ID: 2777667

Novel Oxysterol Sulfates Alleviate Injured Liver Function and Decrease Mortality in Mouse Models

Shunlin Ren, Yanxia Ning, Jin Kyung Kim, Hae-Ki Min, James Brown*, and WeiQi Lin*

Department of Internal Medicine, Virginia Commonwealth University/McGuire VA Medical Center, Richmond, VA 23249

***Durect Corporation, Cupertino, CA 95014**

Introduction

: Acute liver failure (ALF) is one of the most dramatic and devastating diseases. ALF often results in severe hepatocyte injury and apoptosis, leading to massive necrosis in the liver and the sudden death of otherwise healthy individuals. Endotoxin- and drug-induced liver injury and viral hepatitis account for most cases of ALF, while severe lipopolysaccharide (LPS)- and acetaminophen (ATMP)-induced hepatotoxicity as the most frequent causes of ALF in the United States, and the leading causes of death in non-coronary intensive care unit (ICU) patients in the world. Currently, treatment options for ALF are extremely limited, and in severe cases, liver transplantation is the only treatment option available. Therefore, there is a dire need for the development of effective therapeutic strategies for ALF. Novel oxysterol sulfates, 25-hydroxycholesterol 3-sulfate (25HC3S) and 25-hydroxycholesterol 3, 25-disulfate (25HCDS), have been demonstrated to be potent regulators of lipid metabolism, inflammatory response, cell apoptosis, and cell survival.

Aim

: In the present study, we tested these products' potential to treat LPS- or ATMP-induced acute liver failure in a mouse model.

Methods:

Acute liver failure mouse model was established by intravenous injection with LPS or ATMP. The injured liver function was treated with intraperitoneal administration of 25HC, 25HC3S or 25HCDS. Serum enzymatic activities were determined in our clinic laboratory. ELISA assays were used to detect pro-inflammatory factor levels in sera. Western blot, Real-time Quantitative PCR and RT^2 Profiler PCR Array analysis were used to determine levels of gene expression.

Results:

Administration of 25HC3S/25HCDS decreased serum liver-impaired markers; significantly reduced of cytokines and inflammatory cell infiltration in the tissues, and alleviated liver, lung, and kidney injury. Subsequently, the administration increased the survival rate in the LPS- or ATMP-induced mouse model, only 10% of the animals survived in 96 hours without 25HC3S versus 90% survival with the 25HC3S. These effects resulted from the inhibition of the expression of genes involved in the pro-inflammatory response and apoptosis and the simultaneous induction of the expression of genes involved in cell survival. Compared to 25HC, 25HC3S and 25HCDS exhibited significantly stronger effects in these activities, indicating that the cholesterol metabolites play an important role in the inflammatory response, cell apoptosis, and cell survival in vivo.

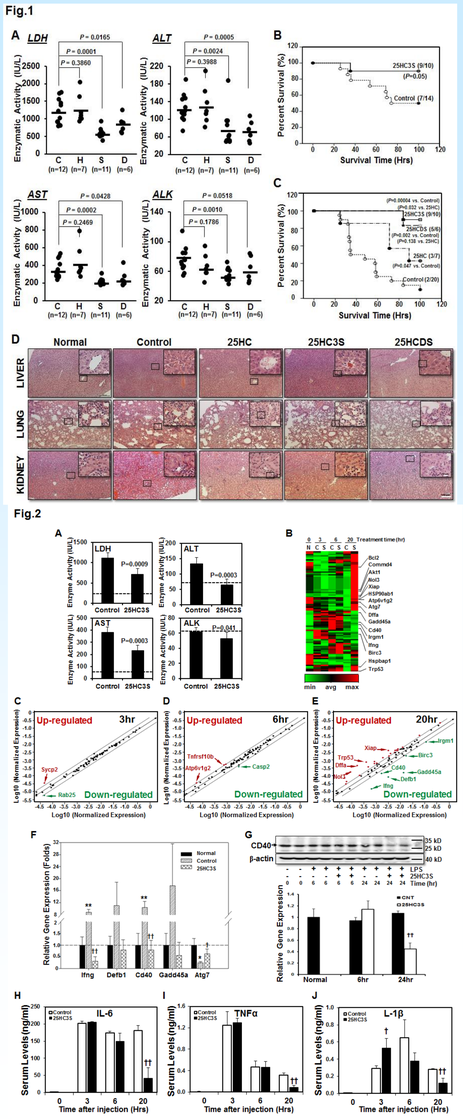
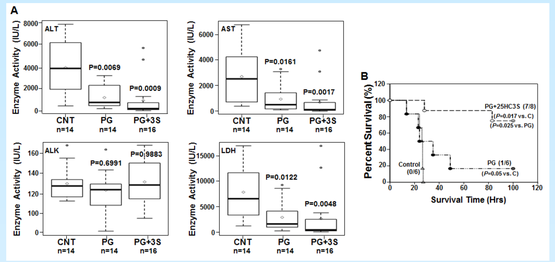


Fig.1. 25HC3S or 25HCDS treatment improves organ function and survival rates in lipopolysaccharide (LPS)-induced endotoxemic mice. **Panel A**: Total 36 21-week-old male C57BL/6J mice were randomly divided into four groups (CNT, n = 12; H, n = 7; S, n = 11 and D, n = 6), received vehicle or 50mg/kg 25HC or 25HC3S or 25HCDS via intraperitoneal. Two hours later, all mice were intravenously injected with 4mg/kg LPS. Twenty four hours following LPS injection, serum was collected and activities of LDH, ALT, AST and ALK were determined in clinical laboratory. Compared to the control group, serum LDH, ALK, AST and ALK levels were decreased in 25HC3S and 25HCDS pretreated mice. Each point represents an individual mouse and data are pooled from three independent experiments. Solid bar shows the average value of each group. *P* value is shown in each part. C: represents vehicle treated control mice; H, 25HC pretreated mice; S, 25HC3S pretreated mice; D, 25HCDS pretreated mice. **Panel B**: 12-week-old male C57BL/6J mice were administered either vehicle (control, n = 14) or 25HC3S (50 mg/kg; n = 10); **Panel C**: 22-week-old male C57BL/6J mice were administered vehicle (control, n = 20), 25HC (40 mg/kg; n = 7), 25HC3S (50 mg/kg; n = 10) or 25HCDS (50 mg/kg; n = 6) as indicated, two hours before LPS (5 mg/kg) challenge. Mouse survival was observed and recorded up to 100 hours. *P* value versus Control group is shown in each part. **Panel D**: 22-week-old male C57BL/6J mice were administered vehicle (control, n = 4), 25HC (40 mg/kg; n = 4), 25HC3S (50 mg/kg; n = 4) or 25HCDS (50 mg/kg; n = 4) as indicated, two hours before LPS (5 mg/kg) challenge. The liver, lung and kidney tissue were harvested after 24 hr following LPS injection for morphological study. Tissue from age-matched mouse without any treatment were used as normal control (Normal, n = 4). The paraffin-embedded tissue sections were stained using H&E method and photographed for evaluation. Representative photos are shown at x100 magnification (bar = 100 μm). Inserts are shown at x400 magnification of the boxed areas (bar = 10 μm). Normal represents normal mice without any treatment (n=4); Control, vehicle-treated control mice; 25HC, 25HC-pretreated mice; 25HC3S, 25HC3S-pretreated mice; 25HCDS, 25HCDS-pretreated mice.

Fig.2. 25HC3S treatment regulates hepatic apoptosis-related gene expression and suppresses secretion of pro-inflammatory factors in a time-dependent manner in LPS-induced endotoxemic mice. 22-week-old male C57BL/6J mice were pretreated with vehicle or 25HC3S two hours before LPS (4 mg/kg) administration. Mice without any treatment were used as a normal control. Each group contained four mice. Liver tissues and serum were collected and harvested at the indicated times (3, 6 and 20 hr) following LPS injection. The activities of LDH, ALT, AST and ALK were determined in clinical laboratory. Total mRNAs from liver tissue were extracted and gene expressions were determined by the RT2 Profiler PCR Array and Q-PCR assay. Hepatic CD40 expression level was detected by Western blot. Meanwhile, the serum levels of IL-6, TNFα and IL-1β were determined by a quantitative ELISA kit. **Panel A** shows serum enzymatic activity at 20 hr following LPS injection; dashed line shows the normal level. **Panel B** shows results of Clustergram analysis: the gene expression profile at different time points following LPS administration; **Panels C**, **D**, and **E**, scatter plot analysis: gene expressions with a greater than 2-fold change at 3, 6 and 20 hr, respectively, following LPS administration. **Panel F**, Q-PCR analysis of hepatic Ifng, Defb1, CD40, Gadd45a and Atg7 mRNA expression at 20 hr following LPS stimulation (mean ± S.D., n=4); **Panel G**, Western blot assay of hepatic CD40 protein expression at the indicated times after LPS stimulation. Upper panel: representative bands of CD40 expression; Lower panel: densitometric analysis from 4 samples of each group (mean ± S.D.). β-Actin was used as the loading control. **Panels H**, **I**, and **J**, ELISA analysis of pro-inflammatory cytokines IL-6, TNFα, and IL-1β, respectively, in mouse sera. The data are presented as the mean ± S.D., n = 4. N or Normal: Normal 22-week-old male mice without any treatment; C or control: control mice receiving vehicle; S or 25HC3S: 25HC3S-pretreated mice. *, $P < 0.05$; **, $P < 0.01$ vs. Normal group; †, $P < 0.05$; ††, $P < 0.01$ vs. control group.



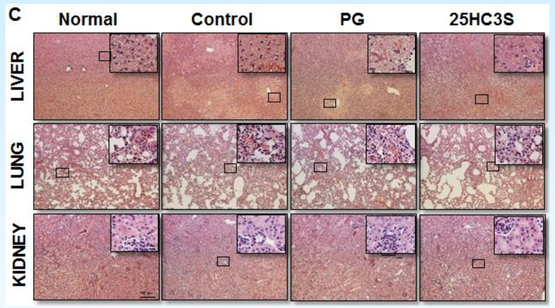


Fig. 3. 25HC3S treatment improves organ function and survival rates in ATMP overdose mice. **Panel A**: Serum activities of LDH, ALT, AST and ALK were determined by a clinical laboratory after 350 mg/kg ATMP injection for 24 hr. CNT: represents control mice with ATMP injection only; PG: represents vehicle with PG treated control mice; 3S, 25HC3S treated mice. Each point represents an individual mouse and data are pooled from three independent experiments. Solid bar shows the average value of each group. **Panel B**: 12-week-old female C57BL/6J mice were administered either with control (n = 6), vehicle (n = 6) or 25HC3S (n = 8) 2 hr before ATMP (600 mg/kg) treatment. Mouse survival was observed and recorded up to 100 hours. **Panel C**: Morphological study of liver, lung, and kidney tissues. The representative sections are shown at x 100 magnification (bar = 100 μm). Inserts are shown at x 400 magnification of the boxed areas (bar = 10 μm). Normal represents normal mice without any treatment (n=3); Control, control mice; PG, vehicle with PG pretreated mice; 25HC3S, 25HC3S-pretreated mice.

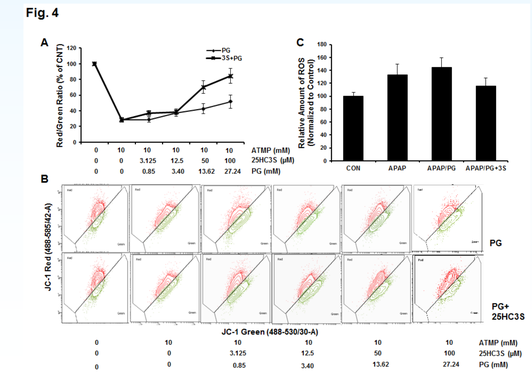


Fig. 4 25HC3S restores the mitochondria membrane potential destroyed by ATMP in a dose-dependent manner in Huh7 cells. Huh7 cells were seeded in 60mm dishes at 6.5x10^5/dish and cultured overnight before different dosage of 25HC3S (3.125, 12.5, 50,100 μM in PG) were added, the relative amount PG were added as control (0.85, 3.40, 13.62, and 27.24 mM). Two hours later, cells were treated with10mM ATMP for 24 hours and harvested for mitochondria membrane potential (MMP) assay. Cells without any treatment were used as control. MMP was analyzed by JC-1 staining on flow cytometer using 488nm excitation with 530/30 nm (Green) and 585/42 nm (Red) band-pass emission filters. MMP was indicated by red/green fluorescence ratio. Data were represent the mean ± SD for three independent experiments and presented as percentage of control.

Conclusions: 25HC3S/25HCDS have potential to serve as novel biomedicines in the therapy of acute liver failure and acute multiple organ failure

Conflicts of Interest: The study is supported by pending patent applications and granted patents; and has also been supported and licensed by DURECT Corporation, Cupertino, CA. S.R.'s significant financial interest in the sponsor of this research, DURECT Corporation, in the form of stock ownership was divested on 4/15/2015.