Reduction of Hepatocyte Injury by SRT-015, a Novel Inhibitor of Apoptosis Signal-Regulating Kinase 1 (ASK1)

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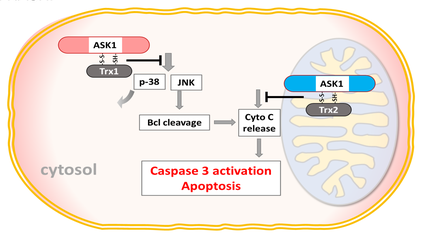
Background

SRT-015 is a novel, small molecule inhibitor of the apoptosis signal-regulating kinase 1 (ASK1)[1]. ASK1 is a redox-sensing kinase that is involved in hepatocyte injury caused by drug toxicity, alcohol exposure, bacterial toxins, oxidative stress, and lipotoxicity. ASK1 is localized in the cytoplasm and the mitochondria and normally bound to, and repressed by antioxidant proteins, including thioredoxin 1 in the cytosol and thioredoxin 2 in the mitochondria[2].

As illustrated below, activation of cytosolic or mitochondrial pool of ASK1 signals either through the JNK and p38 MAP kinase[3] or inflammasome cascades but eventually converge on execution of hepatocyte injury and death. Such non-overlapping ASK1 signaling suggests that activation of each ASK1 pool might be pathology-specific.

Here we show that the mitochondrial pathway appears to be more relevant to the chronic ASK1 activation found in NASH, and that, SRT-015 interrupts this pathway, resulting in therapeutic efficacy in a diet-induced obese (DIO) mouse model of NASH.



Aims

We investigated mechanism(s) of hepatoprotective efficacy of the novel ASK1 inhibitor, SRT-015, in acute rodent model of liver injury and in therapeutic DIO-NASH mouse model of NASH with liver fibrosis.

The mechanism of action of SRT-015 was compared to other ASK1 inhibitors, including selonsertib.

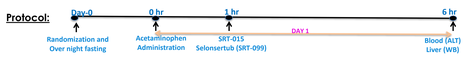
Methods

In vitro methods

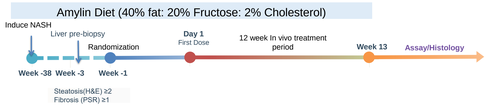
- SRT-015, Takeda 19[4], Pfizer 18[5], Pfizer 38[5] were synthesized by Seal Rock Therapeutics. Selonsertib (GS-4997) was obtained from ChemieTek, GS-444217 from ProbeChem.
- Compound IC_{50}s were determined by the inhibition of ASK1 catalytic domain enzymatic activity (ADP-Glow Kinase assay; Promega).
- Apoptosis (Caspase 3/7; Promega) was induced in HepG2 cells obtained from ATCC (Manassas, VA) by 1 mM H2O2 treatment for 20 hrs in the presence of compounds. Most values presented as Mean ± SEM.

In vivo methods

- **Acute APAP-induced rodent liver injury model**
- Acetaminophen (APAP, 300 mg/kg) or vehicle IP. SRT-015 and selonsertib dosed PO 1 hr after APAP. Six hours after APAP blood was sampled for ALT and liver for WB.
- Primary antibodies: Rabbit Anti-p38, Rabbit anti-phospho-p38, Rabbit Anti-ASK1,Rabbit anti-phospho-ASK1 Rabbit anti β-Actin (1:1000 dilution for all antibodies from CST)
- Secondary antibody: HRP-linked anti-rabbit IgG (1:1000 dilution)



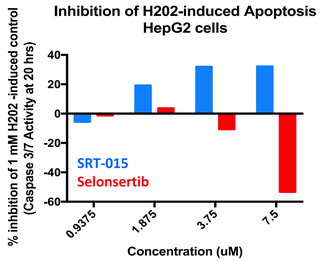
- **Therapeutic Gubra DIO-NASH mouse model**
- Compound concentration in plasma and liver tissue determined by fit for purpose LC-MS/MS.
- Study design:



- After 38 weeks on AMLN diet, in mice with biopsy-confirmed hepatosteatosis and fibrosis, SRT-015 or selonsertib dosing in AMLN chow (ad libitum) was initiated for an additional 12 weeks.
- Compound dosing concentrations were chosen to match compound liver exposures (see figure 3) with SRT-015 and selonsertib.
- Histological, serum chemistry and biochemical analysis was performed at study termination.
- Phosphorylation of p38 and JNK in liver tissue was measured using MSD assay.
- RNAseq analysis was performed on NASH vehicle, 0.5% SRT-015 and selonsertib groups
- Statistical analysis by ANOVA followed by Tukey Multiple Comparisons Test; * P<0.05, ** P< 0.01, ***P < 0.001, ****P < 0.0001.

Results

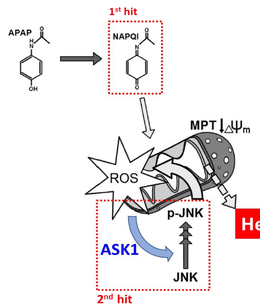
1) Anti-apoptotic activity of SRT-015 and other ASK1 inhibitors in vitro



Compound	ASK1 IC50 (nM)	Inhibition of H2O2-induced apoptosis @ 7.5 uM
SRT-015	17	34%
Selonsertib	8.4	None (pro-apoptotic)
GS-444217	23.1	none
Takeda 19[4]	14.4	none
Pfizer 18[5]	8.4	28%
Pfizer 38[5]	9.6	12%

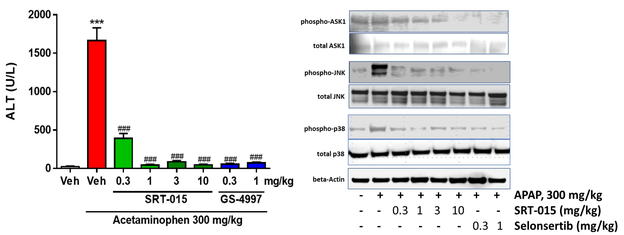
- All literature identified ASK1 inhibitor compounds demonstrated enzymatic activity against the ASK1 kinase.
- Under HepG2 assay conditions, inhibition of apoptosis was not observed with selonsertib, GS-444217 or Takeda 19.

2) Reduction of acute liver injury by SRT-015 and selonsertib



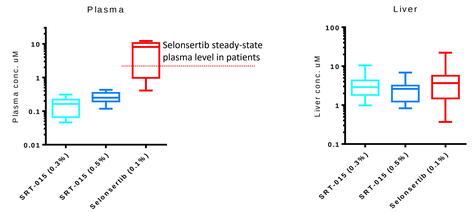
- APAP reactive intermediate, NAPQI, induces a mitochondrial oxidative stress (1st hit).
- Mitochondrial oxidative-stress activates cytosol and mitochondrial ASK1-JNK pathway (2nd hit).
- Mitochondrial JNK translocation amplifies the oxidative stress and leads to collapse of mitochondrial membrane potential and hepatic cell death[6].

- SRT-015 and selonsertib (GS-4997) significantly reduced liver injury due to APAP overdose, and dose-dependently decreased ASK1-JNK/p38 signaling activated by APAP.

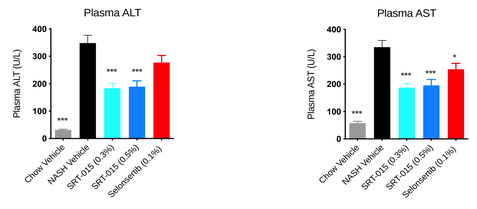


3) Anti-fibrotic efficacy of SRT-015 in DIO-NASH mouse model

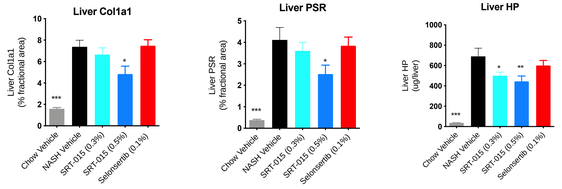
- Biopsy-confirmed therapeutic DIO-NASH mouse model was used to investigate chronic effects of SRT-015 and selonsertib on lipotoxic liver injury and fibrosis. Selonsertib was administered in chow at the dose intended to match human plasma concentration at 18 mg[7] (a top dose in phase 2 and 3 clinical trials in NASH).
- SRT-015 was dosed at matching liver concentrations to allow direct comparison between the two ASK1 inhibitors.



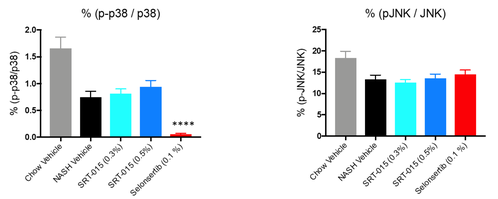
- After 12 weeks of administration, SRT-015 significantly decreased plasma ALT and AST, whereas selonsertib had a modest effect, and only reduced plasma AST.



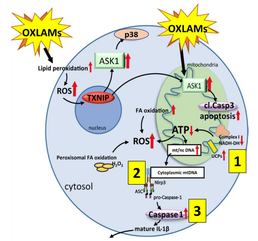
- SRT-015 significantly decreased liver fibrosis as measured by IHC staining for Col1a1, and picro-Sirius red (PSR), and content of hydroxyproline (HP).
- In contrast, selonsertib had no significant effect on liver Col1a1, PSR or HP. (Detailed efficacy results are discussed in Poster #1658 AASLD 2020)



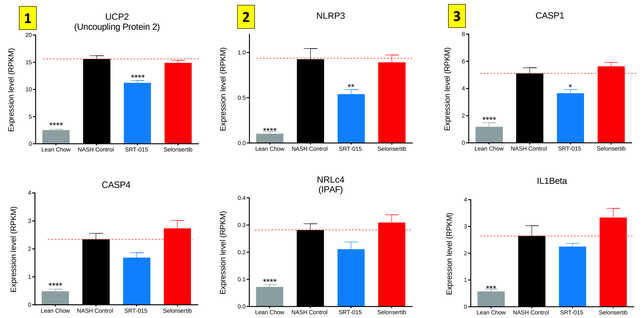
- Despite significant liver pathology, phospho-p38 and phospho-JNK were not elevated in livers of mice chronically fed the AMLN diet.
- Consistent with phase 3 clinical results[8], selonsertib reduced hepatic levels of phospho-p38 in the DIO-NASH model but did not demonstrate hepatic efficacy. Selonsertib had no effect on phospho-JNK.
- SRT-015 did not affect either biomarker, yet demonstrated substantial efficacy (decreased fibrosis, inflammation and hepatoxicity) in the DIO-NASH model.



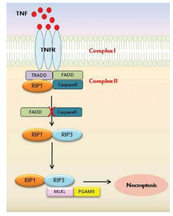
4) SRT-015 suppressed inflammasome/pyroptosis pathway, a key mechanism of lipotoxic liver injury, in DIO-NASH mouse model (RNAseq).

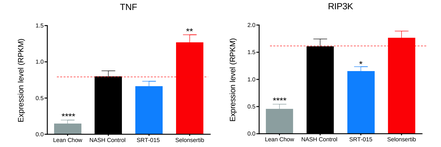


- From the literature[9], oxidized metabolites of fatty acids, including linoleic acid (OXLAM), are increased in NASH patients and their levels correlate with disease severity.
- OXLAM-induced mitochondrial dysfunction is associated with activation of mitochondrial ASK1 and elevated levels of NLRP3 inflammasome components and caspase-1 activation.
- As shown below, the inflammasome pathway is upregulated in the DIO-NASH mouse model and is inhibited by SRT-015 but not selonsertib.



5) An increased expression of TNF by selonsertib could nullify its effects on ASK1 inhibition (RNAseq data).





- The observed increased expression of TNF induced by selonsertib again differentiates it from SRT-015 and likely represents an off-target effect unrelated to ASK1 inhibition.

Summary and Conclusion

- Acute hepatocyte injury, due to APAP or H2O2, is mediated by ASK1 acting on the p-p38 or p-JNK pathways. SRT-015, in contrast to selonsertib and literature ASK1 inhibitors, provides hepatoprotection against both agents.
- In the chronic DIO-NASH mouse model, liver injury does not lead to elevation of p-p38 or p-JNK; rather the NASH lipotoxicity appears to be associated with activation of the mitochondrial ASK1/inflammasome cascade (RNAseq).
- In contrast, and in agreement with clinical studies, selonsertib significantly inhibited p-p38 in the DIO-NASH mouse model but was not efficacious.
- SRT-015 shows superior efficacy to selonsertib in the therapeutic DIO-NASH model demonstrating decreased fibrosis, inflammation, and hepatoxicity, despite having no appreciable effect on p38 or JNK phosphorylation.
- **SRT-015, with its distinct in vitro and in vivo pharmacology, warrants further development for NASH, alcoholic hepatitis, and drug-induced acute liver failure. Phase 1 clinical trials are expected to initiate in 2021.**

References and Acknowledgements

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