ROLE OF THE beta-D-ENDOGLUCURONIDASE HEPARANASE IN SEPTIC CARDIOMYOPATHY

Role of the β-D-endoglucuronidase heparanase in septic cardiomyopathy

Background: Septic cardiomyopathy (SC) is frequently recognized in patients with septic shock and indicates a worse prognosis. Disturbed calcium homeostasis characterizes the pathophysiology of SC. Heparanase is an endogenous immune modulator, however the role of heparanase in SC is fully unknown.

Objective: To evaluate (i) activity of heparanase in patients with septic shock, (ii) role of heparanase gene single nucleotide polymorphisms (SNPs) on heparanase activity, (iii) role of heparanase in the induction of SC, and (iv) therapeutic effects of heparanase inhibition on SC in mice.

Methods: (i) After permission (EK:206_09), serum heparanase activity was measured in patients with septic shock (n=20) and after abdominal surgery (n=9) at day 1 (D1) and 2 (D2) after onset/surgery. (ii) SNPs (rs4693608; rs4364254) were determined in these patients. (iii) Murine cardiomyocytes (HL-1 cells) were stimulated with heparanase (15mU/ml) for 24h. After permission (70/7348), C57BL/6J-mice (n=8 per group) were challenged with heparanase (1U, *i.v.*), lipopolysaccharide (LPS) and peptidoglycan (PepG) (6mg/kg, 1mg/kg; *i.p.*) or vehicle (150µl saline). 1h after LPS/PepG-challenge mice were treated with a specific heparanase inhibitor (N-desulfated re-N-acetylated heparin; 500µg; *i.v.*) or vehicle. Serum heparanase activity and ejection fraction (EF) was quantified 24h later and signaling events on calcium hemostasis in were investigated by immunoblot in HL-1 cells and heart tissue. Statistics: 1-way or 2-way-ANOVA and Bonferroni test (significance $p<0.05$).

Results: Heparanase activity at D1 and D2 was significantly higher in patients with septic shock compared to surgical control patients. In patients with heparanase genotypes CT-GG, TT-GG, CT-AG or CT-AA (n=11), heparanase activity significantly decreased at D2 compared to D1. Patients

with heparanase genotypes TT-AA or TT-AG (n=9) showed no significant difference in heparanase activity between D1 and D2. Stimulation of HL-1 cells with heparanase significantly reduced the expression of sarcoplasmic calcium ATPase (SERCA2) and phosphorylation of the SERCA2-modulator phospholamban. When compared to vehicle-challenged animals, heparanase or LPS/PepG-challenge resulted in a significantly reduced EF, a significantly elevated heparanase activity, and a significant reduction of cardiac SERCA2 expression and phospholamban phosphorylation. Inhibition of heparanase subsequent to LPS/PepG-challenge resulted in a significantly attenuated reduction of EF, a significantly attenuated elevation of heparanase activity, and a significantly attenuated reduction of SERCA2-expression and phospholamban phosphorylation compared to untreated animals.

Conclusions: Heparanase activity is elevated during septic shock in a genotype-dependent manner. The therapeutic inhibition of heparanase attenuates SC. Thus, heparanase may serve as a potential therapeutic target to treat SC in men.

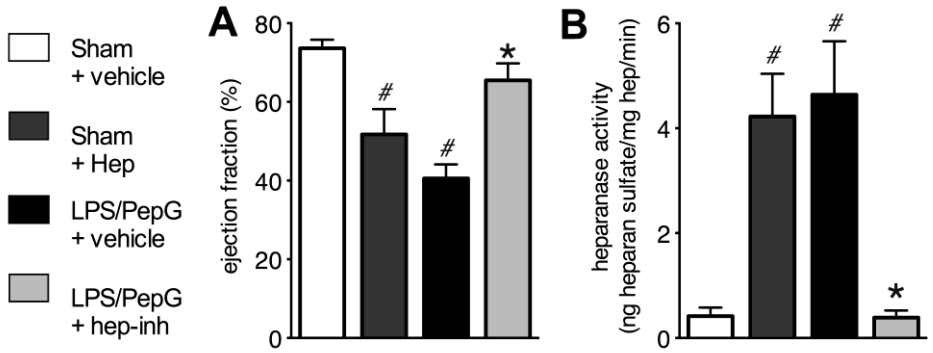

Fig 1 (A) percentage ejection fraction, (B) serum heparanase activity. Means ± SD for n = 8 per group observations. $^{\#}P < 0.05$ vs sham + vehicle; $^{*}P < 0.05$ vs LPS/PepG + vehicle. Hep = heparanase, hep-inh = heparanase inhibitor.