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

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Background

Circulating cytokines induce inflammatory changes in brain vascular endothelial cells that promote monocyte adhesion and transmigration across the blood brain barrier. This process contributes to the initiation and exacerbation of neuroinflammation, which ultimately leads to neuronal injury and neurodegeneration. Bromodomain and extraterminal domain (BET) proteins are histone acetylation readers that activate cytokine-dependent transcription in monocytes and endothelial cells in chronic vascular inflammation models. Targeting BET proteins with epigenetic therapies may reduce endothelial activation during neuro-inflammation.

Objective

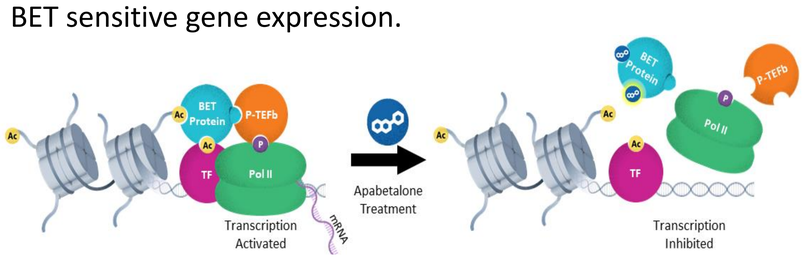
To evaluate the anti-inflammatory properties of apabetalone, a clinical stage small molecule that inhibits the transcriptional activity of BET proteins, in cellular models of brain inflammation.

Results

In THP-1 monocytes, apabetalone suppressed the expression of genes induced by TNFα, including IL-1β, the chemokine MCP-1, chemokine receptors CCR1 and CCR2, and the adhesion molecule VLA-4 (40% to 90% reduction, $p<0.05$). In hCMEC/D3 endothelial cells, cytokine stimulated secretion of key inflammatory chemokines involved in monocyte attraction and vascular inflammation was reduced by apabetalone, including granulocyte-macrophage colony-stimulating factor, fractalkine, MCP-3, IP-10, and IL-6 (40% to 90% reduction, $p<0.05$). In TNFα and IFNγ stimulated human brain microvascular endothelial cells (HBMVECs), apabetalone inhibited the mRNA levels and the surface abundance of the cell adhesion proteins VCAM-1 (80% reduction) and E-selectin (50% reduction). In agreement with this inflammatory marker downregulation, apabetalone treatment countered THP-1 adhesion to HBMVECs in laminar flow assays. In mice, apabetalone treatment attenuated the LPS-induced mRNA expression of inflammation markers in the brain including *SELE*, *ICAM1*, *CCR2*, and *CD68*.

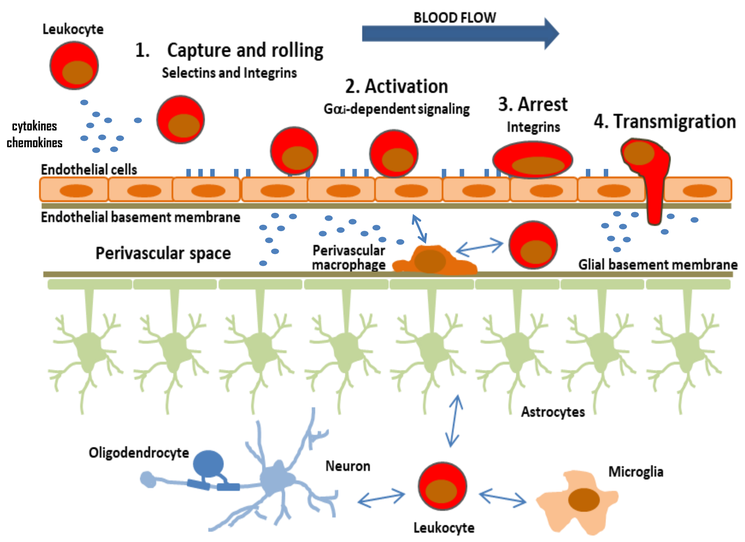
Drug Mechanism of Action

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 Inflammatory Genes in Monocytes

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

Gene Expression		TNFα Fold Induction	Apabetalone % Reduction
Cytokines	IL-1β	3.5	75
	TNFα	3.8	ns
Chemokines and Their 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: Student's t-test; bold: p<0.05

Apabetalone Downregulates Inflammatory Protein Secretion in Brain Endothelial Cells

hCMEC/D3 cell monolayers cultured on suspended filters were stimulated for 24h with cytokines (100ng/mL) ± 25μM apabetalone. Luminal protein secretion was analyzed by bead based immunoassays.

Luminal Cytokines	TNFα+IFNγ Fold Induction	Apabetalone % Reduction	IL1β Fold Induction	Apabetalone % Reduction
MCP-3	305	93	101	81
Fractalkine	101	89	5	47
GM-CSF	11	85	64	42
MCP-1	54	68	13	48
IL-1RA	7	52	5	28
G-CSF	8	52	21	9
TNFa	n/a	n/a	47	45
IL-6	49	52	236	45
IL-8	16	41	31	39
IP-10	4450	30	23	55

Statistical significance: ANOVA with Tukey's multiple comparisons test; bold: p<0.05

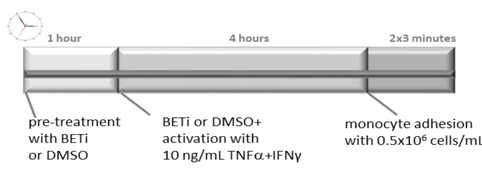
Apabetalone Reduces Monocyte Adhesion to Cytokine-Activated Brain Endothelial Cells

Primary human brain microvascular endothelial cells were stimulated for 4h with 10ng/mL TNFα/IFNγ ± apabetalone and gene expression and surface protein was analyzed (PCR, FACS).

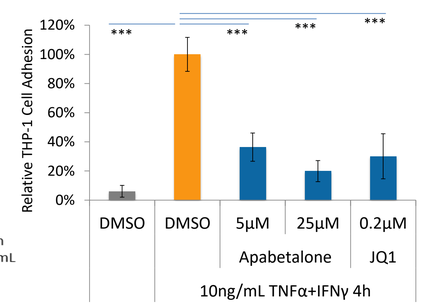
	mRNA Expression			Surface Protein Expression		
	TNFα+IFNγ	5μM Apa	25μM Apa	TNFα+IFNγ	5μM Apa	25μM Apa
	Induction	% Reduction		Induction	% Reduction	
VCAM-1	355	-45	-89	12	-53	-81
E-selectin	32	-16	-43	372	n/s	-53

Statistical significance: ANOVA with Tukey's multiple comparisons test; bold: p<0.05

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



Quantification of cell-cell adhesion

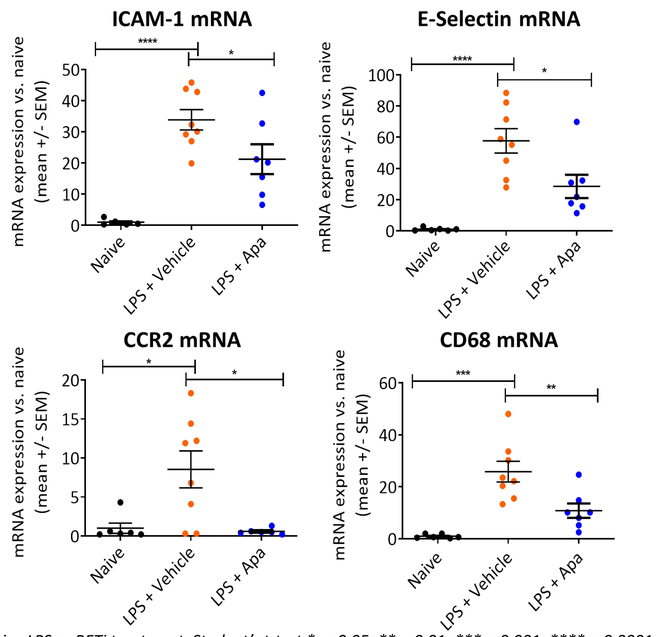


*Statistical significance: ANOVA withTukey's multiple comparisons test; *** p<0.001*

Apabetalone Reduces Inflammation Marker Expression in the Brain of Endotoxemic Mice

C57BL/6 mice pretreated with 150 mg/kg b.i.d. apabetalone for 7 days received 10 μg of lipopolysaccharide (LPS) i.p. mRNA from brain cortex was analyzed 24h post LPS injection.

Endothelial Adhesion Markers



Monocyte and Macrophage Markers

*"Naïve" mice did not receive LPS or BETi treatment. Student's t-test * p<0.05; **p<0.01; ***p<0.001; ****p<0.0001*

Conclusions

- Apabetalone decreased neuroendothelial activation and interaction with monocytes, potentially reducing immune cell transmigration into the brain in neuroinflammatory conditions.
- The effect of apabetalone treatment on the cognition of diabetic patients ≥70 years old with acute coronary syndrome is being evaluated through repeat MoCA testing in the phase 3 BETonMACE trial.