Apabetalone, a BET Bromodomain Inhibitor, Suppresses Inflammatory Mediators in Microglia that Contribute to Neurodegenerative Disease

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Introduction

Chronic inflammation is prevalent in neurodegenerative disorders. Activated microglia produce proinflammatory cytokines as well as complement components C3 and C1q, which promote aberrant synapse loss and dysfunction. Apabetalone is a small molecule in Phase 3 trials for cardiovascular disease. As an inhibitor of bromodomain and extraterminal domain (BET) proteins, apabetalone regulates gene expression through epigenetics. Clinical trials in cardiovascular patients and preclinical models demonstrate anti-inflammatory effects on factors in the periphery that can infiltrate the brain, suggesting therapeutic potential in neurodegenerative disease. Here we characterize apabetalone's effects on microglia to mitigate inflammation and processes contributing to neurodegenerative pathology.

Objective

Determine whether apabetalone affects proinflammatory activation of microglia and subsequent expression of factors that promote neurodegeneration.

Methods

BV-2 microglial cells were stimulated with LPS and interferon-gamma. Apabetalone's effect on expression of proinflammatory cytokines & genes associated with synaptic pruning were examined by real-time PCR. Cellular proliferation and morphology were monitored. 8 week old C57BL/6 male mice received 150 mg/kg apabetalone 2x daily PO for 6 days. On day 7, LPS was administered IP and apabetalone was administered 4h prior to LPS & again with LPS treatment. Mice were euthanized 24h post LPS.

Results

After proinflammatory stimulation, microglia acquired the condensed morphology associated with a proinflammatory phenotype. Treatment with apabetalone reversed microglia back to a ramified, resting phenotype. Stimulation of microglia induced expression of interleukin-6, interleukin-1β, as well as complement C3, and complement C1q. Apabetalone dose dependently opposed induction of these key contributors to neurodegenerative processes. Apabetalone was not cytotoxic and did not impact proliferation. In vivo, apabetalone reduced expression of markers of activated macrophages & microglia, endothelium, or monocytes in the brain of mice.

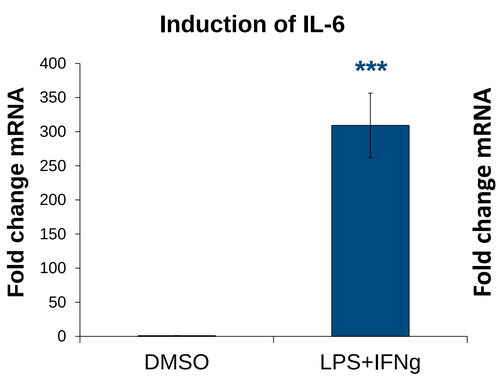
Conclusions

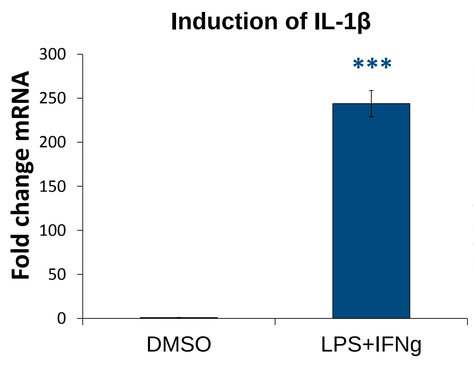
Apabetalone reduced activation of microglia and suppressed expression of proinflammatory factors that drive chronic neuroinflammation and overactive synaptic pruning associated with cognitive decline. Apabetalone reduced expression of inflammatory markers in distinct cell types in the brain of a mouse model of inflammation. BET inhibition offers a new frontier for neurodegenerative therapeutics through simultaneous regulation of multiple pathogenic processes.

Apabetalone Suppresses Expression of Select Proinflammatory Cytokines in Stimulated BV-2 Microglia

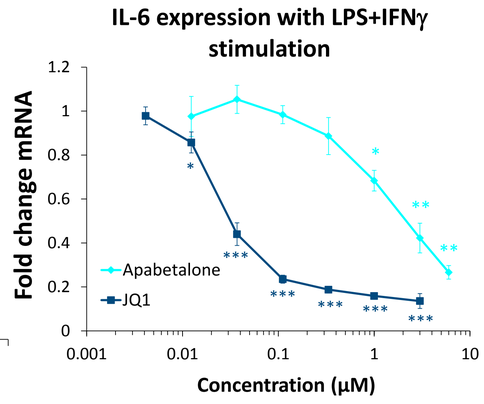
BV-2 cells were stimulated with 10 ng/mL LPS & 5 ng/mL IFNγ. Gene expression was analyzed by real-time PCR

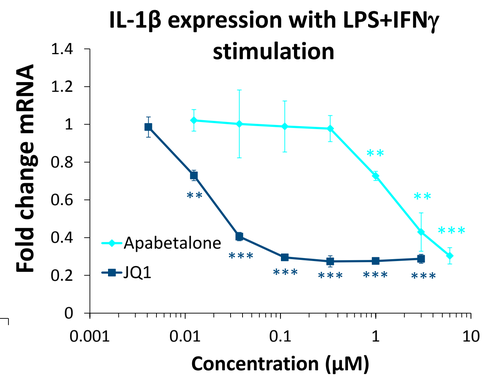
Induction of gene expression with LPS+IFNγ stimulation





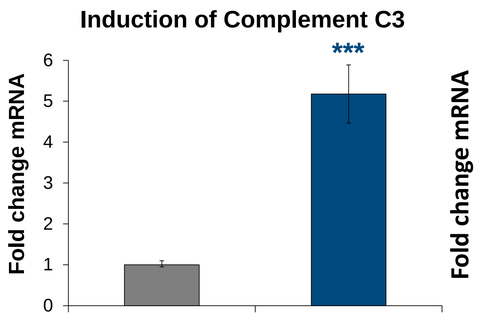
Regulation of LPS+IFNγ stimulated gene expression by BETi

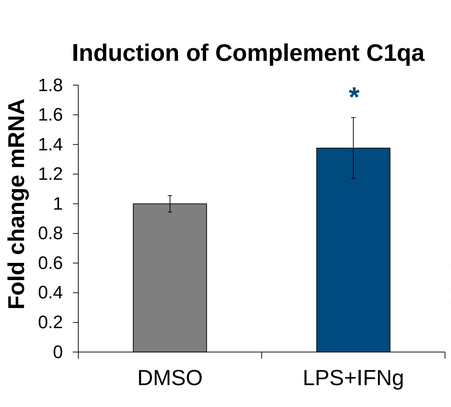


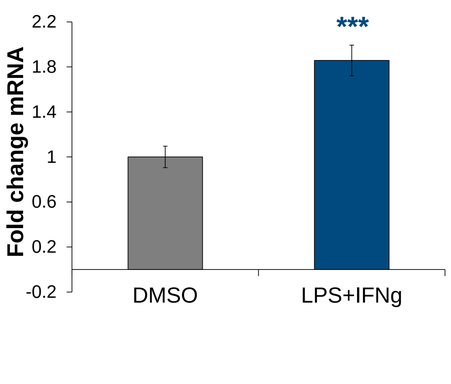


Apabetalone Suppresses Expression of Complement Components Associated with Synaptic Pruning in Stimulated BV-2 Microglia

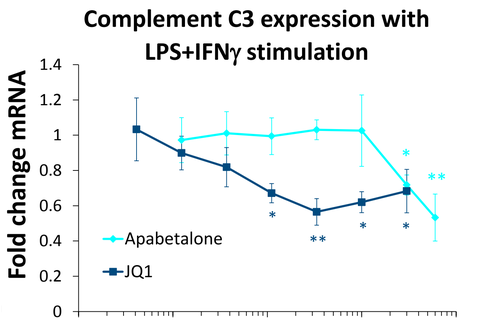
Induction of gene expression with LPS+IFNγ stimulation

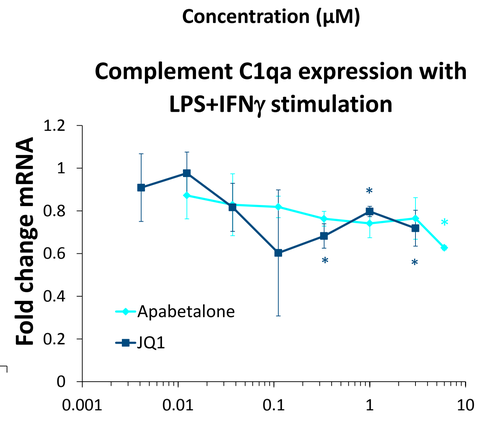


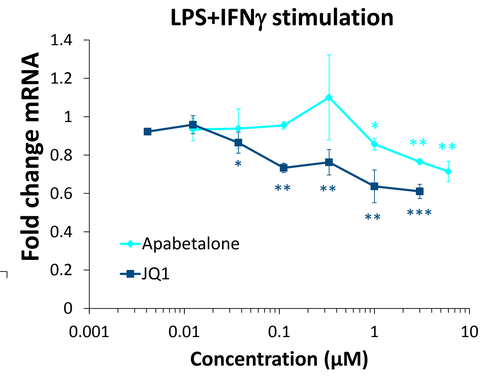




Regulation of LPS+IFNγ stimulated gene expression by BETi





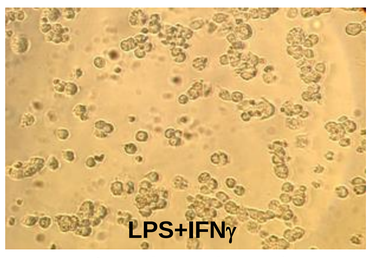


Statistical significance determined with a Student's *t*-test * p<0.05; **p<0.01; ***p,0.001

Proinflammatory Stimuli Promote Condensed Morphology Associated with Inflammation Which is Reversed by Apabetalone in BV-2 Microglia

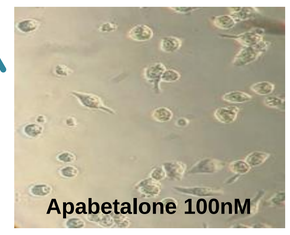
Reversal of activated morphology by apabetalone

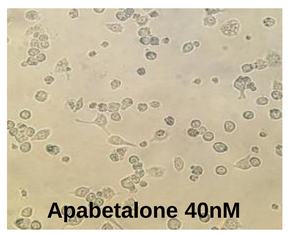
Ramified morphology with branching processes associated with resting state

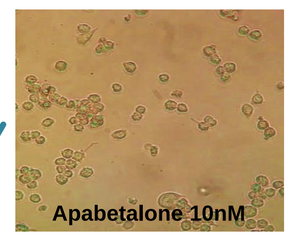


Round, condensed morphology associated with inflammation

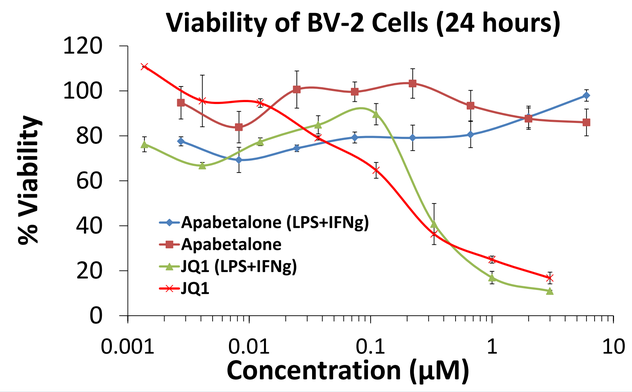
LPS+IFNγ



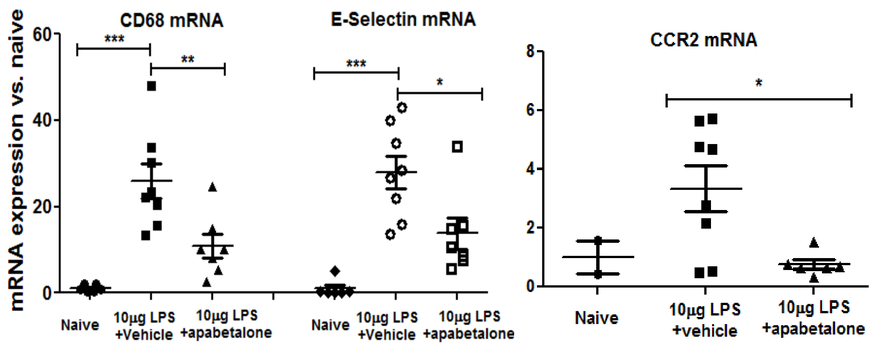




Apabetalone Promotes Survival vs. Comparator That Reduces Viability



Reduction in Markers of Activated Macrophages & Microglia (CD68), Endothelium (E-Selectin), or Monocytes (CCR2) in the Brain of a Mouse Model of Inflammation



Summary

- Apabetalone counters induction of select cytokines & complement components under inflammatory conditions in microglia. Apabetalone may counter neuroinflammation & overactive synaptic pruning associated with cognitive decline.
- Apabetalone reverses the inflammatory morphology acquired by stimulated microglia, consistent with suppression of the inflammatory response.
- Apabetalone promotes survival of microglia versus a comparator molecule.
- Apabetalone reduces expression of markers of activated macrophages & microglia, endothelium, or monocytes in the brain of a mouse model of inflammation.
- BET inhibition is a promising therapy that modulates multiple processes contributing to neurodegenerative disease.