Apabetalone Epigenetically Inhibits Monocyte Adhesion To Brain Endothelial Cells By Downregulating Key Neuroinflammation Markers *In Vitro* And *In Vivo*

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Background

Cytokines activate inflammatory pathways in brain endothelial cells that promote the recruitment and transmigration of monocytes across the blood brain barrier. This process contributes to the initiation and exacerbation of neuroinflammation, leading to neuronal injury. Epigenetic dysregulation exacerbates inflammatory signaling in monocytes and vascular endothelial cells. Thus, epigenetic factors constitute attractive therapeutic targets.

Study objective: Using cellular and animal models of neuroinflammation, we evaluate anti-inflammatory properties of the clinical stage small molecule apabetalone that inhibits acetylated histone readers bromodomain and extraterminal domain (BET) proteins.

Methods

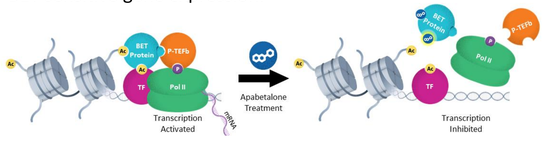
- **THP-1 monocyte** transcriptional responses to TNFα +/- apabetalone were examined.
- **Human brain microvascular endothelial cells** (HBMVECs), stimulated with TNFα and IFNγ +/- apabetalone, were assayed for gene expression, cytokine secretion, surface adhesion protein level, and THP-1 adhesion under flow conditions.
- ***In vivo* brain inflammation** was assessed in C57BL/6 male mice pretreated with 150 mg/kg apabetalone for 7 days and then injected with 10 µg lipopolysaccharide (LPS) intraperitoneally. Brain mRNA was analyzed 24h post LPS injection.

Results

- In **THP-1 cells**, apabetalone suppressed the expression of genes induced by TNFα, including IL-1β, chemokine MCP-1, chemokine receptors CCR1 and CCR2 and cell-cell adhesion molecule VLA-4.
- In cytokine stimulated **HBMVECs**, apabetalone reduced the mRNA induction of vascular activation markers IL-6, MCP-1, VCAM-1, and E-selectin. Surface expression of adhesion proteins VCAM-1 and E-selectin as well as secretion of IL-6 and MCP-1 were also reduced. Consequently, apabetalone countered THP-1 adhesion to HBMVECs in laminar flow assays.
- In **mice**, apabetalone attenuated the LPS-induced brain mRNA expression of inflammatory markers including E-selectin, ICAM, CCR2, and CD68.

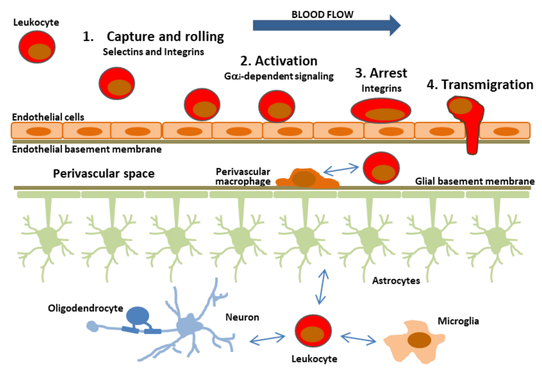
Drug Mechanism of Action

BET proteins control gene transcription through interactions with transcription factors and recruitment of RNA polymerase II. Apabetalone binds to bromodomains in BET proteins, causing their release from chromatin and downregulation of BET sensitive gene expression.



BET: bromodomain and extraterminal proteins
ac: acetylated lysine residue on DNA associated proteins
BD: bromodomain
TF: transcription factor

Leukocyte Infiltration Into the Central Nervous System Contributes to Neuroinflammation



Adapted from Rossi et al., 2011, Journal of Leukocyte Biology

Apabetalone Downregulates the Inflammatory Genes in Monocytes

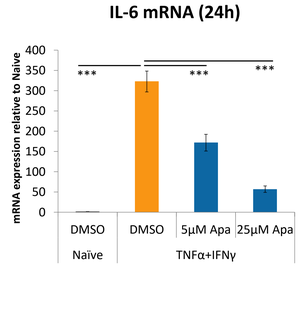
The THP-1 cell line was stimulated for 4h with 10ng/mL TNFα ± 20µM apabetalone.
Gene expression was analyzed by real-time PCR.

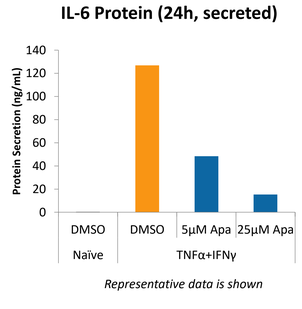
Gene Expression		TNFα (fold change)	Apabetalone (% change)
Cytokines	**IL-1β**	3.5	75
	TNFα	3.8	NS
Chemokines and Receptors	**CCR1**	1.4	51
	CCR2	0.5	50
	MCP-1	3.7	77
Adhesion molecules	**CD44**	1.8	26
	VLA-4	0.9	35

Statistical significance determined with a Student's t-test; significant: p<0.05

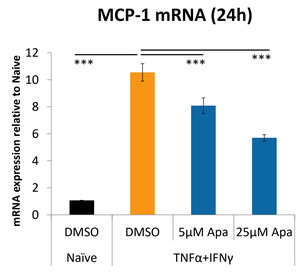
Apabetalone Suppresses the Expression of Inflammatory Mediators in Brain Endothelial Cells

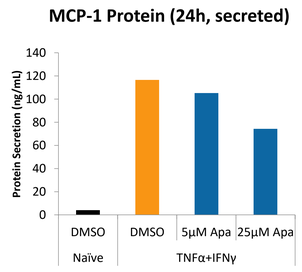
Primary human brain microvascular endothelial cells - stimulated for 4-24h with 10ng/mL TNFα/FNγ ± apabetalone.
Gene expression was analyzed by real-time PCR.
Cytokine secretion was examined by Multi Analyte Profiling.
Adhesion protein surface expression was measured by FACS.





Representative data is shown





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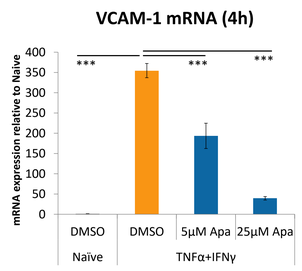
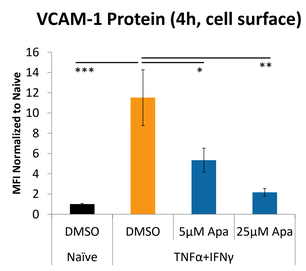
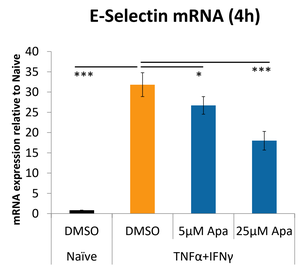
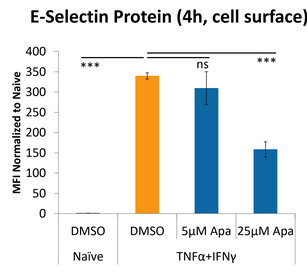




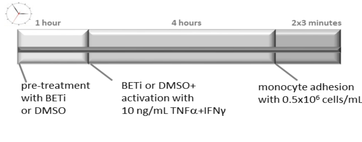


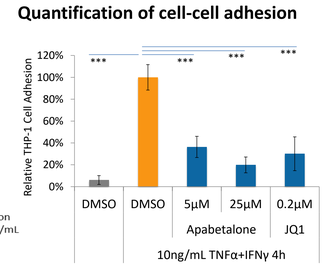


*Statistical significance: One-way ANOVA followed by Tukey's multiple comparisons test; *p<0.05, **p<0.01, ***p<0.001. Error bars represent standard deviation*

Apabetalone Reduces Monocyte Adhesion to Cytokine-Activated Brain Endothelial Cells

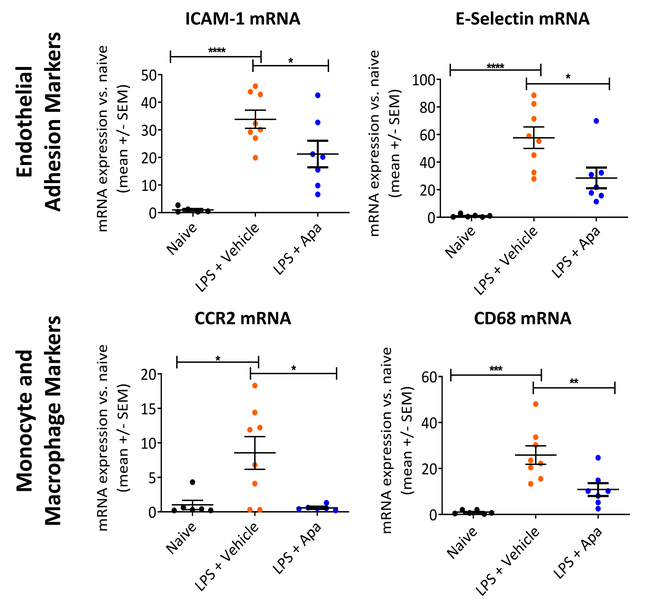
Adhesion of THP-1 cells to cytokine treated HBMVECs was assessed in parallel flow conditions.





*Statistical significance: One-way ANOVA followed by Tukey's multiple comparisons test; *** p<0.001*
Error bars represent standard deviation

Apabetalone Reduces the Expression of Inflammation Markers in the Brain of Endotoxemic Mice



*Naïve cells did not receive LPS or BETi treatment. Statistical significance determined with a Student's t-test * p<0.05; **p<0.01; ***p<0.001; ****p<0.0001*

Summary

- **Apabetalone decreased neuroendothelial activation and interaction with monocytes, potentially reducing immune cell transmigration into the brain in neuroinflammatory conditions.**
- **Apabetalone's effect on cognition in diabetes patients following acute coronary syndrome ≥70 years old is being evaluated by repeat MoCAs in the phase 3 BETonMACE trial (Results Q4 2019).**