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AGGRESSIVE FLUID RESUSCITATION IN SEPSIS TO RESTORE A NORMODYNAMIC STATE: ECHOCARDIOGRAPHY IN A MURINE MODEL.

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Introduction: Early fluid resuscitation and antibiotic administration are critical for treatment of sepsis. Hypothesis: We evaluated the impact of three different early resuscitation regimens on cardiac performance in a murine model of sepsis.

Methods: 3 groups of 10 C57Bl/6 mice were made septic byecal ligation and puncture (CLP); 5 controls had sham ligation. After CLP animals received antibiotics (ceftriaxone 30mg/kg and clindamycin 25mg/kg q6h) and 1 of 3 fluid regimens: 35mL/kg NaCl 0.9% bolus intraoperatively only (Op Only), 35mL/kg bolus after surgery and then q6h, (Partial) and 100mL/kg after surgery and then q6h, (Full). Echocardiography was done every 3 hours for 36 hours, then every 12, using a high-resolution, 30Mhz scan-head: for each study mice were anesthetized briefly with isoflurane. Stroke Volume (SV, mL) was assessed by Doppler, fractional shortening (FS%) by short axis M-mode and Cardiac Output (CO, mL/min) calculated as SV*HR. Results: CO decreased early, even with Full resuscitation (from 2625±1855mL/min, P<0.05), and decreased even more without it (from 212±2 to 122±2 (Op Only), 24±2 to 14±5 (Partial), p<0.05). SV followed the same trend (58±7 to 34±8mL (Full), 56±6 to 22±4 (Op Only), 51±6 to 28±4 (Partial), P<0.05 vs baseline and sham for all). Full resuscitation increased CO and SV at 12hours (to 212±8 and 44±12 and normalized them at 24hours (CO 25±8, SV 5±13, P=NS from baseline. In underresuscitated animals, SV and CO were still decreased at 24hours (Op Only, CO 15±10, SV 31±14, P=0.05 vs Full for both, Partial CO 16±9, SV 43±9, P=0.02, and 0.10 vs Full) and did not normalize even in animals surviving 72 hours. HR did not change significantly and was similar among the groups. FS was unchanged as well. Conclusions: Early and aggressive resuscitation is mandatory to restore a normodynamic state in sepsis. In this murine model, which replicates clinical sepsis, early underresuscitation can lead to a sustained hypodynamic state persisting over 72 hours.

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THE DUFFY ANTIGEN RECEPTOR FOR CHEMOKINES (DARC) IN ACUTE POST-ISCHEMIC RENAL FAILURE (APRF) - A CONDUCTOR OF RENAL CHEMOKINE PRESENTATION.

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Introduction: Both neutrophils (PMN) and chemokines, such as macrophage inflammatory protein 2 (MIP-2), are considered crucial in the development of APRF [1]. Although DARC, a so-called ‘alternative’ chemokine receptor, has long been known, its exact role in vivo continues to be perplexing [2].

Hypothesis: DARC modulates post-ischemic renal PMN recruitment and thereby the development of APRF. Methods: We compared wild-type (WT) and DARC gene-deficient (DARC−/−) mice in a PMN-dependent model of APRF [3]. At 24 and 48h after renal ischemia-reperfusion (IRI), blood samples were taken, and both kidneys were removed. Further analyses included: plasma creatinine concentrations (Crea, indicator of renal function), renal myeloperoxidase activities (MPO, indicator of renal PMN content), DARC-specific RT-PCR, renal MIP-2 concentrations, and immunostaining for renal MIP-2 expression. Sham-operated mice served as controls. Statistical analyses included ANOVA, Student-Newman-Keuls test, and two-sided Fisher’s exact test. Data are given as mean±SEM (n=6-8).

Results: Following IRI, WT developed severe APRF (at 48h: 40% mortality, Crea 1.23±0.24 mg/dl) and extensive renal PMN recruitment, accompanied by a strong up-regulation of DARC mRNA expression. DARC−/− showed no renal dysfunction (at 48h: 0% mortality, Crea 0.15±0.04 mg/dl, both p<0.05 vs. WT) and no post-ischemic PMN infiltrations. Total renal MIP-2 concentrations were only mildly different between WT and DARC−/− (at 24h: 68.8±17.0 pmol/g vs 55.7±2.9 pmol/g, respectively). But as opposed to WT, immunostaining failed to detect any MIP-2 staining in endothelial cells of DARC−/− after IRI, making MIP-2 unlikely to be presented to rolling PMN in DARC−/−. Conclusions: We propose that DARC mainly exerts its drastic effects by controlling spatial chemokine distribution within the kidney, which in turn regulates PMN recruitment and subsequent APRF. References: 1.) J Mol Med 2004; 82:91-101 2.) Blood 2002; 100:3853-60 3.) Crit Care Med 2000; 28:2507-14

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EFFECT OF CYTOKINE TYROSINE KINASE INHIBITOR, PP1, ON THE CYTOKINE RESPONSE INDUCED BY LIPOPOLYSACCHARIDE.

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Introduction: Lipopolysaccharide (LPS) stimulates inflammation and cytokine production. LPS actions on intracellular signaling result in cytokine synthesis and are poorly understood, but phosphorylation plays a key role. Src tyrosine kinases with PP1 attenuates the cytokine response to LPS and improves survival in a murine model of sepsis.

Methods: Sprague-Dawley rats (14-18wk) were operated mice served as controls. Statistical analyses included ANOVA, Student-Newman-Keuls test, and two-sided Fisher’s exact test. Data are given as mean±SEM (n=6-8).

Results: Following IRI, WT developed severe APRF (at 48h: 40% mortality, Crea 1.23±0.24 mg/dl) and extensive renal PMN recruitment, accompanied by a strong up-regulation of DARC mRNA expression. DARC−/− showed no renal dysfunction (at 48h: 0% mortality, Crea 0.15±0.04 mg/dl, both p<0.05 vs. WT) and no post-ischemic PMN infiltrations. Total renal MIP-2 concentrations were only mildly different between WT and DARC−/− (at 24h: 68.8±17.0 pmol/g vs 55.7±2.9 pmol/g, respectively). But as opposed to WT, immunostaining failed to detect any MIP-2 staining in endothelial cells of DARC−/− after IRI, making MIP-2 unlikely to be presented to rolling PMN in DARC−/−. Conclusions: We propose that DARC mainly exerts its drastic effects by controlling spatial chemokine distribution within the kidney, which in turn regulates PMN recruitment and subsequent APRF. References: 1.) J Mol Med 2004; 82:91-101 2.) Blood 2002; 100:3853-60 3.) Crit Care Med 2000; 28:2507-14

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NELFINAVIR BLOCKS E. COLI INDUCED HUMAN LYMPHOCYTE INDUCED APOPTOSIS.

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Introduction: The anti-retroviral protease inhibitor, Nelfinavir (NFV) has been shown to decrease apoptosis and improve survival in a murine model of sepsis. No studies, though, have been done to evaluate this effect in human T cells. Hypothesis: NFV will prevent bacterial induced human lymphocyte apoptosis in vitro. Methods: Human peripheral blood mononuclear cells were obtained from three healthy human volunteers by Ficol® gradient density separation. 1.8x10⁶ Cells were plated in 0.02 µm Anopore® transwells with E. Coli (4x10⁶ CFU) in log phase growth. NFV or the ethanolic diluent was added in dose dependent concentrations of 1, 2, and 5 µM to separate trans-wells and the cells were incubated overnight. Each sample was done in duplicate. After being labeled with human anti-CD3, percent apoptosis in these lymphocytes was evaluated by flow cytometry for active caspase 3 and TUNEL. Results: NFV causes a dose dependent decrease in E. Coli induced apoptosis. Conclusions: NFV at high dose completely prevents human lymphocyte death in dose dependent concentrations. Anti-retroviral PI may represent a novel therapy to prevent lymphocyte death in sepsis.