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.

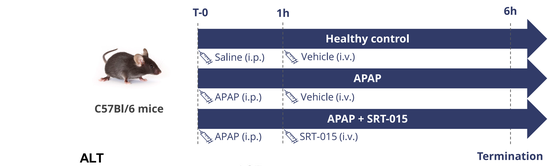
For non-normally distributed variables:

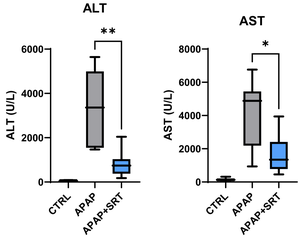
*: p<0.05; **: p<0.01; ***: p<0.001 using a two-tailed Mann-Whitney test

§: 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

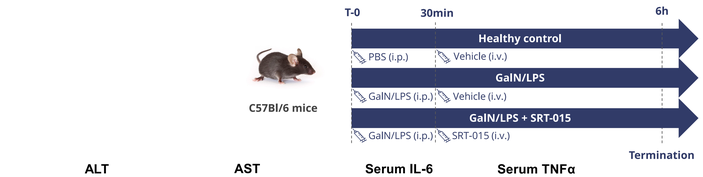


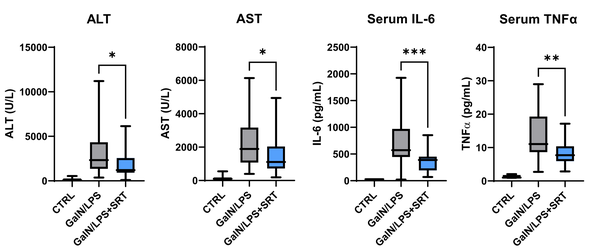


ALT and AST were measured in sera of mice 6h after APAP or Tween-80/saline intraperitoneal (i.p.) injection. Mice received an intravenous (i.v.) injection of SRT-015 or vehicle 1h after APAP injection.

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 GaIN/LPS INJECTION ALLEVIATES LIVER INJURY AND SYSTEMIC INFLAMMATION

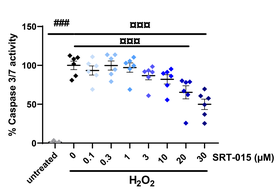




ALT, AST, IL-6 and TNFα were measured in sera of mice 6h after GalN/LPS or PBS intraperitoneal (i.p.) injection. Mice received an intravenous (i.v.) injection of SRT-015 or vehicle 30 min after GalN/LPS injection.

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.

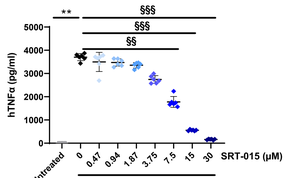
ADDITION OF SRT-015 AFTER H_2O_2 CHALLENGE REDUCES OXIDATIVE STRESS-INDUCED APOPTOSIS IN HEPATOCYTES



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.**

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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.

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