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COMMENTARY

Physalin B attenuates liver fibrosis via suppressing LAP2α-HDAC1 mediated deacetylation of glioma-associated oncogene 1 and hepatic stellate cell activation

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Liver fibrosis is an excessive scarring process resulting in progressive disruption of the normal tissue architecture and impaired organ function. It comprises many different etiologies and its worldwide consequences present a substantial medical and economic burden (Weiskirchen et al., 2018). Mechanistically, fibrosis is initiated by parenchymal cell destruction resulting in tissue damage that is associated with an inflammatory response. This in turn provokes the local activation of mesenchymal cells, which have the capacity to produce extracellular matrix complexes such as collagens. Most important in this process are hepatic stellate cells that are pericytes in the perisinusoidal space of the liver. Upon activation, these cells lose their quiescent phenotype and transit, a process called trans-differentiation, into proliferative fibrogenic α-smooth muscle actin (α-SMA) positive myofibroblasts. These are the central cellular drivers of hepatic fibrogenesis in experimental and human liver injury. Although the cellular and molecular mechanisms of hepatic fibrosis including important pro-inflammatory and pro-fibrogenic soluble mediators (chemokines, cytokines) and the signalling pathways have been identified, there exist no specific and effective antifibrotic pharmacological therapies.

Beside synthetic drugs, complementary and alternative medicine are presently focused on in basic and clinical research. In particular, a number of herbal remedies and active ingredients show promising effects against hepatic fibrosis experimentally in cell culture and preclinical animal models or even in the first clinical trials (Weiskirchen, 2016). These compounds counteract the intercellular production or activity of ROS, prevent hepatic infiltration of circulating inflammatory producing blood cells, interfere with the relevant signalling pathways or mediators involved in the production or turnover of the extracellular matrix.

The present study by Zhu and colleagues investigated the antifibrotic activities of physalin B in two models of hepatic fibrosis, primary cultures of mouse hepatic stellate cell and the well-established immortalized human hepatic stellate cell line LX-2 (Zhu et al., 2021).

Physalin B possesses an unique 13,14-*seco*-16,24-*cyclo*-steroidal skeleton and a H-ring with a C_{14}-O-C_{27} bond forming an intriguing cage-shaped structure. It is a main active physalin of *Physalis angulata L.* belonging to the nightshade family (*Solanaceae*) of plants. The authors gave physalin B to mice that were either simultaneously subjected to repeated applications of carbon tetrachloride or to a bile duct surgery, both established experimental models of hepatic fibrosis. The *seco*-steroid ameliorated hepatic fibrosis as assessed by overall reduction in the deposition of hepatic collagen, lowered blood markers of liver damage (alanine aminotransferase and aspartate aminotransferase) and diminished expression of fibrogenic markers (collagen type I, **tissue inhibitor of metalloproteinase 1 (TIMP1)**, α-SMA, and **transforming growth factor-β1 (TGF-β1)**). In cultured hepatic stellate cells, physalin B decreased α-SMA expression during spontaneous activation and trans-differentiation. Moreover, physalin

Abbreviations: α-SMA, α-smooth muscle actin; GLI1, glioma-associated oncogene 1; HDAC1, histone deacetylase 1; LAP2α, lamina-associated polypeptide 2α; TGF-β, transforming growth factor-β; TIMP1, tissue inhibitor of metalloproteinase 1.

B was effective in blunting TGF-β-induced activity of a luciferase-based **alpha-1 type I collagen (COL1A1)** reporter in human hepatic stellate cell line LX-2. Similarly, physalin B repressed glioma-associated oncogene 11 (GLI1) mRNA expression and activity of a GLI1-dependent reporter construct in primary mouse hepatic stellate cells and in the immortalized human hepatic stellate cell line LX-2, while the expression of *GLI2* and *GLI3* were unaffected. Interestingly, the drug effectively blocked GLI1-induced expression of collagen and α-SMA in cultured hepatic stellate cells, most likely by hindering GLI1 nuclear localization that in turn resulted in reduced mRNA expression of typical GLI1 target genes such as *Hedgehog Interacting Protein (Hhip)*, *Cyclin D*, *Cyclin E* and *c-myc*. Moreover, physalin B induced GLI1 acetylation by blocking complex formation between lamina-associated polypeptide 2α (LAP2α) and **histone deacetylase 1 (HDAC1)**, which normally promotes GLI1 deacetylation. The same stimulatory effect on GLI1 acetylation was induced by the HDAC inhibitor **vorinostat** confirming the protective effect of the LAP2α/HDAC1 complex on GLI1 deacetylation.

The finding that physalin B treatment mitigated liver fibrosis via inhibiting the interaction between LAP2α and HDAC1, which provoke increased acetylation and nuclear translocation of GLI1 is novel. In a related study, the same authors have recently shown that physalin B ameliorates experimental non-alcoholic steatohepatitis in mice by stimulating autophagy and p62-**Keap1-Nrf2** anti-oxidative signalling, suggesting that this drug has also some general anti-inflammatory and hepatoprotective activities (Zhang et al., 2021). In line with a previous report, physalin B was shown to inhibit **nitric oxide** production by **lipopolysaccharide (LPS)** or **interferon-γ**-activated macrophages, and to protect mice against a lethal LPS challenge. Thus, physalin B has the potential to counteract a broad range of biological processes that contribute to the pathogenesis of hepatic fibrosis. In particular, the inhibition of collagen, α-SMA, TGF-β and TIMP1 provides evidence that physalin B exerts anti-fibrogenic properties in the liver by inhibiting hepatic stellate cell activation. During hepatic fibrosis, the up-regulation of these pro-fibrogenic marker genes is a hallmark that indicates activation and trans-differentiation of quiescent hepatic stellate cells into proliferative, profibrogenic and extracellular matrix-producing myofibroblasts.

This study provides novel molecular insights into the biological activity of physalin B and in principle opens up new avenues for the implementation of novel anti-fibrotic therapies. However, the study has still some limitations. Although, the authors investigated the anti-fibrotic activities in *in vitro* and *in vivo* models of ongoing hepatic fibrogenesis showing that the drug efficiently impacts disease initiation and progression. It will be essential in future studies to prove the therapeutic effectiveness in other suitable models. Moreover, previous evaluation of different physalins in a panel of 10 human and murine cancer cell lines demonstrated that physalin B has a broad cytotoxic activity towards most of the cell lines tested, with a half-maximal inhibitory concentration (IC_{50}) in the range of 0.6–2.7 μM. In the present study, Zhu and colleagues determined an IC_{50} of 5 μM for the immortalized human hepatic stellate cell line LX-2 and used in their animal experiments concentrations of 1 to 5 $mg \cdot kg^{-1}$ body weight given intraperitoneally for 14 (bile duct ligation model) or 28 consecutive days (carbon tetrachloride model). The concentrations used in luciferase reporter assays and cell apoptosis testing were in the range of 0.25–1.0 μM, concentrations that might be toxic to other cell types. Moreover, inhibition of GLI1 by physalin B might have several unwanted side effects. GLI1 is a transcriptional effector of the Hedgehog signalling pathway playing key roles in the development and homeostasis of many organs and tissues. Transcriptional suppression, aberrant post-translational modification such as hyper-acetylation or untargeted trafficking of this zinc finger protein will ultimately influence gene transcription and transcriptional output of the Hedgehog pathway. Moreover, the Hedgehog-GLI pathway is relevant for activating cell divisions in undifferentiated progenitor cells and for committing cells to a specific fate.

Effective anti-fibrotic therapies should include the attenuation of excessive matrix synthesis and deposition, the replacement of dysfunctional liver tissue and the restoration of the original tissue architecture. Liver regeneration and remodelling is a well-coordinated process requiring increased cell proliferation and differentiation. Genetic cell lineage-tracing approaches have shown that damaged hepatocytes and biliary epithelial cells are renewed from common liver progenitor cells and liver repair requires the activity of immune cells. Therefore, it will be of fundamental importance to test if the beneficial effects of physalin B in preventing extracellular matrix synthesis interfere with the dynamic and adaptive changes necessary during the process of reconstitution of functional liver tissue. It will be further necessary to evaluate if the observed effects are specific for physalin B or if they can be also imparted by other compounds belonging to the Physalin family. Noteworthy, in a similar study conducted in the same laboratory, physalin D ameliorated experimental liver fibrosis by inhibiting the TGF-β and Yes-associated protein 1 (YAP1) signalling pathways (Xiang et al., 2020), suggesting some overlapping therapeutic activities of Physalins. So, there are still some open questions to be answered in the future.

In summary, the current work has identified a new drug candidate effective in experimental hepatic fibrosis. It will now be necessary to perform well-designed safety and efficacy studies. Future work will show if the observed experimental anti-fibrotic activities of this *seco*-steroid can be translated to the clinic.

NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org.

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CONFLICT OF INTEREST

The author has nothing to declare.

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