Anti-Inflammatory Mechanisms of ASK1 Inhibitor SRT-015

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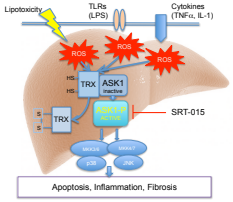
Background

Apoptosis signal-regulating kinase 1 (ASK1) is a ubiquitous redox-sensitive kinase that is activated by pathological stimuli including oxidative stress and lipotoxicity[1]. Activation of ASK1 causes phosphorylation and activation of the downstream kinase cascades to result in inflammation, apoptosis and fibrosis, all key components of liver diseases including NASH and alcoholic hepatitis.

SRT-015 is a novel, liver selective, small molecule inhibitor of ASK1 that recently completed Phase 1 clinical trials[2] (Abstract# 106, D. Burge, oral presentation 11:30 am, Sunday Nov. 6 AASLD, 2022).

We have previously demonstrated the multiple mechanisms of action (MOA) of SRT-015 including decreasing apoptosis, fibrosis and inflammation in vitro and vivo[3]. Here we detail SRT-015 inhibition of inflammation in whole blood from human volunteers and in a DIO-NASH mouse therapeutic model.



SRT-015 inhibits ASK1-induced apoptosis, inflammation and fibrosis.

Aims

Evaluate anti-inflammatory mechanism of action of SRT-015 in whole blood from human volunteers and in a DIO-NASH therapeutic model.

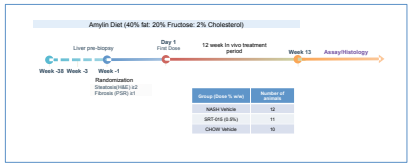
Methods

Whole blood analysis

- Fresh whole blood was collected from human volunteers (N=9; ALLCells) and studies initiated within 3 hours of collection. After a 1 h pre-incubation with SRT-015 (0.9-30uM), blood was challenged with LPS (0.1 ug/ml) to activate the ASK1 pathway. Cells were analyzed 15 min post LPS for p-p38 accumulation by flow cytometry (Beckman CytoFlex Analyzer) or for TNFα secretion after 6 h by ELISA and the IC_{50} determined.
- To evaluate responses from specific immune cell populations, antibodies were used to identify monocytes (CD14+, CD16-), neutrophils (CD16+, CD15+) and lymphocytes (CD3+,CD14-, CD16-) from whole blood by flow cytometry using CytoExpert software. All antibodies from BD Biosciences.

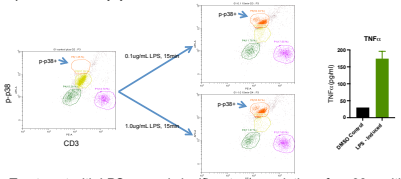
DIO-NASH mouse therapeutic model

- In the DIO-NASH therapeutic model (AMLN diet; Gubra) mice with biopsy confirmed steatosis and fibrosis were treated with SRT-015 (0.5% in chow) for 12 weeks and inflammation evaluated by IHC, plasma cytokines levels by MSD, and pathway expression levels by RNAseq analysis (protocol below).
- Values are expressed as group means of 10-13 (+/- SEM) and statistical significance determined by ANOVA and Tukey's multiple comparison test as follows: *= P<0.05, ** = P<0.01, *** P<0.001.



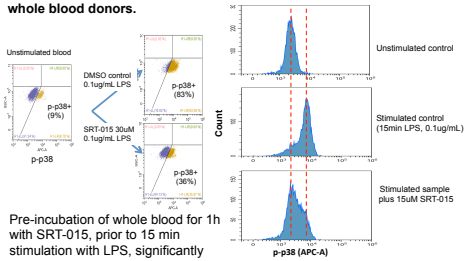
Results

1) LPS-induces p-p38 and TNFα in whole blood from human volunteers.



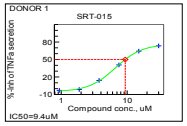
Treatment with LPS caused significant accumulation of p-p38 positive cells in a subpopulation of cells at 15 min and increased secretion of TNFα at 6 hr.

2) SRT-015 decreases LPS-induced p-p38 and TNFα from human whole blood donors.



Pre-incubation of whole blood for 1h with SRT-015, prior to 15 min stimulation with LPS, significantly inhibited accumulation of p-p38 positive cells.

Right panel: Histogram plot demonstrating LPS-induced p-p38 cells accumulation and the inhibitory shift with SRT-015 treatment. IC_{50} results from all donors in section 3.

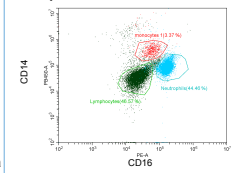


SRT-015 treatment dose-responsively inhibits TNFα in LPS-stimulated whole blood from representative donor. Summary data from all donors below.

3) SRT-015 anti-inflammatory MOA, and downstream target engagement demonstrated by dose-dependent inhibition of LPS-induced TNFα secretion and decreased number of LPS-induced p-p38+ cells in whole blood from nine unique human donors.

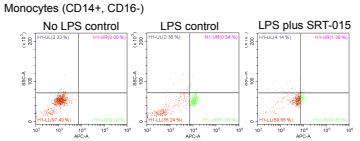
Human Donor Whole Blood	SRT-015 IC_{50} p-p38 inhibition	SRT-015 IC_{50} TNFα inhibition
1	14	9.4
2	5	10.9
3	28	7.6
4	5.5	6.8
5	2.2	7.5
6	2.0	7.5
7	5.4	11.1
9	11.2	6.8
10	4.8	5.4
IC_{50} Mean +/- SEM (uM)	**8.7 +/- 2.6**	**8.1+/- 0.6**

4) SRT-015 strongly inhibits LPS-activated p-p38 monocytes and neutrophils.



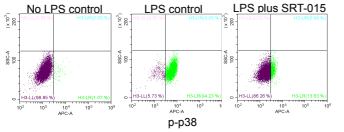
Multiplex analysis using co-staining with anti-CD14, anti-CD16, anti-CD15 and anti-p-p38 antibodies to identify specific immune cell populations as shown in representative samples below.

Monocytes (CD14+, CD16-)



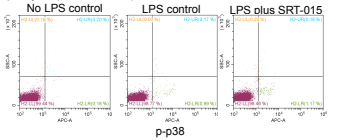
LPS strongly stimulated p-p38+ cells in monocytes (62% positive cells). SRT-015 significantly inhibited effect of LPS in monocytes (23% positive cells @15uM).

Neutrophils (CD15+, CD16+)



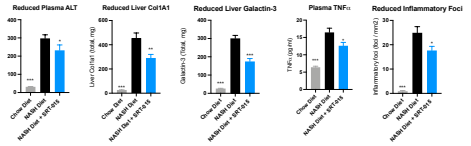
LPS strongly stimulated p-p38+ cells neutrophils (94% positive cells). SRT-015 strongly inhibited effect of LPS in neutrophils (14% positive cells @15uM).

Lymphocytes (CD14-, CD16-)



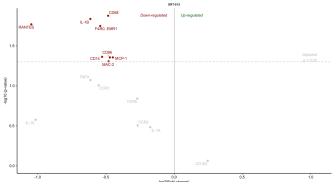
LPS did not stimulate p-p38+ cells in lymphocytes (1% positive cells). SRT-015 did not change effect of LPS in lymphocytes (1% positive cells @15uM).

5) SRT-015 treatment confirmed the in vivo efficacy previously observed in this DIO-NASH model[3], demonstrating anti-inflammatory, anti-fibrotic, and anti-hepatic cell death (section 7) mechanisms.

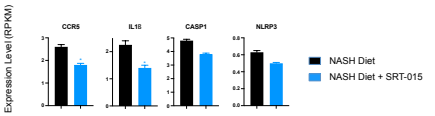


SRT-015 treatment in this therapeutic model significantly decreased liver injury (plasma ALT) and liver fibrosis (Col1A1). Anti-inflammatory activity was confirmed with decreased galectin-3 (inflammatory monocyte marker) and extended with decreased plasma TNFα and inflammatory foci.

6) Anti-inflammatory in vivo effects of SRT-015 in liver was demonstrated by significant down regulation of the monocyte recruitment pathway including IL-1β, CD68, CD86, F4/80, CD14, RANTES, MCP-1, and MAC-2.



7) SRT-015 treatment significantly decreased liver expression of chemokine CCR5 and hepatic cell death factors including members of the inflammasome pathway.



Summary

- In whole blood analysis from human volunteers:
  - LPS induces p-p38 expression and TNFα secretion in monocytes and neutrophils.
  - SRT-015 dose dependently decreases accumulation of p-p38+ cell and inhibits TNFα secretion in these cells.
- In a therapeutic DIO-NASH mouse model SRT-015 treatment:
  - Demonstrated efficacy by significantly reducing liver fibrosis, inflammation and hepatic cell death, all key drivers for NASH.
  - Anti-inflammatory in vivo effects of SRT-015 were demonstrated by significant reduction of:
    - Plasma TNFα, and liver immune cell marker galactin-3, inflammatory foci, pro-inflammatory cytokines, inflammasome and monocyte recruitment factors.

Conclusion

These data demonstrate SRT-015's anti-inflammatory MOA and target engagement in human whole blood assays and anti-inflammatory efficacy in a preclinical therapeutic model.

These data support the continued advancement of SRT-015 as a possible therapeutic for liver diseases including NASH.

References

1. Hattori et al., Cell Communication and Signaling 2009
2. Burge et al., Abstract 106, AASLD Meeting, Nov 4-8, 2022
3. Elias et al., Hepatology. 2020;71:1009A.

Acknowledgements

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Disclosures

All authors are employees of Seal Rock Therapeutics, Inc.