EFFICACY OF THE APOPTOSIS-SIGNAL-REGULATING KINASE 1 (ASK1) INHIBITOR SRT-015 IN *IN VIVO* AND *IN VITRO* PATHOGEN-ASSOCIATED MOLECULAR PATTERNS (PAMPS)-INDUCED DISEASE MODELS

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BACKGROUND & AIM

- Patients with liver cirrhosis have an increased risk of infection and are at high risk of death from sepsis due to severe immune dysfunction, impaired liver function and altered gut microbiota and permeability[1]
- In these patients, immune dysfunction also results in a hyperinflammatory condition in the systemic circulation, as shown by elevation of circulating pro-inflammatory cytokines such as TNFα, IL-6 and IL-1β, which plays a major role in the progression to acute decompensation and acute-on-chronic liver failure (ACLF)[2]
- Apoptosis-signal-regulating kinase 1 (ASK1) is a key mediator of the inflammatory response, activated by reactive oxygen species produced after recognition of pathogen-associated molecular patterns (PAMPs) by toll-like receptor (TLR) family members. Its phosphorylation results in activation of c-Jun N-terminal kinase (JNK) and p38 that regulate cell death and cytokine production[3]

The aim of this study was to investigate the effects of SRT-015, a new investigational drug inhibiting ASK1[4,5]

- **to counteract polymicrobial sepsis in a rodent model**
- **on overt activation of human blood immune cells by a variety of PAMPs**

METHODS

Evaluation of SRT-015 in sepsis mice

- Sepsis was induced through cecal ligation and puncture (CLP) surgery in C57BL/6 male mice (ArtImmune, France). Briefly, under anesthesia, an abdominal incision was performed, and the caecum was tightly ligated at half the distance between distal pole and the base of the caecum. The caecum was punctured once through-and-through and replaced in its original position within the abdomen, which was closed with sutures and wound clips. SRT-015 (10 mg/kg BID) or vehicle was orally administered 30 min before and 5h30 after CLP, then BID for 6 days (n=20/group). Survival was monitored over 7 days

Evaluation of SRT-015 in human blood cells

- Blood from healthy volunteers was collected in lithium heparin tubes at the *Établissement Français du Sang* (EFS, France) and stored at room temperature until use (<20 hours). Blood from several donors of the same blood group genotype were pooled to increase volume and test multiple conditions (Table 1). Whole blood was incubated 4 hours with different TLR agonists (Human TLR agonists, InvivoGen, Table 2) in presence of a dose range of SRT-015 (0 – 1.9 – 3.8 – 7.5 – 15 µM). In a first design, SRT-015 and TLR agonists were added concomitantly (Exp1 and Exp2). In a second design, SRT-015 was added 30 min after TLR agonists (Exp3). Secretion of inflammatory cytokines TNFα, IL-6 and IL-1β was determined by measuring their concentration in the whole blood using ProcartaPlex immunoassay for Luminex (Thermo Fisher Scientific)

Table 1: Description of the human whole blood assays

Experiment	Number of blood donors	Blood group genotype	Condition tested
Exp1	3	O+	Dose range of SRT-015 concomitant to TLR agonists
Exp2	4	A+	Dose range of SRT-015 concomitant to TLR agonists
Exp3	3	O+	SRT-015 (15 µM) added 30 min after TLR agonists

Table 2: List of the TLR agonists used for the *ex vivo* studies

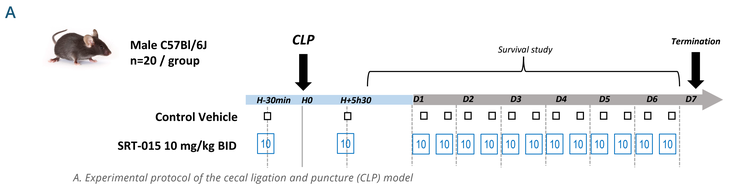
TLR	Agonist	Nature	Final concentration
TLR2	PAM3CSK4	Synthetic triacylated lipopeptide (LP), a bacterial cell wall component in Gram+ and Gram	1 µg/mL
TLR4	LPS EK	Lipopolysaccharide from Escherichia coli K12	10 µg/mL
TLR5	FLA-ST	Flagellin from Salmonella typhimurium	1 µg/mL

STATISTICS

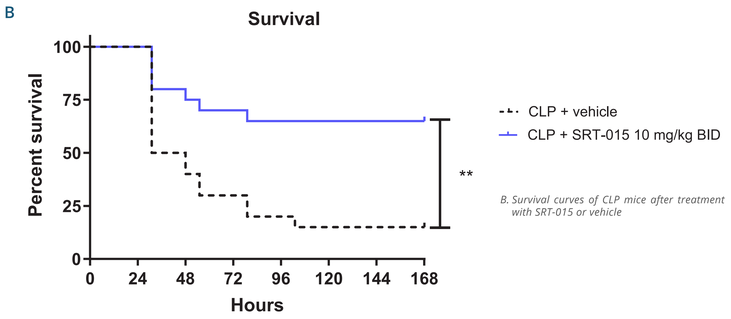
- Sepsis model: survival curves were compared using Gehan-Breslow-Wilcoxon test (**p<0.01)
- Whole blood assay: in dose response study, each dose of SRT-015 was compared to the control condition (vehicle (DMSO) in presence of TLR agonist) using Kruskal-Wallis test with Dunn's multiple comparisons test (*p<0.05, **p<0.01, ***p<0.001). When SRT-015 was added after the TLR agonists (Exp3), SRT-015 was compared to the control condition (vehicle in presence of TLR agonist) using one-tailed Mann-Whitney test (# p<0.05, ## p<0.01, ### p<0.001)

RESULTS

SRT-015 IMPROVES SURVIVAL OF MICE WITH SEPSIS



A. Experimental protocol of the cecal ligation and puncture (CLP) model



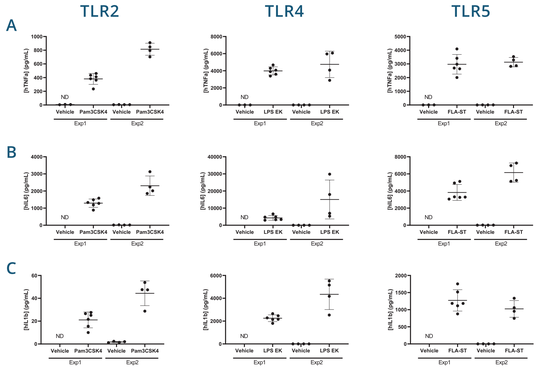
B. Survival curves of CLP mice after treatment with SRT-015 or vehicle

→ In CLP-induced sepsis mice, SRT-015 administration significantly improved the survival curves, with 65% surviving mice in SRT-treated mice *vs* 15% of mice in the control group, 7 days post-CLP (p=0.004)

→ Although its mechanism of action in this model remains to be explored, SRT-015 may likely act by inhibiting PAMPs-induction of reactive oxygen species and activation of ASK1, thereby alleviating cytokine storm and organ dysfunction in sepsis

SRT-015 REDUCES PRO-INFLAMMATORY CYTOKINE RELEASE INDUCED BY TLR ACTIVATION OF HUMAN WHOLE BLOOD

EFFECT OF *EX VIVO* TLR ACTIVATION ON CYTOKINE PRODUCTION IN HUMAN WHOLE BLOOD

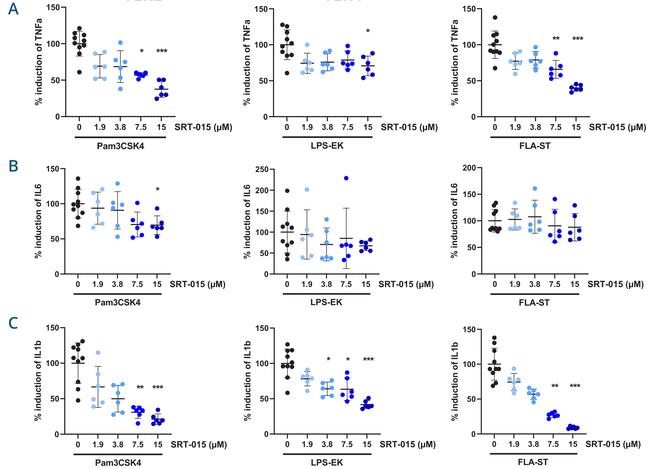


A) TNFα, B) IL-6, C) IL-1β secretion 4 hours after TLR2 (Pam3CSK4), TLR4 (LPS-EK) and TLR5 (FLA-ST) stimulation in human whole blood assays (Exp1 and Exp2). ND: not detected

→ Without TLR activation (vehicle), cytokines levels were almost not detectable in blood from healthy volunteers in these experimental conditions

→ *Ex vivo* TLR activation in this whole blood assay induces pro-inflammatory cytokines secretion in a reproducible manner

DOSE RESPONSE OF SRT-015 IN HUMAN WHOLE BLOOD AFTER TLRS ACTIVATION



*Effect of a dose range of SRT-015 on A) TNFα, B) IL-6 and C) IL-1β secretion 4 hours after TLR2 (Pam3CSK4), TLR4 (LPS-EK) and TLR5 (FLA-ST) stimulation in human whole blood assays (Exp1 and Exp2). *p value compared to the control condition (vehicle + TLR agonist)*

→ SRT-015 dose-dependently inhibits TNFα, IL-6 and IL-1β secretion in response to TLR2, TLR4 and TLR5 activation

EFFECT OF SRT-015 ON CYTOKINE SECRETION WHEN ADDED AFTER TLR ACTIVATION IN HUMAN WHOLE BLOOD

Table 3: % inhibition of cytokines secretion by SRT-015 (15 µM) added concomitantly or after TLR agonists (Exp3)

	SRT-015 treatment	TLR2	TLR4	TLR5
TNFα	Concomitant	62%***	29%*	60%***
	30 min after agonist	54%*	11%	34%*
IL-6	Concomitant	30%*	32%	12%
	30 min after agonist	23%*	14%	20%
IL-1β	Concomitant	79%***	58%***	91%***
	30 min after agonist	76%*	65%*	76%*

**p value compared to the control using Dunn's test*
 # p value compared to the control using Mann-Whitney test

→ SRT-015 also reduced 4-hour cytokines secretion when added 30 minutes after the TLR agonists

CONCLUSION

- **Although further experiments are needed to fully understand how SRT-015 improves survival in septic mice, one potential mechanism may be by regulation of the innate immune response**
- **These results further support the development of SRT-015 in advanced liver disease and ACLF**

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