. If hepatocyte damage occurs chronically, as with hepatitis C infection or due to a number of genetic diseases (e.g. primary biliary cirrhosis), the persistence of fibrogenic myofibroblasts generates an accumulation of scarring fibrosis that exacerbates the liver disease and with time results in cirrhosis (see Figures 1 and 2). However, the process of HSC transdifferentiation and fibrogenic myofibroblast proliferation, fibrogenesis and apoptosis is intimately linked with the activity of other cell types and the factors released from them [1]. In particular, Kupffer cells appear to play a pivotal role in fibrogenic myofibroblast activity, in both the formation and resolution of liver fibrosis [1,3]. Oxidative stress and cytokines released from Kupffer cells have been suggested to regulate the transdifferentiation of HSCs and their subsequent proliferation [4,5]. Persistence of fibrogenic myofibroblasts is likely to promote fibrosis since they direct several positive regulatory feedback mechanisms through their secretion of extracellular matrix proteinases (matrix metalloproteinases) that degrade the normal matrix [6]. Extracellular matrix re-modelling also releases a pool of growth factors and cytokines that may increase proliferation and inhibit the apoptosis of fibrogenic myofibroblasts [1].

Therapeutic options for treating liver fibrosis
At present, there are no treatments for liver fibrosis. Several approaches aim to inhibit specific pro-fibrogenic factors produced by the various cells involved [7]. However, accumulating data suggest that there may be a high degree of redundancy in the factors modulating fibrogenic myofibroblasts. Because so many factors are able to modulate fibrogenic...
myofibroblasts in experimental systems, targeting one factor and/or its pathway of action may be ineffective. This laboratory has demonstrated that targeting the fibrogenic myofibroblasts and reducing their numbers by stimulating apoptosis is an effective antifibrogenic approach [8,9]. We have also examined the effectiveness of inhibiting HSC transdifferentiation and fibrogenic myofibroblast proliferation via the PXR (pregnane X receptor), and provide evidence that this may be a realistic and effective antifibrogenic approach [10,11].

The PXR
The PXR belongs to the nuclear receptor gene superfamily of transcription factors whose activity (in most cases) is modulated by ligand hormones [12,13]. Included within this superfamily are receptors for steroid hormones, thyroid hormone and retinoids. Orphan nuclear receptors are defined as nuclear receptors for which the endogenous ligand effector has yet to be firmly established. The PXR is an orphan nuclear receptor (NR1I2) that has been shown to regulate the expression of specific genes in hepatocytes (e.g. CYP3A subfamily genes; where CYP is cytochrome P450) [14–16]. The receptor binds pregnane steroids, bile acids and drug ligands such as rifampicin (human only), hyperforin, lovastatin, clotrimazole and metyrapone [14–19].

Mechanism(s) of action of the PXR in fibrogenesis
The isolation and culture of HSCs on plastic in a medium containing serum results in a phenotypically similar transdifferentiation of HSCs to fibrogenic myofibroblasts [1]. This model system provided the first indication that the PXR may affect fibrogenesis. Addition of PXR activators to human HSC cultures resulted in an inhibition in their transdifferentiation and proliferation [20]. Although these effects could be observed with lower efficacy in rodent HSC cultures (i.e. by reducing the growth stimulus in culture through reductions in serum levels), the PXR could not be detected in rodent fibrogenic myofibroblasts (in contrast with human cells) and the role of the PXR was unclear [10].

To test the hypothesis that the PXR could mediate antifibrogenic effects of PXR activators, an in vivo model of fibrosis was used. Rodents were treated with carbon tetrachloride (CCl4) and the rodent PXR activator PCN (pregnenolone 16α-carbonitrile). Without interacting with the damage mechanism(s) of carbon tetrachloride, PCN markedly reduced the levels of fibrosis in rats and mice [10]. Using mice with a disrupted PXR gene, the antifibrogenic effects of PCN were shown to be mediated via the PXR [10]. More recent results indicate that rodent HSCs express the PXR, although the levels fall rapidly as the cells transdifferentiate in culture, whereas human HSC PXR expression is maintained ([11]; and C.J. Marek and M.C. Wright, unpublished work).

The antifibrogenic effects of PXR activators are therefore primarily mediated through their ability to activate the PXR. Using human HSCs as a model system, we have now shown that PXR activation results in an inhibition of proliferation and transdifferentiation [11]. Moreover, PXR activation results in the down-regulation of TGF-β1 (transforming growth factor-β1) expression, a major factor in the promotion of fibrogenicity (e.g. through its stimulation of collagen type I expression) [11] (summarized in Table 1).

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### Table 1 | Mechanistic evidence for PXR activator effects in fibrogenesis

<table>
<thead>
<tr>
<th>Cell type (response in vitro)</th>
<th>Species (PXR activator)</th>
<th>Hepatocytes</th>
<th>qHSCs</th>
<th>aHSCs</th>
<th>q → a HSCs</th>
<th>Monocytes</th>
<th>Macrophages</th>
<th>Kupffers</th>
<th>Endothelial oval BECs</th>
<th>Whole liver</th>
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<tbody>
<tr>
<td></td>
<td>Rat (PCN)</td>
<td>Express PXR</td>
<td>Express PXR</td>
<td>PXR not detectable</td>
<td>PCN inhibits transdifferentiation</td>
<td>?</td>
<td>?</td>
<td>Express PXR</td>
<td>?</td>
<td>PCN inhibits CCl4-induced liver fibrosis</td>
</tr>
<tr>
<td></td>
<td>Mouse (PCN)</td>
<td>Express PXR</td>
<td>Induction of CYP3A</td>
<td>PCN inhibits transdifferentiation</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>PCN inhibits CCl4-induced liver fibrosis in PXR+/+ mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human (rifampicin)</td>
<td>Express PXR</td>
<td>Express PXR</td>
<td>Express PXR</td>
<td>Rifampicin and other PXR activators inhibit transdifferentiation</td>
<td>Express PXR</td>
<td>Express PXR</td>
<td>Express PXR</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Transcriptionally functional PXR activators inhibit proliferation and TGF-β, siRNA-mediated PXR knockdown reversal</td>
<td>Rifampicin increases IL-6 secretion</td>
<td>Rifampicin inhibits IL-1β secretion/ increases IL-6 secretion/ induces IL-10 secretion</td>
<td>Rifampicin inhibits IL-1β secretion/ inhibits IL-6 secretion/ inhibits LPS-induced TNFα secretion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aHSC, activated HSC; BEC, biliary epithelial cell; IL, interleukin; qHSC, quiescent HSC; siRNA, small interfering RNA; TNFα, tumour necrosis factor α.
Figure 2 | The transdifferentiation of centrilobular HSCs generates fibrosis

Typical serial liver sections from control rats or rats treated with carbon tetrachloride and immunostained for α-smooth-muscle actin (α-sma, brown) with subsequent haematoxylin staining (left panels) or stained for collagens (red) using Sirius Red stain (right panels). CV, central vein. Scale bar, 100 µm.

CONTROL

α-sma

sirius red

CV

CCl₄

α-sma

sirius red

CV

Conclusions

Finding practical solutions to clinical problems includes identifying new uses for existing therapeutics. Much is known about the PXR and the compounds that activate it through its important role in regulating drug-metabolizing enzymes. It remains to be seen whether a treatment for liver fibrosis is effective when it could also result in an increase in the metabolism of certain drugs. However, the PXR also stimulates liver growth, a feature likely to aid liver recovery.

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References


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