SZN-043, a Hepatocyte-targeted R-spondin mimetic, stimulates hepatocyte proliferation in an acute alcoholic hepatitis model

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Introduction

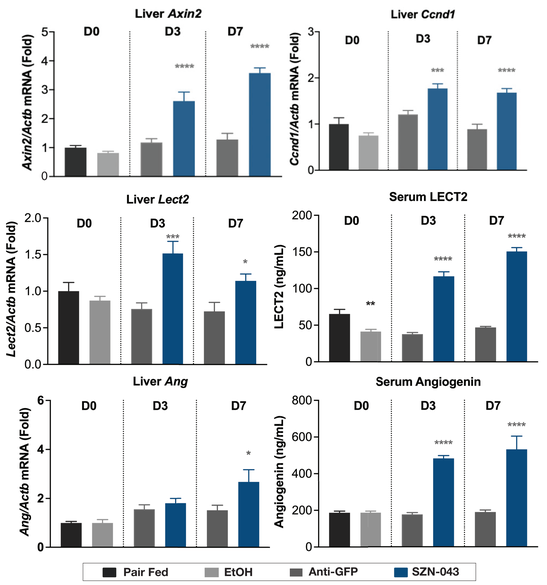
Wnt signaling plays a central role in hepatocyte expansion during development and tissue repair. R-spondins (RSPOs) are known enhancers of Wnt signaling, via stabilization of Frizzled and LRP co-receptors. SZN-043 is a bispecific fusion protein and hepatocyte-specific R-spondin mimetic. Severe alcoholic hepatitis (AH) is characterized by reduced hepatocyte proliferation and impaired hepatic regeneration. Since improved hepatocyte proliferative capacity has been linked to increased survival in AH, therapies that can stimulate hepatocyte proliferation may have substantial benefits in these patients. Therefore, we tested the effect of SZN-043 on hepatocyte expansion and liver function in a commonly used AH-induced liver injury model.

Methodology

Eleven-month-old female C57BL/6J mice were fed the control Lieber-DeCarli diet ad libitum for 5 days to acclimatize them to liquid diet. Mice were then allowed free access to a Lieber-DeCarli diet containing 5% (vol/vol) EtOH for 7 weeks. Control groups were pair-fed with an isocaloric control diet. In the second week, and for the remainder of the EtOH feeding, mice were gavaged twice weekly with EtOH (5.23 g/kg body weight) or isocaloric maltose dextrin, respectively. Mice were then randomized and injected intraperitoneally with either SZN-043 (30 mg/kg daily) or anti-GFP control (10 mg/kg twice weekly) for 7 days. Blood and liver tissue samples were collected at Days 0, 3 or 7 of treatment and analyzed for serum chemistry, gene expression, immunostaining and histopathology.

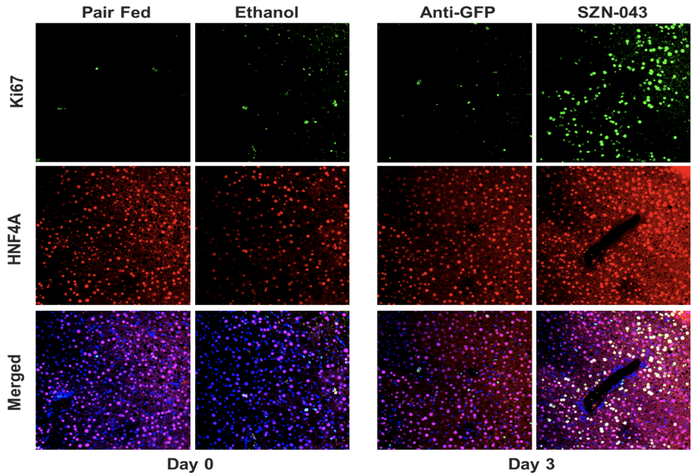
Results

SZN-043 Activated the Wnt Signaling Pathway



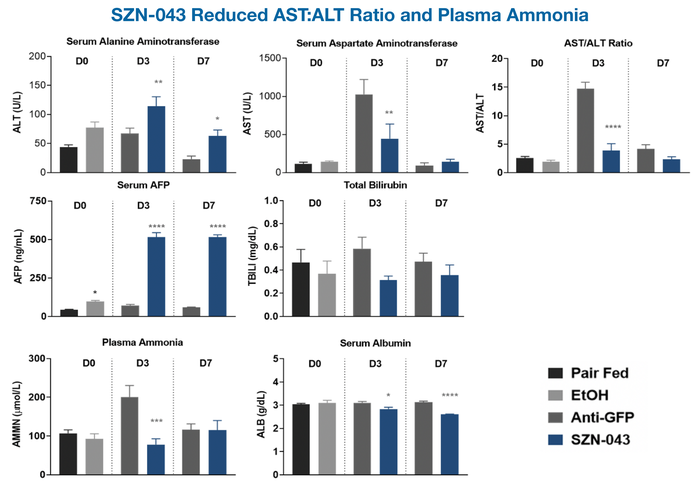
Elevated *Axin2* and *Ccnd1* expression, 2 direct Wnt/b-catenin target genes were observed in response to SZN-043. SZN-043 induced also an increase in liver mRNA and circulating protein concentrations of the hepatokine LECT2 and angiogenin, two additional Wnt target genes, encoding for secreted proteins.

SZN-043 Induced Hepatocyte-Specific Proliferation



Double staining with the proliferation marker Ki67 (green) and the hepatocyte-specific marker HNF4A (red) show that SZN-043 and RSPO stimulates hepatocyte cell growth. Note the presence of doubly stained Ki67+HNF4A+ hepatocytes (white nuclei) in the SZN-043-treated group (merged with DAPI).

SZN-043 Reduced AST:ALT Ratio and Plasma Ammonia



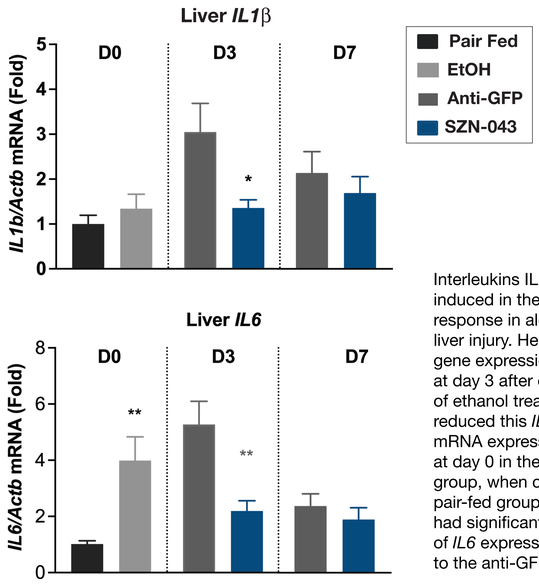
ALT was modestly increased, and AST was greatly reduced, after SZN-043 treatment when compared to control group resulting in a large reduction in AST/ALT ratio. SZN-043 significantly reduced plasma ammonia concentration. Serum albumin was mildly decreased in response to SZN-043. There was a reciprocal transient upregulation of alpha-fetoprotein (AFP) expression, sometimes referred to as “fetal albumin”, in response to SZN-043.

SZN-043 Induced Multiple Proliferation Markers

	Pair Fed D0	EtOH D0	Anti-GFP D3	SZN-043 D3	Anti-GFP D7	SZN-043 D7
Mki67	1.0 *(0.09)*	3.3 *(0.44)*	2.16 *(0.38)*	18.2**** *(1.62)*	2.7 *(0.87)*	6.08* *(0.64)*
Ccnd1	1.0 *(0.14)*	0.8 *(0.06)*	1.2 *(0.09)*	1.8*** *(0.10)*	0.9 *(0.11)*	1.7**** *(0.09)*
Cdc20	1.0 *(0.09)*	3.4 *(0.58)*	1.8 *(0.28)*	14.4**** *(2.20)*	4.1 *(1.62)*	5.8 *(0.94)*
Rrm2	1.0 *(0.04)*	2.0 *(0.18)*	1.8 *(0.26)*	5.8**** *(0.46)*	1.8 *(0.32)*	3.2* *(0.39)*
Cdk1	1.0 *(0.14)*	3.3 *(0.56)*	1.7 *(0.30)*	19.0**** *(0.99)*	3.2 *(1.25)*	5.7* *(0.40)*
Ccnb1	1.0 *(0.11)*	6.9 *(1.66)*	2.5 *(0.67)*	38.6**** *(4.59)*	6.5 *(3.19)*	9.6 *(1.59)*

Gene of interest/Actb mRNA fold (SEM) normalized to the pair-fed group mean value.
* $p < 0.05$, ** $p < 0.01$, *** $p<0.001$, **** $p<0.0001$.

SZN-043 Reduced Interleukins, IL1β and IL6 mRNA



Interleukins IL1β and IL6 are induced in the inflammatory response in alcohol-associated liver injury. Here, the level of *IL1b* gene expression was elevated at day 3 after discontinuation of ethanol treatment. SZN-043 reduced this *IL1b* increase. *IL6* mRNA expression was elevated at day 0 in the ethanol-treated group, when compared to the pair-fed group. At day 3, SZN-043 had significantly reduced the level of *IL6* expression, when compared to the anti-GFP group.

Conclusions

- **SZN-043 can effectively activate the Wnt signaling pathway in aged mice after a prolonged chronic-binge ethanol treatment**
- **SZN-043 stimulates hepatocyte-specific proliferation**
- **ALT was modestly increased after SZN-043 treatment and AST was greatly reduced when compared to control group at Day 3 resulting in a large reduction in AST/ALT ratio**
- **SZN-043 mitigated the increase in plasma ammonia and IL-1β and IL-6 expression after the last ethanol binge**
- **Altogether, these data provide proof-of-concept that SZN-043 stimulates hepatocyte expansion and improves liver function under conditions of impaired proliferation due to age and alcohol use**

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