SZN-043 Improves Liver Metabolic Function in a Mouse Preclinical Model

Mehaben Patel*#, Trevor Fisher#, Chenggang Lu, Darshini Shah, Wen-Chen Yeh, Geertrui F. Vanhove, Jay Tibbitts, Helene Baribault

Surrozen Inc., South San Francisco, CA 94080, USA; *Presenting author, #contributed equally.

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. SZN-043 is a hepatocyte-targeted antibody-based R-spondin mimetic that has been shown to stimulate hepatocyte proliferation and improve hepatic function in preclinical models of acute and chronic liver injury.

The methacetin breath test provides a rapid, non-invasive assessment of liver metabolic function in liver disease of diverse etiologies. CYP1A2 converts orally administered ^{13}C-methacetin via O-dealkylation to acetaminophen and $^{13}CO2$. Since the collection of exhaled CO_2 in small animals is technically challenging, we assessed the effect of SZN-043 on methacetin clearance in mice following IV dosing of non-labeled methacetin and quantified methacetin and its major metabolite, acetaminophen, in serum.

Methods

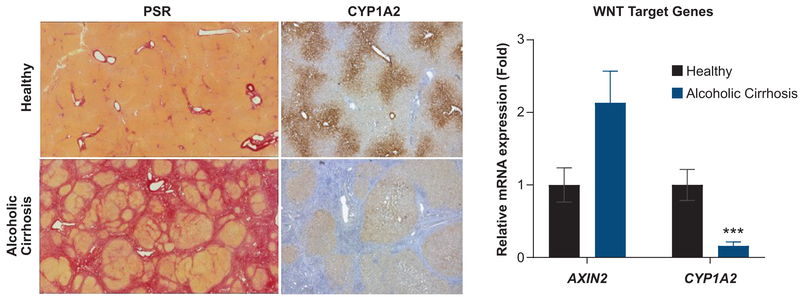
Protein expression of a key metabolic enzyme, CYP1A2, was evaluated in human liver tissue remnants from resections from patients with alcohol-associated liver disease and non-tumorous liver tissue from healthy controls. Samples were used for histological processing and total mRNA extraction, n=12 per group. Picrosirius Red staining and immunohistochemical analyses of CYP1A2 were also performed. *AXIN2* and *CYP1A2* expression levels were analyzed by qPCR. In mice, in a first study, we determined the pharmacokinetic properties of methacetin and acetaminophen. C57BL/6J females, 30-weeks old, received a single IV dose of methacetin (10 mg/kg) or acetaminophen (25 mg/kg). Serum was collected at selected time points (n=5 mice per time point) over a 8-hour period. Serum concentrations of methacetin and acetaminophen were measured using Liquid Chromatography-Mass Spectrometry (LC-MS) and relevant pharmacokinetic (PK) parameters estimated. In a second study, C57BL/6J males, 10-weeks old, were dosed with SZN-043 at either 3, 10, 30 or 100 mg/kg on Day 0 and methacetin (25 mg/kg) was injected IV one hour prior to terminal blood collection on Days 1, 2, 3, 4, 5, 6 and 14 (n=4 per group). A control group without SZN-043 treatment was included on Day 0 (n=4). Serum samples were collected for LC-MS analysis and liver samples were snap-frozen for Cyp1a2 mRNA analysis by qPCR. Graph values are presented ± SEM. Statistical analysis was done by comparing values to expression at time = 0: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. For AUC, values were calculated using the trapezoidal method and normalized to baseline (B) at time t=0, assuming constant values during the course of the study.

Conclusions

Methacetin quantification after IV dosing in mice can be used to show that SZN-043 can increase liver function in small animals, and that the duration of this effect can last beyond effects on gene regulation.

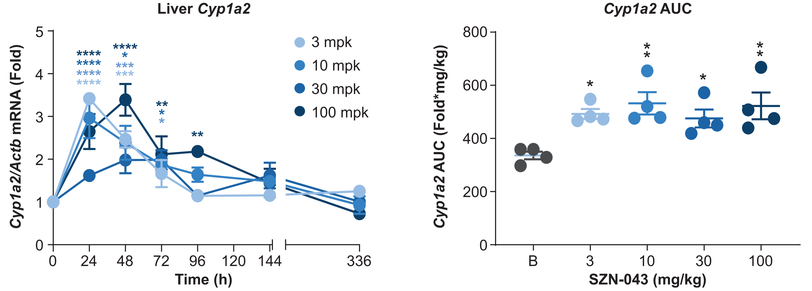
Results

CYP1A2 Protein is Strongly Reduced in Alcohol-Associated Liver Disease



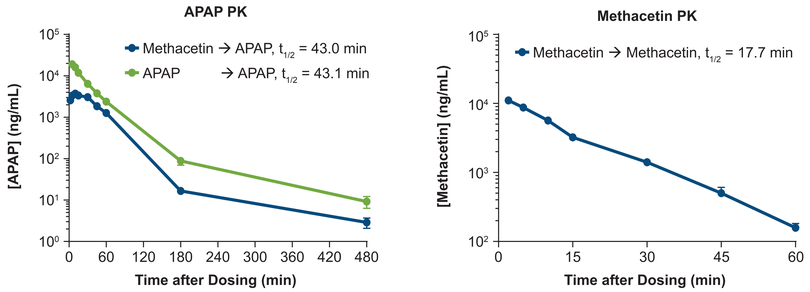
- Explants from patients with decompensated alcoholic cirrhosis were used for histological processing and total mRNA extraction. Non-tumorous liver tissue from hepatic resections was used as a healthy control, (n=12 per group). Picrosirius Red (PSR) staining shows the presence of extensive fibrosis in cirrhotic livers.
- CYP1A2 was strongly downregulated in diseased livers as shown by immunohistochemistry (left) and qPCR (right). Axin2 was not significantly differentially expressed.

SZN-043 Induced *Cyp1a2* mRNA Expression



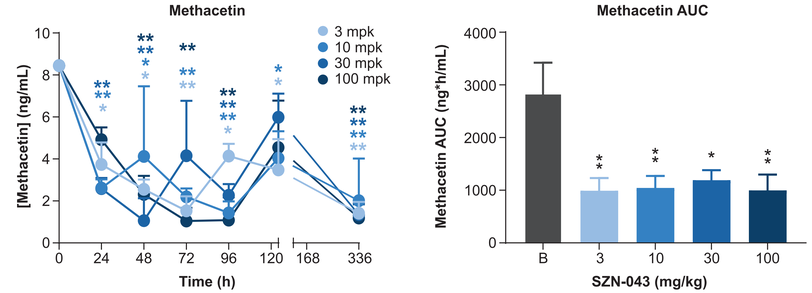
- SZN-043 induced *Cyp1a2* mRNA expression at all doses tested with a maximal response already achieved at 3 mg/kg, as shown by a time course of *Cyp1a2* expression after dosing (left) and quantification of the total area under the curve for the entire period (right).

Pharmacokinetics Properties of Methacetin and Acetaminophen (APAP)



- (Left) APAP or methacetin were dosed IV in C57BL/6J mice, followed by quantification of serum APAP. After methacetin administration, APAP serum level peaked at 10 minutes (T_{max}) — a result consistent with the rapid conversion of methacetin to APAP by CYP1A2 — and was cleared with a half-life of 43 minutes. APAP was cleared with a similar half-life after APAP IV injection, 43.1 minutes.
- (Right) Methacetin was injected IV and methacetin serum concentrations were measured. Methacetin half-life was 17.7 minutes.

SZN-043 Increased Methacetin Clearance



- SZN-043 increased methacetin clearance at all tested doses (3 to 100 mg/kg), consistent with an increase of CYP1A2 activity after treatment with SZN-043.

Presenting author: Mehaben Patel, email: meha@surrozen.com
Surrozen Inc., South San Francisco, CA 94080
www.surrozen.com

SURROZEN