Effect of BAM15 on LPS-induced Septic BMM and Neuronal Cell

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Background

- Sepsis, a life-threatening organ dysfunction in response to systemic infection from infection, burn, or other factors, is a world-wide healthcare burden with long-lasting and perhaps life-long complications. Sepsis and its complications, including sepsis-associated encephalopathy (SAE), are one of the most frequent causes of high mortality in the intensive care unit (ICU). Despite extensive research, the pathophysiology of sepsis remains obscure. Currently, no specific therapy exists for sepsis except for seeking to treat the underlying cause and complications.
- During bacterial invasion, the LPS on bacterial surface binds to Toll-like receptors (TLR) on immune cell surface, initiating signaling cascade which results in increased gene expression on proinflammatory cytokines (IL6, IL10 and TNF-α).
- BAM 15, a novel mitochondrial uncoupler, acts directly to enhance ATP synthesis for a short period followed by MMP impairment reducing further ATP synthesis which results in overall attenuating inflammation effect.
- Our goal is to determine the effect of BAM15 during LPS-induced sepsis by examining the inflammatory cytokine concentration, their associated gene expression and mitochondrial function in PC12 cells and bone marrow-derived macrophages, comparing neural versus systemic infections, respectively.

Methods

Lipopolysaccharide (LPS) stimulated PC12 Cells and Bone marrow-derived macrophages (BMM) for Sepsis Model along with BAM15 Treatment

Harvested PC12 cells (American Type Culture Collection) and Bone marrow-derived macrophages (BMM) from wild-type (WT) mice were treated with 100 ng/mL of LPS from Escherichia coli 026: B6 (Sigma-Aldrich) with or without 10 nM BAM15.

qRT-PCR and ELISA Analysis

Quantitative polymerase chain reaction (qPCR) was performed using SYBR Green master mix with cDNA template and target primers based on ΔΔCT method with β-actin as a housekeeping gene to convert RNA into cDNA. The nucleotide sequences of primers for the analysis of inflammatory cytokine genes (IL-6, IL-10, TNF-α, NF-κB, iNOS, IL-1β, FIZZ, Arg1, and TGFβ). Cytokines in the supernatant (inflammatory cytokines: IL-6, IL-10, TNF-α) were measured by ELISA assay (Biolegend) with IL-6 and TNF-α being diluted at 50-100 fold and IL-10 being diluted at 10 fold.

Mitochondrial Function

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR were analyzed by using Seahorse XFp Analyzers (Agilent) per protocol for treated PC12 cells and BMM at each condition. The ECAR graph was calculated from OCR data, and all data was analyzed by using Seahorse Wave 2.6 software. Mitochondrial membrane potential (MMP) was investigated using MitoTracker Red CMXRos (Thermo Fisher Scientific) as a red fluorescent color on mitochondria with active membrane potential in accordance with the manufacturer's protocol. Then read on the microplate reader at excitation 361 nm and emission 487 nm.

Statistical Analysis

Graph establishment and data analysis were performed using the Statistical Package for Social Sciences software and GraphPad Prism 7.0. The differences between multiple groups were examined for statistical significance by one-way analysis of variance (ANOVA) with Tukey's analysis. A p-value < 0.05 was considered statistically significant.

Future Research

- Further studies targeting cell energy status interference as a part of sepsis adjuvant therapy
- Determining other factors (not cytokine production) that drive energy production in neuronal cells during sepsis encephalopathy

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Results

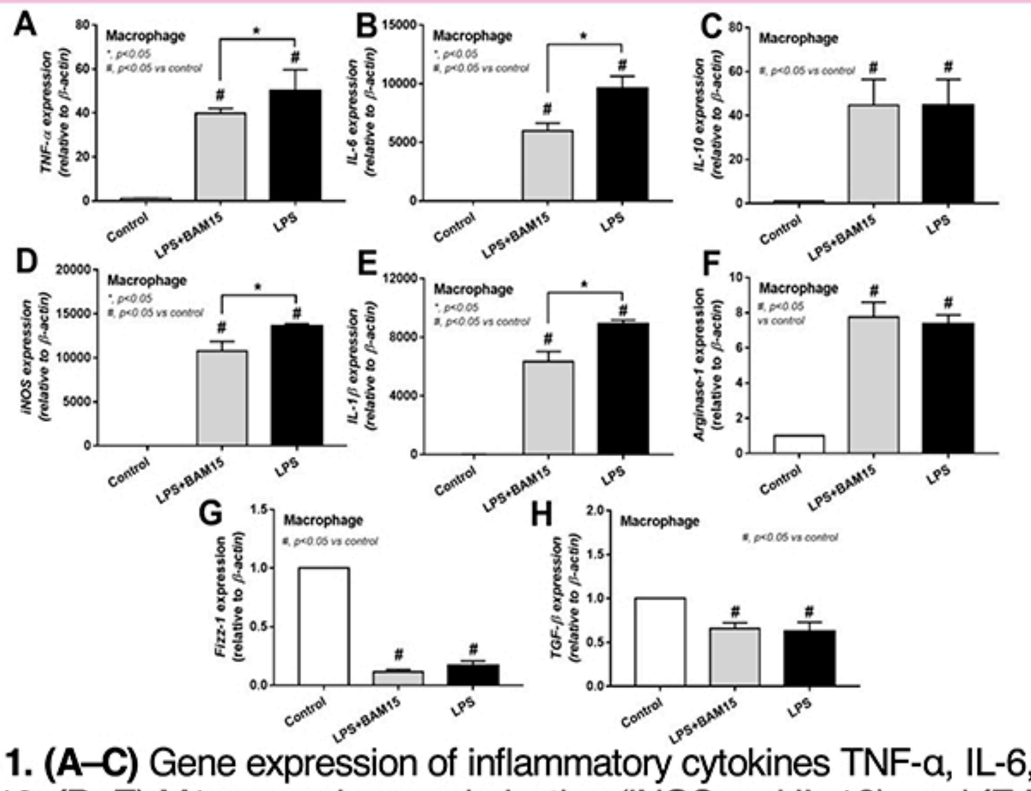

Figure 1. (A–C) Gene expression of inflammatory cytokines TNF-α, IL-6, and IL-10, **(D, E)** M1 macrophage polarization (iNOS and IL-1β), and **(F-H)** M2 macrophage polarization (Arginase-1, Fizz-1, and TGF- β) of BMM after 24 h LPS induction, with or without BAM15 illustrated in comparison with the BMM in control media.

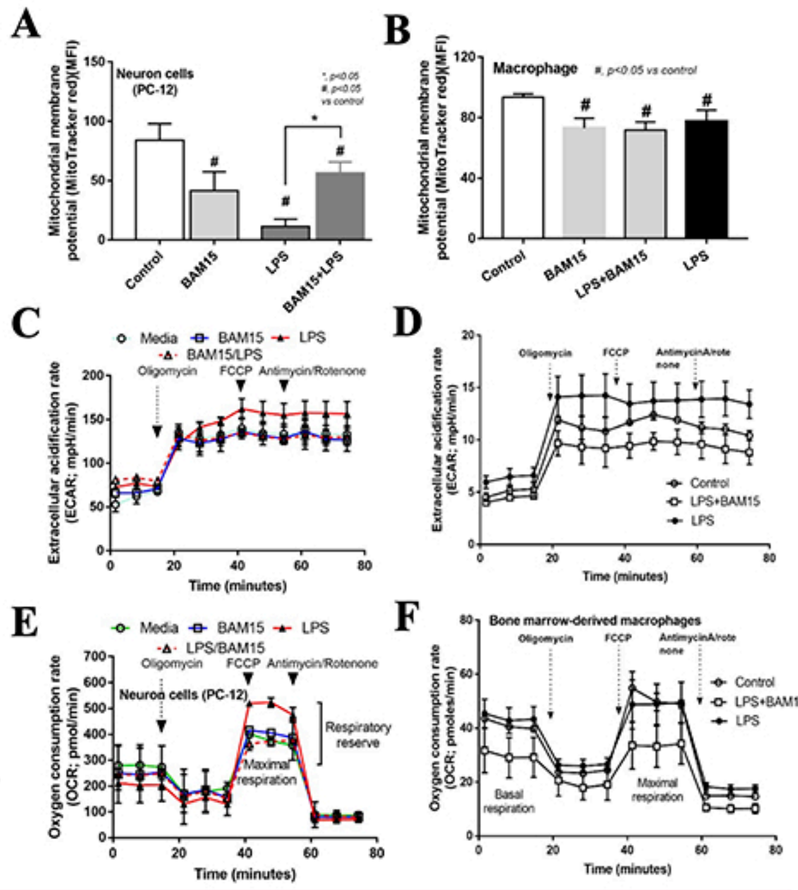

Figure 2. PC12 cell line and Bone marrow-derived macrophage (BMM) (A-B) Characteristics of PC12 cells and BMM after 48 h stimulation by LPS, with or without BAM15, in comparison with the control media (media) or BAM15 alone, as evaluated by mitochondrial membrane potential, **(C-F)** cell energy status through mitochondria (oxygen consumption rate; OCR) and glycolysis (extracellular acidification rate; ECAR).

Conclusion

- In PC-12, LPS could not induce cytokines possibly due to low neuronal TLR4 expression (data not shown) implying lesser influence of neurons in sepsis hyper-cytokine production. However, LPS reduced PC-12 mitochondrial activities that was attenuated by BAM15.
- In BMM, LPS reduced mitochondrial function and increased glycolysis which facilitated pro-inflammatory macrophages which were attenuated by BAM15 through the normalization of cell energy status and cytokine reduction.
- Sepsis-induced activation in both neurons and macrophages, mainly through mitochondrial damage (reduced MMP) (PC-12) and enhanced glycolysis (macrophages) which were attenuated by BAM15.
- BAM15 attenuated sepsis and SAE by reducing the cell energy status to prevent the damage from sepsis-induced overwhelming stimulation (prevent excessive ROS in both neurons and macrophages).
- Notably, BAM-15 reduced mitochondrial membrane potential in macrophages but not in neurons which might be due to i) more potent glycolysis activity in macrophages for glycolysis-dependent cytokine production than neurons (neurons have less ability on cytokine production) and ii) the differences in the number of mitochondria as macrophages have fewer mitochondria than neurons, thus making the reduced MMP in macrophages easier than in the neurons.

References

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