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

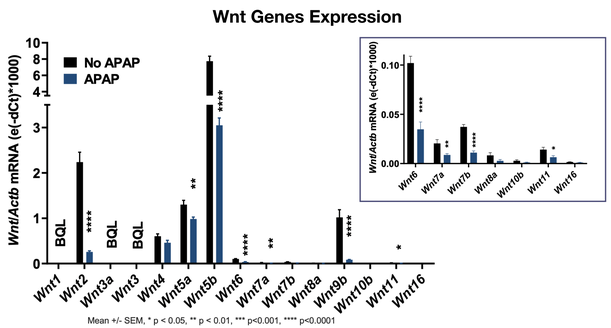
Wnt signaling plays a central role in hepatocyte expansion during development and tissue repair. R- spondins (RSPOs) amplify Wnt signaling via stabilization of Frizzled and LRP co-receptors and their function depends on the presence of Wnt ligands, which are upregulated in injured tissue. The acetaminophen (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

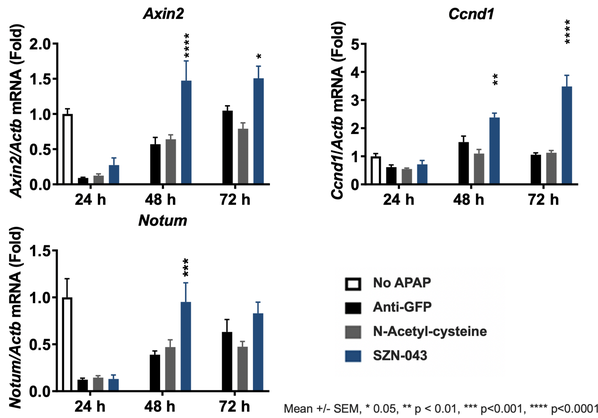
APAP Reduces Wnt Ligand Expression"



Mean +/- SEM, * p < 0.05, ** p < 0.01, *** p<0.001, **** p<0.0001

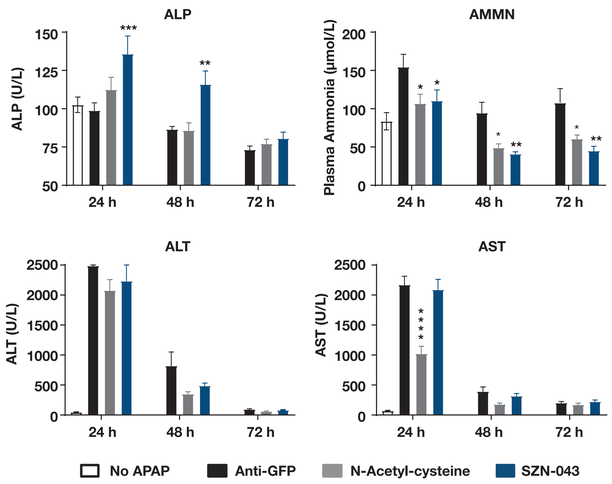
Wnt2, *Wnt5a*, *Wnt5b* and *Wnt9b* were highly expressed in mouse livers and reduced significantly at 24 hours after APAP dosing.

SZN-043 Activates the Wnt Signaling Pathway



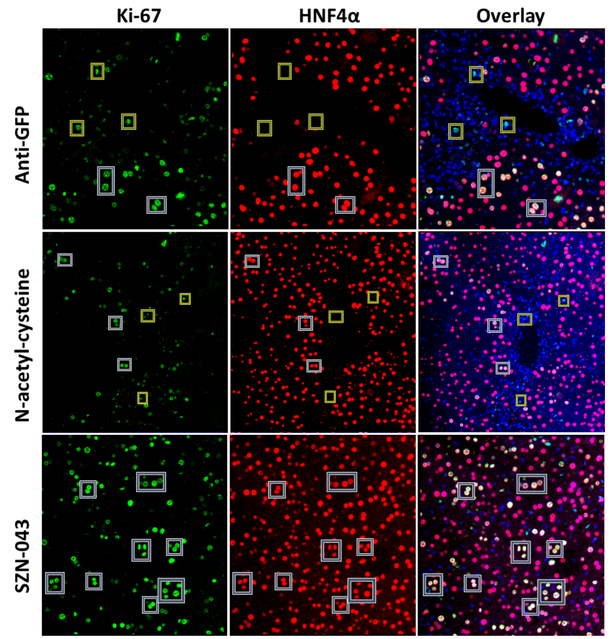
Elevation of Wnt/b-catenin target genes expression after a single dose of SZN-043. Physiological feedback inhibitory mechanism of Wnt activity is preserved in response to SZN-043 with increased notum expression.

SZN-043 Reduces Plasma Ammonia



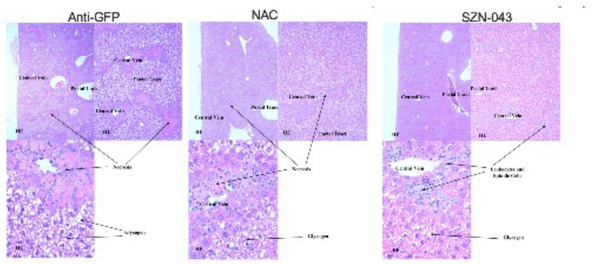
SZN-043 reduced plasma ammonia compared to negative control. ALP levels showed significant elevation after SZN-043 dosing, consistent with its engagement with ASGR1, known to mediate elimination of circulating ALP protein. No effect was observed on AST or ALT

SZN-043 Stimulates Hepatocyte Proliferation



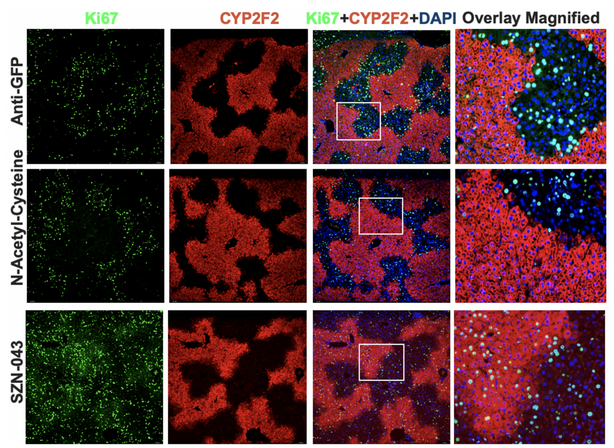
SZN-043 increased Ki67+HNF4α+ doubly-positive hepatocytes (white squares) after SZN-043 treatment (72 h). In contrast, a substantial proportion of non-hepatocytes appeared to proliferate in the anti-GFP and NAC groups, based on the presence of Ki67+ nuclei with undetectable HNF4A (yellow squares).

SZN-043 Improves Liver Histology at 72 hr.



APAP treatment showed large regions of diffuse necrosis in the pericentral regions of the liver samples in mice treated with anti-GFP. In contrast, livers from mice treated with NAC and SZN-043 displayed reduced necrosis.

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



With SZN-043 treatment, proliferating hepatocytes are observed through all hepatic zones.

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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