A POLYMER CONJUGATE NANOMEDICINE INHIBITS LPS-INDUCED MAPK ACTIVATION AND REDUCES ENDOTOXEMIA-MEDIATED KIDNEY INFLAMMATION

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INTRODUCTION AND OBJECTIVES

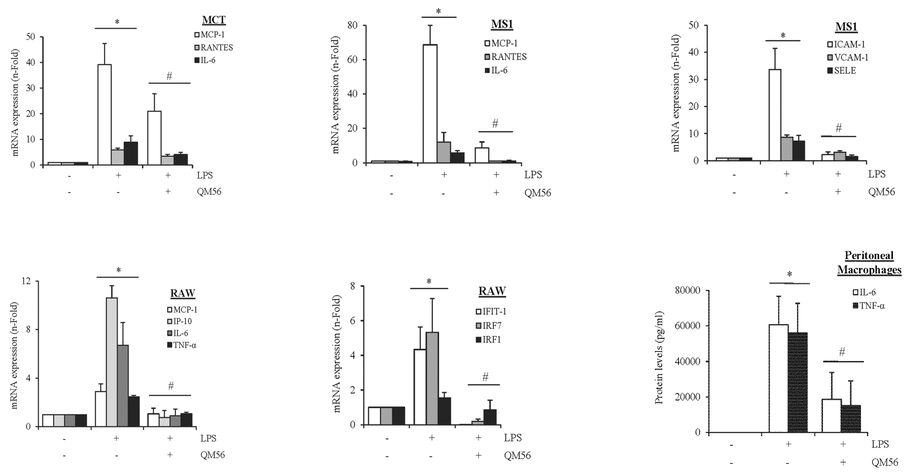
Endotoxemia-associated acute kidney injury during sepsis is a major cause of mortality in ICU patients by contributing to multiple organ failure. The systemic and kidney deregulated inflammatory response provoked by endotoxemia involves a widespread activation of the innate immune system. Toll-like receptor 4 (TLR4) is a key mediator of innate immunity and amplifies inflammation in AKI. Moreover, kidney TLR4 expression is increased in experimental endotoxemia and TLR4 targeting is protective. However, no drugs focused on TLR4 are in clinical use. We previously reported that the experimental nanomedicine QM56 inhibits NF-κB-dependent proinflammatory responses and protects from experimental nephrotoxic AKI *(1,2).* We have now explored whether QM56 interferes with LPS-induced TLR4 signaling and protects from endotoxemia-induced renal inflammation and injury.

METHODS

Cell culture assays were done in the mouse cell lines of kidney tubules (MCT), endothelium (MS1) and macrophages (RAW264.7) and also in primary peritoneal macrophages. Cells were treated with LPS (1µg/ml) for 6h or cotreated 1h with QM56 (20 µM) and then LPS (1µg/ml) and further analyzed for both TLR4-dependent proinflammatory pathway regulation and NF-κB-dependent cytokine expression. Results from cell culture assays were validated in C57BL/6 mice injected with LPS (0.4 mg/kg, i.p.) alone or cotreated with LPS and QM56 (2.5 mg/mouse, r.o., added 5 hours before or along with LPS). The control group was treated with drug vehicle. After treatment, mice were euthanized and kidneys analyzed for gene (qRT-PCR) and protein (IHC) expression.

RESULTS 1

QM56 prevents the synthesis of proinflammatory soluble factors in key cell types involved in kidney endotoxemia

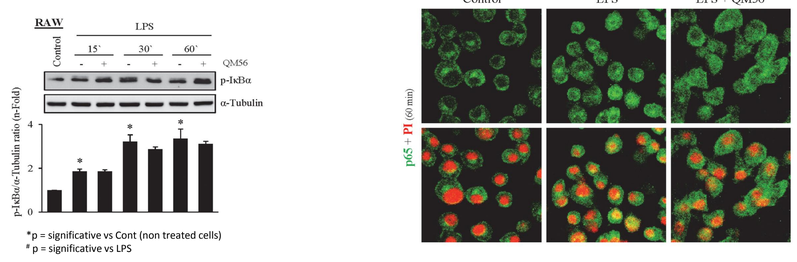


MCT: Murine Tubular Kidney Cells
MSI: Murine Endothelial Cells
RAW264.7: Murine Macrophages

*p = significative vs Cont (non treated cells)
p = significative vs LPS

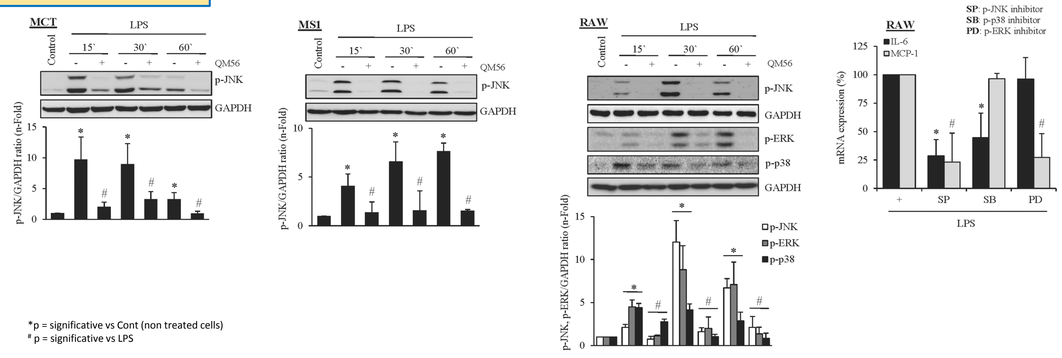
RESULTS 2

QM56 does not impede NF-κB activation asessed by IκBα phosphorylation and p65 nuclear translocation



*p = significative vs Cont (non treated cells)
p = significative vs LPS

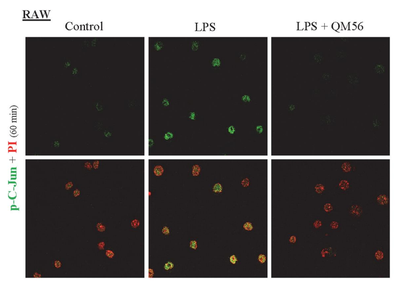
RESULTS 3



*p = significative vs Cont (non treated cells)
p = significative vs LPS

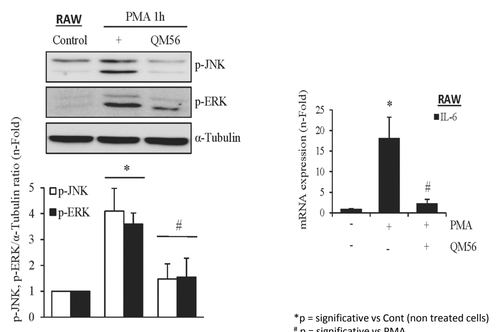
RESULTS 4

QM56 blocks c-Jun/AP-1 nuclear traslocation



RESULTS 5

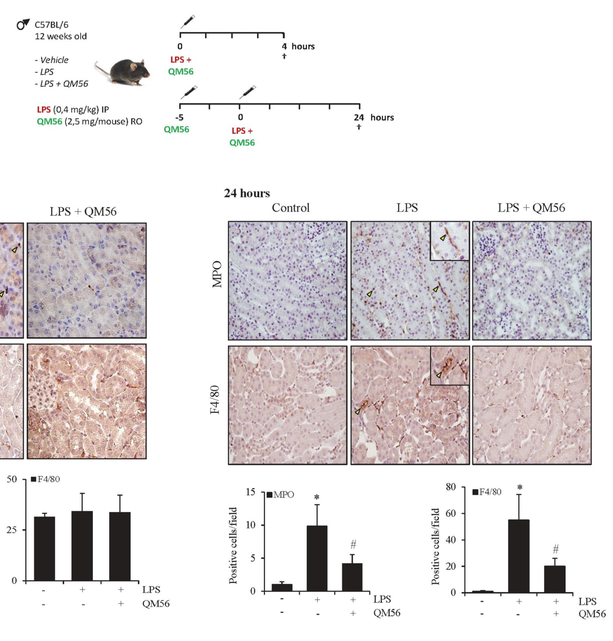
QM56 inhibits JNK and ERK canonical signaling and cytokine synthesis induced by PMA



*p = significative vs Cont (non treated cells)
p = significative vs PMA

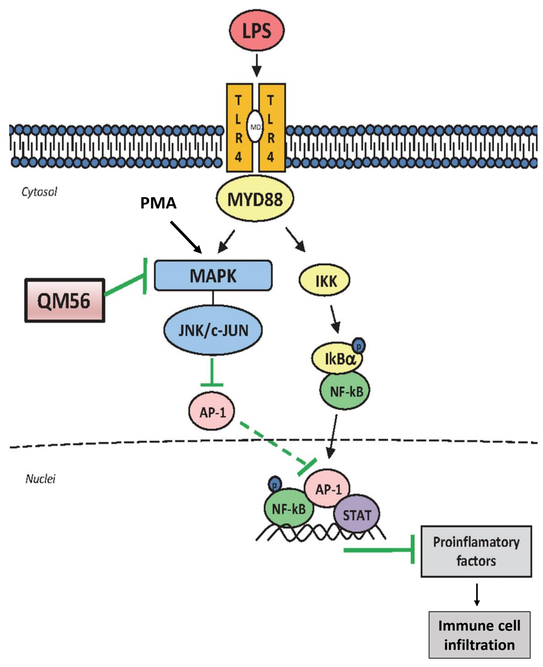
RESULTS 6

QM56 inhibits LPS-triggered inflammation in kidney mice



*p = significative vs Cont (non treated mice)
p = significative vs LPS treated mice

GRAPHICAL ABSTRACT



CONCLUSIONS

➢ QM56 inhibits the activation and signaling of MAPKs, known transcriptional NF-κB partners, induced by both the classical (TLR4) and the canonical (PMA/PKC) signaling pathways in relevant renal cell types.

➢ QM56 does not preclude NF-kB activation but downregulates the NF-κB proinflammatory transcriptional activity.

➢Overall, this study identifies QM56 as a potential therapy for endotoxemia and sepsis by inhibiting the TLR4-dependent activation of the MAPKs/NF-κB pathway and dependent inflammatory responses.

BIBLIOGRAPHY

1. Vicent M. et al., 2006. Poly-L-glutamic acid (PGA) aided inhibitors of apoptotic protease activating and factor 1 (Apaf-1): an antiapoptotic polymeric nanomedicine. *J Med Chem*. 49(13), 3763-3765.
2. Ucero A et al., 2013. A Polymeric Nanomedicine Diminishes Inflammatory Events in Renal Tubular Cells. *PLoS ONE*, 8(1).

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