ROLE OF MTOR INHIBITOR IN A MURINE MODEL OF LPS-INDUCED ACUTE KIDNEY INJURY

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Introduction and Aims: Sepsis remains a serious cause of morbidity and mortality without an effective pharmacotherapy. Among the several disorders encountered in sepsis, Acute Kidney Injury (AKI) is characterized by endothelial and tubular dysfunction. The aim of this study is to evaluate the effects of Rapamycin (Rp) in this setting.

Methods: C57BL/6 mice were randomized into following groups: Control (CTR, PBS infusion i.p.), Septic (LPS, 10mg/Kg i.p.), Rp (Rp, 5mg/Kg i.p.) and Rp/LPS groups. After 24h from infusion, renal tissue sections were evaluated by immunohistochemical (IHC) and immunofluorescence (IF) analysis. Endothelial cells (EC) and tubular cells were stimulated in vitro with LPS (4ug/ml) and Rp (5 nM) and analyzed by FACS and Western Blot (WB).

Results: Masson Thricrome staining revealed an early fibrosis, in LPS mice, characterized by collagen deposits at tubulointerstitial level compared to CTR (LPS: 38.5±4.54 vs CTR:8.7±1.54, p=0.01). Interestingly, Rp infusion induced a dramatic decrease of extracellular matrix deposits (Rp: 10.24±2.22, vs LPS, p=0.02). Then we examined the presence of activated myofibroblasts on renal tissue by the expression of α-SMA marker. Rp pre-treatment hampered the α-SMA expression, found otherwise increased in LPS mice (Rp:1.34±0.55 vs LPS: 9.02±2.59 p=0.001). Moreover, EC after LPS stimulation underwent to deep remodeling, acquiring a fibroblastic-like phenotype (CD31+/α-SMA+, LPS:30.16±26.83 vs CTR 0.66±0.13;p=0.01). Rp protected EC from dysfunction, restoring specific endothelial markers (CD31+ α-SMA+ Rp/LPS: 7.95 ±1.02 vs LPS; p=0.01). As in vivo, LPS led to EC dysfunction in vitro with a reduction of endothelial markers and an increase of fibroblast markers. Both LPS and Rp did not affect tubular and endothelial viability (caspase3neg) in endotoxemic AKI. Considered as novel biomarker for renal disease, tubular Klotho expression was evaluated by IHC for this marker. LPS administration significantly reduced tubular klotho expression and its restoration by Rp was clear evident both in vivo (HIC, CTR: 93.33±1.044 vs LPS: 24.26±10.19, p=0.001; Rp/LPS: 71.60±11.88 vs LPS, p=0.01) and in vitro (WB, LPS 24h:0.36±0.02 vs Basal, p=0.02; Rp/LPS 24h:0.80±0.07).

Conclusions: These data suggest that LPS acts directly on EC and tubular cells, promoting renal dysfunction. Rp treatment may represent a possible therapeutic strategy to counteract sepsis induced AKI.