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Anti-fibrotic and Anti-Inflammatory Mechanisms of Best-in-Class ASK1 Inhibitor SRT-015

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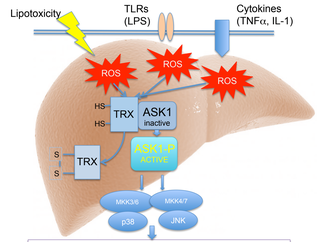
Background

SRT-015 is a novel, small molecule inhibitor of the apoptosis signal-regulating kinase 1 (ASK1). ASK1 is a ubiquitous redox-sensitive kinase that is activated by pathological stimuli including oxidative stress and lipotoxicity[1]. ASK1 is localized in the cytoplasm and the mitochondria and normally bound and repressed by antioxidant proteins, including thioredoxin 1 in the cytosol and thioredoxin 2 in the mitochondria[2].

As shown below, different stress stimuli induce reactive oxygen species (ROS) that result in the oxidation and dissociation of thioredoxin from ASK1 leading to ASK1 activation. In turn, activated ASK1 induces phosphorylation and activation of the JNK and p38 MAP downstream kinase cascades[3] to result in apoptosis, inflammation and fibrosis, all key components of NASH.



Aims

1) Compare SRT-015 cellular mechanisms with other ASK1 inhibitors
2) Demonstrate in vivo efficacy and mechanisms of action in a biopsy-confirmed DIO-NASH mouse model with SRT-015 and compare with the clinical entity selonsertib.

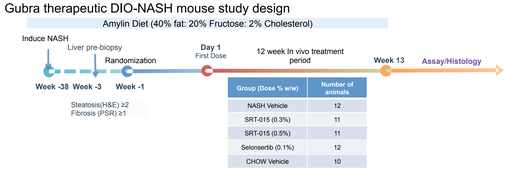
Methods

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, and the JNK inhibitor SP600125 and p38 inhibitor Losmapimod from Selleckchem.
- Compound IC_{50}s were determined by the inhibition of ASK1 catalytic domain enzymatic activity (ADP-Glow Kinase assay; Promega).
- All cells lines were obtained from ATCC (Manassas, VA) and human primary PBMCs were obtained from ALLCELLS (Alameda, CA). Cells were challenged using stress protocols to induce ROS and compounds evaluated in dose-responsive manner to determine the IC_{50}. Most values presented as Mean +/- SEM (n).
- To evaluate the TLR4- ASK1 pathway[1], human PBMCs (up to 9 different donors) were challenged with 100 ng/ml LPS (Sigma) for 30 min to determine p-p38 (HTRF P-p38 Kit; CisBio) and 6 hrs for TNFalpha levels (TNFa; BD OptEIA).
- Apoptosis (Caspase 3/7; Promega) was induced in HepG2 cells by 1 mM H2O2 treatment for 20 hrs in the presence of compounds.
- The myofibroblast marker alpha-SMA (a-SMA) was induced in human fibroblasts by TGFbeta1 (TGFb; R&D) with compound co-treatment for 48 hrs. Alpha-SMA quantified by in-Cell ELISA (Thermo) using mouse anti-alpha smooth muscle Actin antibody (1A4; ABCAM).
- Cytotoxicity (Cell-Titer Glo; Promega) of compounds using fibroblasts and HepG2 cells were measured after 72 hr exposure to compounds alone (no stress protocol). Compound cytotoxicity in HepG2 cells induced with 1 mM H2O2 was determined at 20 hrs.

In vivo methods

- PK performed at 10 mg/kg PO in mice.
- Drug compound concentration in plasma, liver and other tissues determined by fit for purpose LC-MS/MS
- Gubra therapeutic DIO-NASH mouse study design

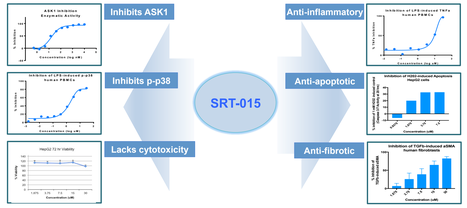


Group (Dose % w/w)	Number of animals
NASH Vehicle	12
SRT-015 (0.3%)	11
SRT-015 (0.5%)	11
Selonsertib (0.1%)	12
CHOW Vehicle	10

- After 38 weeks on AMLN diet, in mice with biopsy confirmed 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.
- RNAseq analysis of liver tissue was performed on NASH vehicle, 0.5% SRT-015 and selonsertib groups; historical lean control group was used for comparison.
- Statistical analysis by ANOVA followed by Tukey Multiple Comparisons Test; * P<0.05, ** P< 0.01, ***P < 0.001, ****P < 0.0001.

Results

1) SRT-015 inhibits ASK1 pathway, decreases apoptosis, fibrosis and inflammation in cellular models with no off-target cytotoxicity



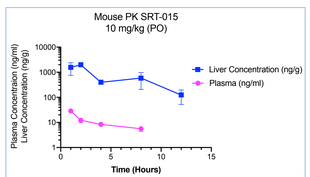
- SRT-015 inhibits ASK1 pathway (ASK1 biochemistry; p-p38 inhibition), decreases apoptosis (H2O2-induced caspase 3/7), fibrosis (TGFa-induced myofibroblast marker a-SMA) and inflammation (LPS-induced TNFa) in cellular systems with no off-target cytotoxicity.

Desired Phenotype	ASK1 inhibition	Lack hERG Inhibition	Downstream Pathway Acute Inhibition	Anti -inflammatory	Anti – fibrotic	Anti-apoptotic	Lack cytotoxicity	Lack cytotoxicity
Assay	ASK1 Enzymatic IC50 (nM)	% inhibition @ 10 uM	hPBMC LPS-induced p-p38 inhibition IC50 (uM)	hPBMC LPS-induced TNFa inhibition IC50 (uM)	hFibroblasts TGFb-induced a-SMA inhibition IC50 (uM)	HepG2 H2O2-induced apoptosis inhibition @ 7.5 uM	hFibroblasts Cytotoxicity (72 hrs) IC50 (uM)	HepG2 Cytotoxicity (72 hrs) IC50 (uM)
SRT-015	17	10%	2.8 +/- 0.4 (5)	9.1 +/- 1.4 (9)	13.7 +/- 2.7 (4)	34%	>30 (3)	>30 (3)
Selonsertib	8.4	39%	28.7 +/- 1.3 (8)	6.1 +/- 0.6 (11)	23.6 +/- 2.0 (5)	none	20.8 +/- 2.6 (3)	20.6 +/- 2.8 (4)
GS-444217 (Gilead)	23.1	80%	5.1 +/- 1.6 (4)	5.6 +/- 0.8 (4)	20.6 (2)	none	>30	26.2 (2)
Takeda 19[4]	14.4	45%	0.4 (1)	1.1 (2)	1.2 (2)	none	7.3 +/- 2.9 (3)	23.1 (2)
Pfizer 18[5]	8.4	ND	>30 (2)	13.8 (2)	>30 (2)	28%	>30 (2)	>30
Pfizer 38[5]	9.6	49%	1.49 (2)	1.9 (2)	6.4 (2)	12%	>30 (2)	>30
p38 inhibitor Losmapimod	>10,000	ND	0.002 +/- .001 (3)	0.19+/-0.1 (3)	30 (1)	28%	>30 (1)	>30
JNK inhibitor SP600125	397	ND	>30 (1)	7.8 (2)	6.9 (2)	67%	8.5 (2)	29.9 +/- 0.1 (3)

Cellular responses of ASK1 inhibitors and standards

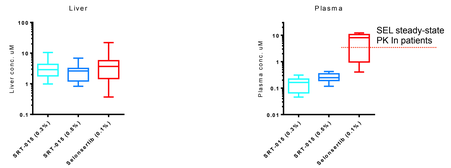
- All literature identified ASK1 inhibitor compounds demonstrated enzymatic activity against the ASK1 kinase but varied by lacking acute p-p38 inhibition, not inducing anti-ASK1 effects or displaying off-target cytotoxicity.
- SRT-015 and select ASK1 inhibitors decreased both p-p38 and TNFa. All compounds inhibited TNFa release at 6 hrs suggesting inhibition via a different mechanism.
- Significant levels (>20%) of hERG inhibition at 10 uM were identified in many compounds, but not SRT-015
- Inhibition of apoptosis was not observed with selonsertib, GS-444217 or Takeda 19.
- Cytotoxicity of compound treatment alone for 72 hrs (no stress induction) was observed with Takeda 19 and selonsertib in fibroblasts and with selonsertib and SP600125 in HepG2 cells.

2) SRT-015 PK: Liver-selective compound



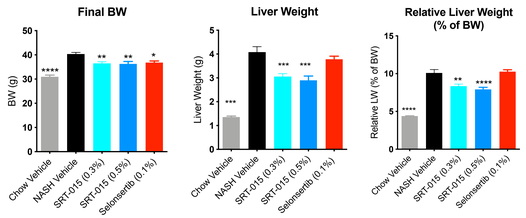
SRT-015 uniquely exhibits a liver-selective distribution profile with >10x liver:plasma ratio.

3) SRT-015 and selonsertib in vivo: Matched liver concentrations in therapeutic DIO-NASH mouse model

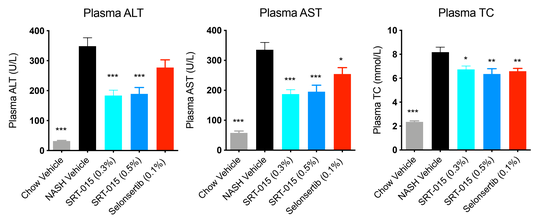


- Well-matched compound liver concentrations were observed in the study
- As expected, SRT-015 plasma levels are lower than selonsertib. Selonsertib plasma levels in this study were within range of human steady state level at 18 mg: 1.3 uM

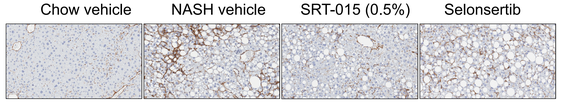
4) SRT-015 treatment decreased DIO-induced BW and hepatomegaly in therapeutic DIO-NASH mouse model



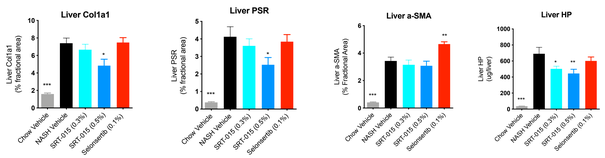
5) SRT-015 treatment improved liver function tests and total cholesterol in therapeutic DIO-NASH mouse model



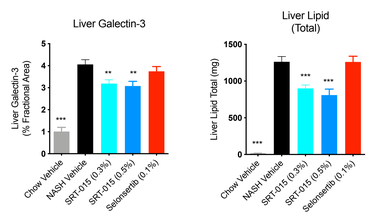
6) SRT-015 treatment demonstrated anti-fibrotic efficacy in therapeutic DIO-NASH mouse model



- Representative IHC images of collagen 1a1 staining (Col1a1, above) and quantification of liver Col1a1, PicroSirius Red (PSR) and a-SMA (below). The liver fraction was estimated as % of total tissue. Liver hydroxyproline (HP) determined by biochemistry.
- SRT-015 significantly decreased Col1a1, PSR, HP and reduced the activated stellate cell biomarker a-SMA. In contrast, selonsertib had no significant effect on liver Col1a1, PSR or HP and significantly increased a-SMA.



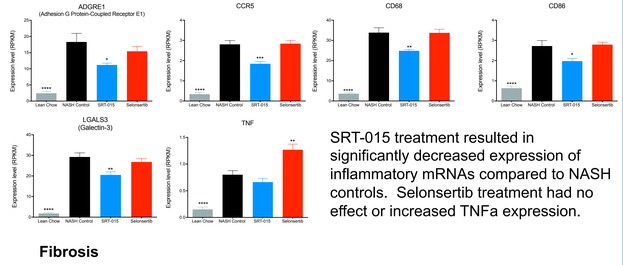
7) SRT-015 treatment demonstrated anti-inflammatory and anti-steatosis activity in therapeutic DIO-NASH mouse model



- SRT-015 significantly decreased galectin-3, an inflammatory monocyte marker and total liver lipids.

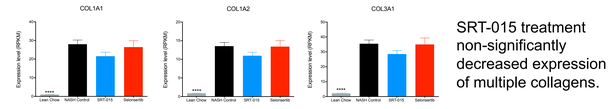
8) RNAseq analysis of liver tissue corroborated and extended SRT-015 anti-inflammatory, anti-fibrosis and anti-hepatotoxicity mechanisms of action in therapeutic DIO-NASH mouse model

Inflammation



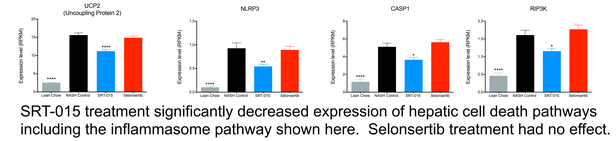
SRT-015 treatment resulted in significantly decreased expression of inflammatory mRNAs compared to NASH controls. Selonsertib treatment had no effect or increased TNFa expression.

Fibrosis



SRT-015 treatment non-significantly decreased expression of multiple collagens.

Hepatocyte death



SRT-015 treatment significantly decreased expression of hepatic cell death pathways including the inflammasome pathway shown here. Selonsertib treatment had no effect. Inhibition of the inflammasome pathway implications is discussed in Poster #1692 AASLD 2020.

Summary

- In cellular studies only SRT-015 demonstrated:
  - Inhibition of ASK1 pathway
  - Decreased apoptosis, fibrosis and inflammation
  - No off-target cytotoxicity
- In a therapeutic DIO-NASH model SRT-015 treatment:
  - Demonstrated efficacy by significantly reducing liver fibrosis, inflammation and hepatic cell death, all key drivers for NASH
  - Decreased plasma AST, ALT and total cholesterol.
  - In contrast, at equivalent liver exposures, selonsertib was not efficacious in vivo with minimal or no significant effects observed.

Conclusion

These data demonstrate SRT-015 is a best-in-class ASK1 inhibitor, with demonstrated efficacy in vitro and in vivo, and supports the evaluation of SRT-015 in NASH patients.

References

1 Hattori et al, Cell Communication and Signaling 2009
2 Zhang et al, Circ. Res. 2004
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Acknowledgements

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