PO-443 — SZN-043, a hepatocyte-targeted R-spondin mimetic, induces hepatocyte proliferation in an acute acetaminophen-induced liver injury model
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Introduction

Wnt signaling plays a central role in hepatocyte expansion during development and tissue repair. R- spondins (RSPOs) amplify Wnt signaling via stabilization of Frizzled and LRP co-receptors and their function depends on the presence of Wnt ligands, which are upregulated in injured tissue. The acetaminophen (APAP) induced liver injury model in mice reproduces all important clinical features of adverse events and toxicity observed with APAP overdose in humans. To test the efficacy of SZN-043 in an acute liver injury model, we tested the ability of SZN-043 to repair APAP-induced liver damage.

Methodology

C57BL/6J mice were randomized based on body weight and fasted overnight to ensure that all animals had an initial, homogeneous, low level of liver glutathione. Mice then received a single i.p. dose of APAP (300 mg/kg) and were returned to their cage with food. One control group received a single i.p. dose of saline. Two hours later, mice were administered a single dose of negative control anti-GFP (10 mg/kg), SZN-043 (10 mg/kg) or positive control N-acetyl cysteine (1200 mg/kg). Blood and liver tissue samples were collected at 24, 48 and 72 hours for clinical chemistry, histopathology, immunostaining and gene expression analysis. To measure the effect of SZN-043 on hepatocyte proliferation, maturation and zonation, liver tissue markers were analyzed using RT-qPCR. Clinical chemistry included ammonium, ALT, AST and total bilirubin levels. Hepatocyte-specific proliferation was analyzed by immunofluorescence staining of Ki67 and HNF4α. Histopathology was done using hematoxylin and eosin staining.

Results

APAP Reduces Wnt Ligand Expression*  

Wnt Genes Expression

<table>
<thead>
<tr>
<th>Wnt Gene</th>
<th>No APAP</th>
<th>Anti-GFP</th>
<th>N-Acetyl-cysteine</th>
<th>SZN-043</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wnt2</td>
<td>100</td>
<td>102</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Wnt5a</td>
<td>99</td>
<td>96</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Wnt5b</td>
<td>102</td>
<td>99</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Wnt9b</td>
<td>101</td>
<td>98</td>
<td>100</td>
<td>97</td>
</tr>
</tbody>
</table>

Mean ± SEM, * p < 0.05, ** p < 0.01, *** p<0.001, **** p<0.0001

Wnt2, Wnt5a, Wnt5b and Wnt9b were highly expressed in mouse livers and reduced significantly at 24 hours after APAP dosing.

SZN-043 Activates the Wnt Signaling Pathway

SZN-043 Stimulates Hepatocyte Proliferation

SZN-043 reduces plasma ammonia compared to negative control anti-GFP. ALP levels showed significant elevation after SZN-043 dosing, consistent with its engagement with ASGR1, known to mediate elimination of circulating ALP protein. No effect was observed on AST or ALT.

Distribution of Proliferating Hepatocytes through all Hepatic Zones After SZN-043 Treatment

Conclusions

• Despite the reduced availability of Wnt ligands in this model, SZN-043 can effectively activate the Wnt signaling pathway
• The physiological feedback inhibitory mechanism of Wnt activity is preserved in response to SZN-043
• SZN-043 stimulates hepatocyte-specific proliferation in all hepatic zones
• SZN-043 reduces plasma ammonia compared to negative control anti-GFP
• SZN-043 improves the histology of injured livers

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