INTRAVENOUS ADMINISTRATION WITH THE ASK1 INHIBITOR SRT-015 ALLEVIATES LIVER INJURY AND SYSTEMIC INFLAMMATION IN DISEASE MODELS OF LIVER FAILURE

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BACKGROUND & AIM

- Acute-on-chronic liver failure (ACLF) is characterized by multiple organ failures in patients with acutely decompensated cirrhosis and is associated with high short-term mortality. The development of ACLF occurs in the setting of systemic inflamation, whose severity increases with the number of organ failures and mortality. ACLF is a complex condition resulting from an uncontrolled activation of the innate immune system associated with an exaggerated systemic oxidative stress due to the translocation of pathogen-associated molecular patterns (PAMPs) from the gut and the release of damage-associated molecular patterns (DAMPs) by dying cells[1].
- SRT-015 is a novel, small molecule inhibitor of the Apoptosis Signal-regulating Kinase 1 (ASK1). ASK1 is a redox-sensitive kinase that is activated by pathological stimuli including oxidative stress, PAMPs and pro-inflammatory cytokines. Previous studies have demonstrated anti-inflammatory and anti-apoptotic effects of SRT-015 *in vitro* in preventive settings, while oral administration of SRT-015 prevents liver injury in various models of liver diseases[2,3,4].

The aim of this study was to evaluate the efficacy of SRT-015 in therapeutic settings (*ie* when added after the pathological induction) using *in vivo* and *in vitro* models.

Therefore, we evaluated

- **the efficacy of intravenous (i.v.) SRT-015 administration in two mechanistic models of liver failure:**
  - **Acetaminophen (APAP) overdose that induces exacerbated oxidative stress and leads to acute liver necrosis;**
  - **D-galactosamine that blocks RNA and protein synthesis, combined with LPS that activates Kupffer cells and macrophages, leading to liver cell apoptosis and necrosis as well as systemic inflammation.**
- **the anti-apoptotic and anti-inflammatory activities of SRT-015 added after oxidative stress or PAMP stimulus in cell-based assays.**

MATERIAL & METHODS

• SRT-015 i.v. administration in APAP mice

C57BL/6 male mice (8-10-week-old) received an intraperitoneal (i.p.) injection of acetaminophen (APAP, 300 mg/kg diluted in 10% Tween-80/90% normal saline) after an overnight fasting. One hour after APAP injection, mice received an intravenous (i.v.) injection of SRT-015 (1 mg/kg) or vehicle (n=7-8/group). Mice were terminated 6h after APAP injection and blood was collected to measure serum alanine (ALT) and aspartate (AST) aminotransferases (Selectra Pro M Lite - Fully Auto Biochemistry Analyzer).

• SRT-015 i.v. administration in GalN/LPS mice

C57BL/6 male mice (8-10-week-old) received an i.p. co-injection of D-galactosamine (GalN, 700 mg/kg) and lipopolysaccharide (LPS E. coli, 25 µg/kg) diluted in phosphate buffered saline (PBS). Mice received intravenous (i.v.) injection of SRT-015 (0.75 mg/kg) or vehicle 0.5h after GalN/LPS injection (n=23-24/group). Mice were terminated 6h after GalN/LPS injection and blood was collected to measure serum ALT and AST using a Daytona plus automate, and cytokines using Luminex technology.

• Effect of SRT-015 in human hepatocytes after H_2O_2-induced stress

Human immortalized hepatocytes (IHH)[5] were stressed with H_2O_2 (300 µM) after 24h starvation. A dose range of SRT-015 (0.1 - 30 µM) was added 15 min after H_2O_2. Caspase 3/7 activity was measured in cell lysates using the Caspase-Glo® 3/7 luminescent assay (Promega) 6h after $H2O_2$ addition.

• Effect of SRT-015 effect in human PBMC after LPS stimulation

Human peripheral blood mononuclear cells (35% $CD3^+$ T cells, 27% $CD4^+$ T cells (helper), 6% $CD8^+$ T cells (cytotoxic), 12% $CD19^+$ B cells, 15% $CD14^+$ monocytes, 4% $CD56^+$ NK cells, TebuBio) were stimulated with LPS (1 ng/ml *Escherichia coli* O111:B4, Sigma Aldrich) 1h after seeding. A dose range of SRT-015 (0.47 – 30 µM) was added 30 min after LPS. TNFα was measured by HTRF (Revvity) in the cell supernatant 6h after LPS stimulation.

• Statistical analyses

Box plots show the median as a line, 25th to the 75th percentile as a box and min and max as whiskers.

For data following a normal distribution:

#: p<0.05; ##: p<0.01; ###: p<0.001 using a two-tailed Student T test

¤: p<0.05; ¤¤: p<0.01; ¤¤¤: p<0.001 using a Dunnett's multiple comparisons test.

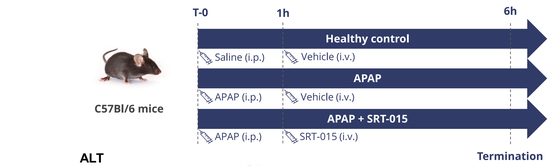
For non-normally distributed variables:

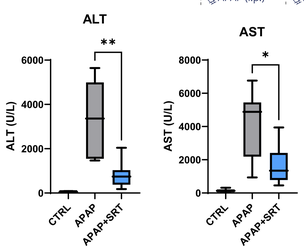
*: p<0.05; **: p<0.01; ***: p<0.001 using a two-tailed Mann-Whitney test

§: p<0.05; §§: p<0.01; §§§: p<0.001 using Dunn's multiple comparisons test.

RESULTS

INTRAVENOUS ADMINISTRATION OF SRT-015 AFTER APAP INJECTION ALLEVIATES LIVER INJURY

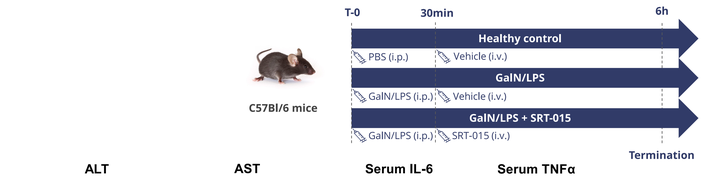


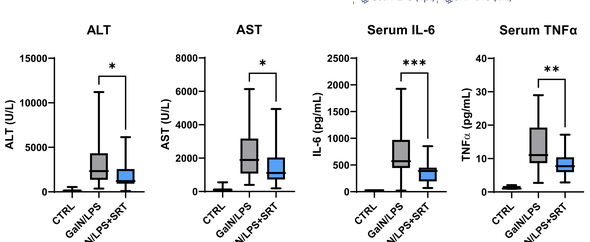


ALT and AST were measured in sera of mice 6h after APAP or Tween-80/saline intraperitoneal (i.p.) injection. Mice received an intravenous (i.v.) injection of SRT-015 or vehicle 1h after APAP injection.

Mice that received intravenous injection of SRT-015 after APAP injection had a significant decrease in ALT and AST levels compared to the vehicle group.

INTRAVENOUS ADMINISTRATION OF SRT-015 AFTER GalN/LPS INJECTION ALLEVIATES LIVER INJURY AND SYSTEMIC INFLAMMATION

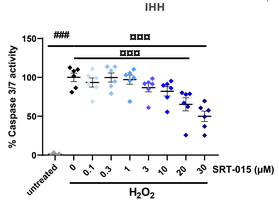




ALT, AST, IL-6 and TNFα were measured in sera of mice 6h after GalN/LPS or PBS intraperitoneal (i.p.) injection. Mice received an intravenous (i.v.) injection of SRT-015 or vehicle 30 min after GalN/LPS injection.

Mice that received intravenous injection of SRT-015 after GalN/LPS challenge had significant decreases in ALT and AST levels, as well as decreased circulatory pro-inflammatory cytokines compared to the vehicle group.

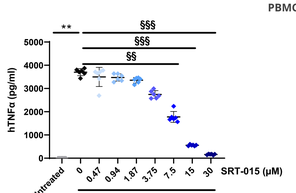
ADDITION OF SRT-015 AFTER H_2O_2 CHALLENGE REDUCES OXIDATIVE STRESS-INDUCED APOPTOSIS IN HEPATOCYTES



Caspase 3/7 activity, a surrogate marker of apoptosis, was measured in cell lysates 6h after H_2O_2 addition. SRT-015 was added 15 min after H_2O_2 stress. Relative luminescence unit (RLU) values were expressed as the percentage of the condition with H_2O_2 without SRT-015.

SRT-015 addition after H_2O_2 stress reduced caspase 3/7 activity in a dose-dependent manner, with and Emax of 50% at the highest dose.

SRT-015 INHIBITS CYTOKINE SECRETION IN HUMAN PBMC, EVEN WHEN ADDED AFTER LPS STIMULUS



TNFα was measured in cell supernatants 6h after LPS stimulation. SRT-015 was added 30 min after LPS.

SRT-015 addition after LPS stimulation reduced TNFa secretion from human PBMC.

This effect was dose-dependent with an IC50 of 7.8 µM and Emax at 96% at the highest dose.

CONCLUSION

- **These results show that iv administration of SRT-015 alleviates liver injury and counteracts systemic inflammation in two models of acute liver failure (ALF), hence demonstrating the therapeutic potential of SRT-015 in ALF and ACLF.**
- **These therapeutic effects might result from both protective effects on hepatocytes (apoptosis) and anti-inflammatory effects on immune cells (cytokine release from PBMC).**
- **These data support the potential of SRT-015 for the treatment of patients with ALF and ACLF.**

REFERENCES

1. Arroyo et al, J Hepatol 2015;62(1 Suppl):S131-43
2. Plonowski et al, Hepatology 2020;72, 1031A
3. Elias et al, Hepatology 2022;76, 5635
4. Elias et al, J Hepatol 2023;78, S139
5. Schippers et al., Cell Biol Toxicol. 1997;13(4-5):375-386

DISCLOSURE

VL, MM, SD, PP, VD, DH, SSJ are employees and stock shareholder of GENFIT S.A.; RH and JC are consultant for GENFIT S.A.; BS is scientific adviser of GENFIT S.A.

We thank the Wetlab team, in particular Philippe Delataille, Perrine Dubois, Morgane Lenne, Elodie Stievenard , Manon Clarisse, Virginie Galoseau and Nathanaël Salty and the Communication team from GENFIT SA, as well as Sai Life Sciences ltd. and Seal Rock Therapeutics, Inc. for their contribution to this work.