SRT-015: Novel ASK1 Inhibitor for the Treatment of NASH

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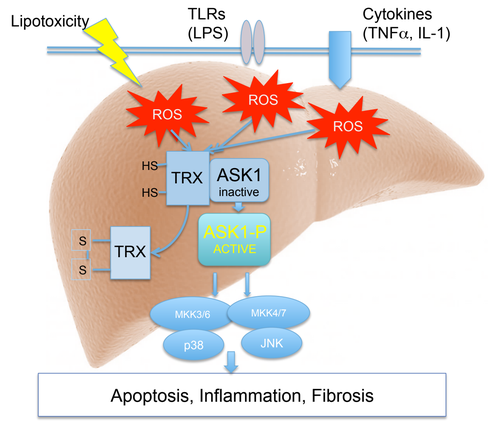
Background

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

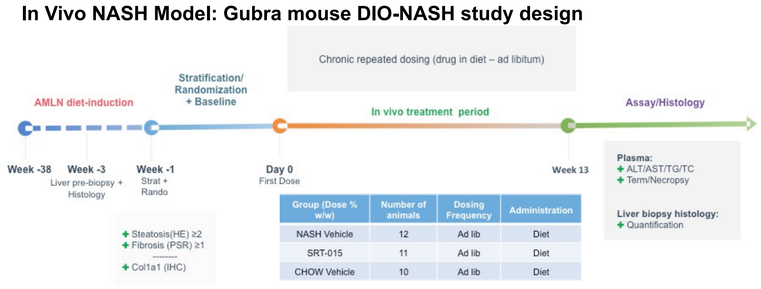
To characterize SRT-015 cellular mechanisms of action and demonstrate in vivo efficacy of SRT-015 treatment in a NASH therapeutic mouse model.

Methods

In vitro methods

- SRT-015, Takeda 19[4], Pfizer 18[5], Pfizer 38[5] were synthesized by Seal Rock Therapeutics. Selonsertib was obtained from Chemietek 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.

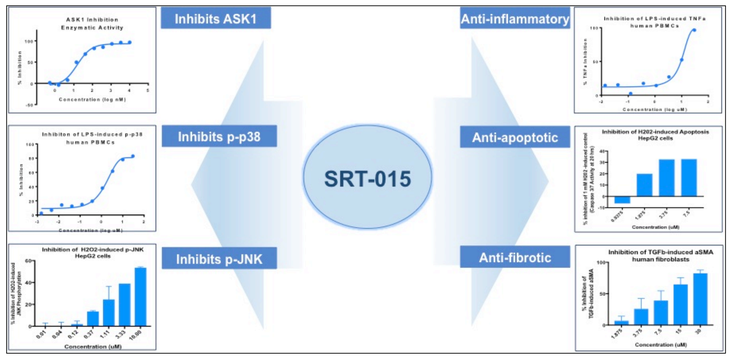
In Vivo NASH Model: Gubra mouse DIO-NASH study design



- After 38 weeks on AMLN diet, SRT-015 treatment was initiated using mice with established fibrosis for an additional 12 weeks.
- Histological, serum chemistry and biochemical analysis were performed at study termination.
- Statistical analysis by ANOVA followed by Tukey Multiple Comparisons Test; # = P< 0.05

Results

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



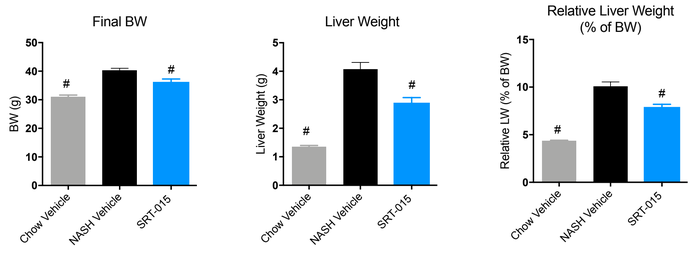
- SRT-015 inhibits ASK1 pathway (ASK1 biochemistry; p-p38 and pJNK inhibition), decreases apoptosis (H2O2-induced caspase 3/7), fibrosis (TGFa-induced myofibroblast marker a-SMA) and inflammation (LPS-induced TNFa) in cellular systems with no off-target cytotoxicity (below).

Desired Phenotype	ASK1 inhibition	Downstream Pathway Acute Inhibition	Anti - inflammatory	Anti – fibrotic	Anti-apoptotic	Lack cytotoxicity	Lack cytotoxicity	Lack cytotoxicity (stressed cells)
Assay	ASK1 Enzymatic IC50 (nM)	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)	HepG2 H202-treated viability at 7.5 uM
SRT-015	17	2.8 +/- 0.4 (5)	9.1 +/- 1.4 (9)	13.7 +/- 2.7 (4)	34%	>30 (3)	>30	98%
Takeda 19[4]	15.7	0.4 (1)	1.1 (2)	1.23 (2)	none	4.4 (2)	>30	70%
Selonsertib	8.4	27.9 +/- 2.1 (5)	5.8 +/- 0.7 (8)	23.6 +/- 2.0 (5)	none	16.2 (2)	22.3 +/- 3.2	66%
Pfizer 18[5]	5.8	>30 (1)	6.3 (1)	>30 (2)	28%	>30 (2)	>30	103%
Pfizer 38[5]	9.6	1.49 (2)	1.9 (2)	6.4 (2)	12%	>30 (2)	>30	74%
p38 inhibitor Losmapimod	>10,000	0.002 +/- .001 (3)	0.19+/-0.1 (3)	30 (1)	28%	>30 (1)	>30	94%
JNK inhibitor SP600125	397	>30 (1)	7.7 (2)	6.9(2)	67%	8.5 (2)	>30	94%

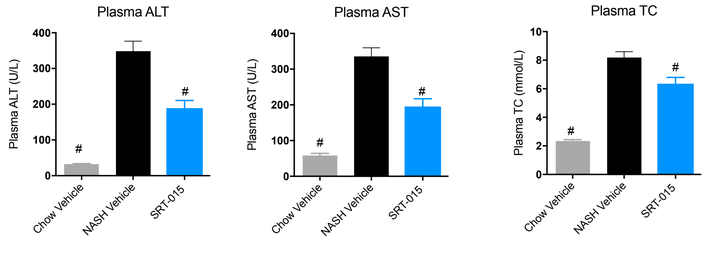
Cellular responses of ASK1 inhibitors and standards

- All literature identified ASK1 inhibitor compounds demonstrated enzymatic activity against the ASK1 kinase but varied by lacking 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 (such as JNK).
- Cytotoxicity of compound treatment alone (no stress induction) was observed with Takeda 19 and selonsertib in fibroblasts and with selonsertib in HepG2 cells. Selonsertib additionally decreased viability in stressed HepG2 cells.

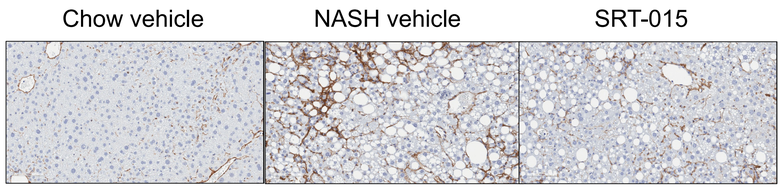
2) In Vivo: SRT-015 treatment significantly decreased DIO-induced BW and hepatomegaly in NASH therapeutic model



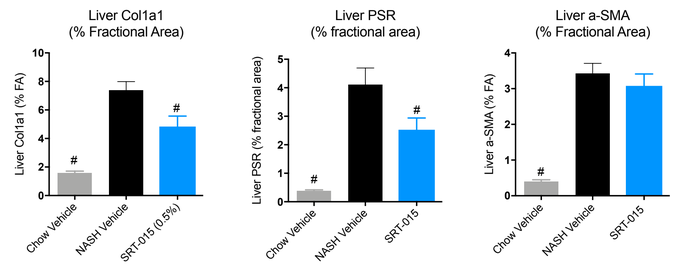
3) SRT-015 decreased DIO-induced liver function tests and total cholesterol in NASH therapeutic model



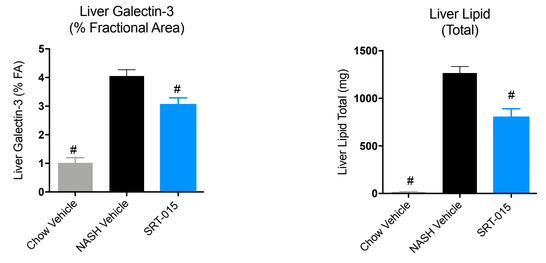
4) SRT-015 anti-fibrotic efficacy in NASH therapeutic model



- 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.
- SRT-015 significantly (P< 0.05) decreased Col1a1 and PSR and reduced the activated stellate cell biomarker a-SMA.



5) SRT-015 demonstrates anti-inflammatory mechanism and decreased steatosis in NASH therapeutic model



- SRT-015 significantly (P< 0.05) decreased galectin-3, an inflammatory monocyte marker and total liver lipids.

Summary and Conclusion

- In cellular studies SRT-015 demonstrated:
  - Inhibition of ASK1 pathway
  - Decreased apoptosis
  - Decreased fibrosis and inflammation
  - No off-target cytotoxicity
- In a therapeutic DIO-NASH model SRT-015 treatment:
  - Resulted in significant effects on both metabolic parameters and liver specific pathology
  - Significantly reduced liver fibrosis, steatosis and inflammation, all key drivers for NASH
  - Decreased plasma AST, ALT and total cholesterol.
- These data demonstrate SRT-015, a novel ASK1 inhibitor, is efficacious in vitro and in vivo and support 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
3 Tobiume et al, EMBO Rep. 2001
4 Lanier et al, ACS Med. Chem. Lett. 2017
5 Lovering et al, European Journal of Medicinal Chemistry 2018

Acknowledgements

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