LPS-MEDIATED RECRUITMENT OF mTOR COMPLEX 1 ENHANCES ENDOTHELIAL DYSFUNCTION IN SEPSIS-INDUCED ACUTE KIDNEY INJURY

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BACKGROUND

The pathophysiology of sepsis induced-Acute Kidney Injury (AKI) is multi-factorial and includes endothelial cell (EC) dysfunction, infiltration of inflammatory cells, intra-glomerular thrombosis, and obstruction of tubules. mTOR complex 1 (mTORC1) has been shown to be activated after LPS binding to Toll-like receptor (TLR)-4, and it may be pivotal in renal cell activation and in the progression of endothelial dysfunction and renal fibrosis. However, the precise signal transduction by which TLR4 activates mTOR in EC is still not known.

METHODS

C57BL/6 mice were randomized into following groups: Control (CTR, PBS intraperitoneal infusion), Endotoxemic (intraperitoneal LPS, 10mg/Kg), Rapamycin (Rp,intraperitoneal Rp, 5mg/Kg) and Rp/LPS groups. After 24h from infusion, renal tissue sections were evaluated by Masson's trichrome staining and immunofluorescence (IF) analysis. EC were stimulated in vitro with LPS (4µg/ml) and Rp (5 nM) and analyzed by FACS and Western Blot (WB).

RESULTS

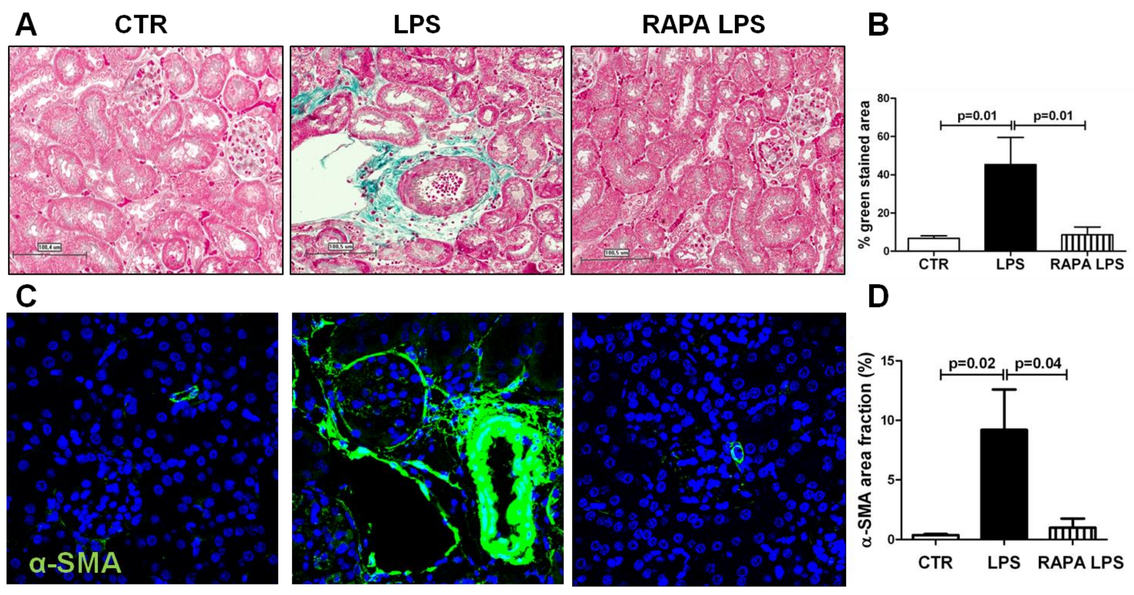

Fig1: **Anti-fibrotic role of Rp in LPS-induced AKI.** Masson's trichrome staining (A-B) revealed an early fibrosis in endotoxemic mice (LPS) respect to control (CTR). An interstitial increase of the myofibroblast marker α-SMA was observed in LPS group. Rp pre-treatment induced a strong decrease of extracellular matrix deposits (RAPA LPS, A-B) and hampered α-SMA expression (RAPA LPS, C-D) .

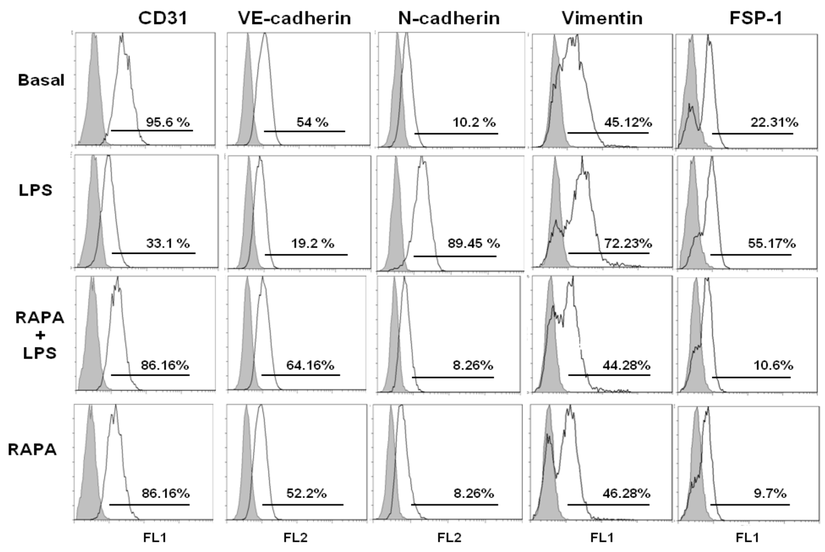

Fig3: **Rp effects on Endothelial Dysfunction *in vitro.*** After LPS stimulation , EC showed a significant reduction of specific EC marker and an increased expression of dysfunctional-fibroblast markers. In the presence of Rp, EC preserved their phenotype.

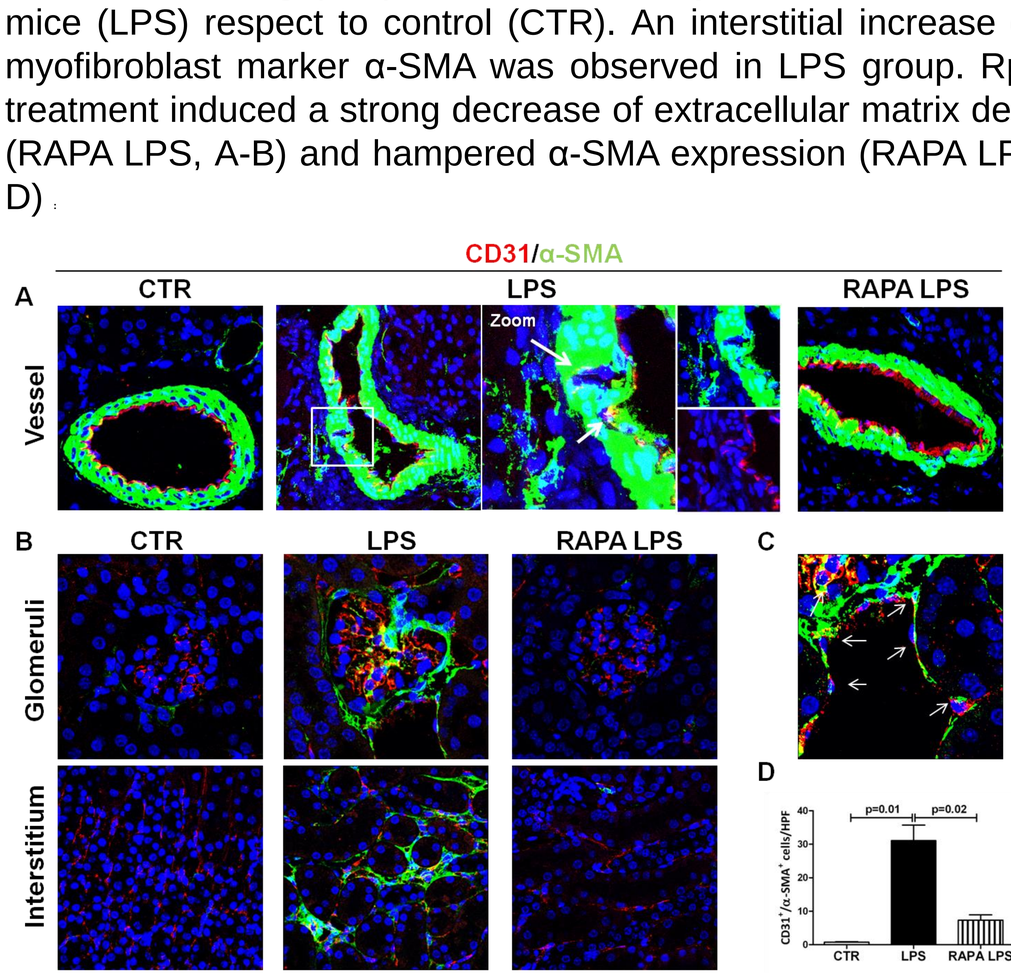

Fig2: **Rp prevented EC dysfunction in endotoxemic AKI.** When activated by LPS, renal CD31+ EC acquired several markers of myofibroblasts (α-SMA, A-C). Along large vessels, $CD31^{+}/\alpha\text{-SMA}^{+}$ EC were found in the media of the vascular wall, showing an invasiveness capacity (A, LPS). Rp pre-treatment restored EC phenotype (RAPA LPS) in all renal compartments (A-D).

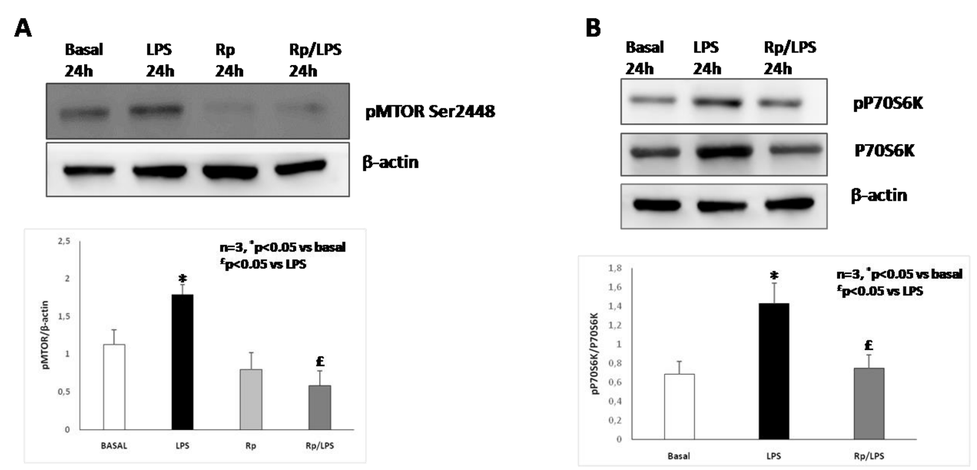

Fig4: **LPS mediated the activation of mTORC1**. LPS administration significantly induced the phosphorylation of mTORC1 and P70S6 kinase ($p<0.05$ vs. basal) that was hampered by Rp.

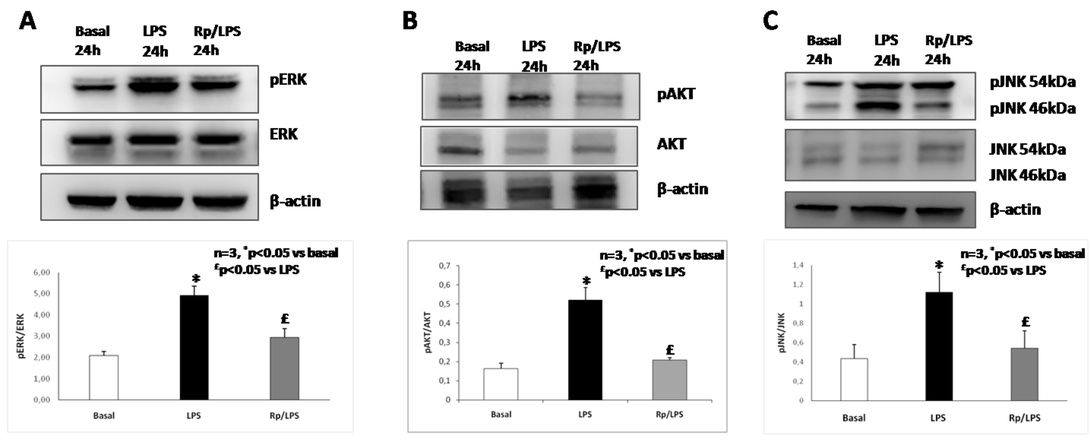

Fig5: **Rp modulated LPS/TLR-4 signaling**. mTOR activation was dependent by the phosphorylation of Erk1/2, JNK1/2, and AKT ($p<0.05$ vs. basal). Rp inhibited the phosphorylation of the entire pathway, suggesting an involvement of mTORC2 in TLR4 signal transduction.

CONCLUSIONS

Our data suggest the requirement of LPS-activated mTORC1 to enhance endothelial dysfunction and renal fibrosis. Rp treatment may represent a possible therapeutic strategy to limit LPS-induced AKI.

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