The Epigenetic Modulator Apabetalone Downregulates Brain Endothelial Activation and Monocyte Adhesion

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Background

Blood brain barrier disruption by proinflammatory cytokines is a putative driver of neuro-degeneration. Stimulated brain microvascular endothelial cells secrete cytokines into the bloodstream (via luminal membranes) and the brain parenchyma (via basolateral membranes). Cytokine-mediated recruitment of monocytes exacerbates neuroinflammation. Bromodomain and extraterminal domain (BET) proteins are histone acetylation readers that activate cytokine-dependent transcription in models of vascular inflammation.

Objective

Here, we demonstrate the impact of apabetalone, a clinical stage BET proteins inhibitor, on inflammatory activation of human brain microvascular endothelial cells.

Methods

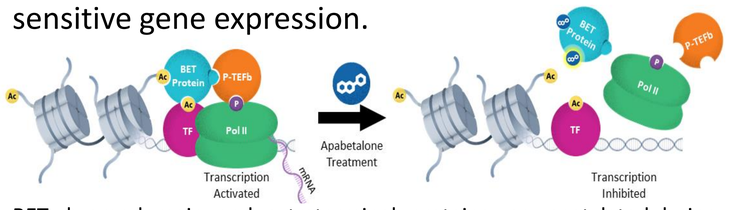
- **Polarized hCMEC/D3 cell monolayers** grown on suspended inserts: cytokine secretion (Milliplex® Multianalyte Profiling) was assessed in response to 25 μM apabetalone or DMSO in the presence of 100 ng/mL IL-1β or TNFα+IFNγ (24h).
- **Primary human brain microvascular endothelial cells (HBMVECs)**: effect of apabetalone on gene expression and adhesion protein surface levels during TNFα+IFNγ stimulation (4h) was assessed by PCR and FACS. THP-1 monocyte adhesion to HBMVECs was measured under laminar flow conditions.

Results

- **hCMEC/D3 cells**: In response to TNFα+IFNγ or IL-1β stimulation, cells had distinct protein secretion profiles across the luminal and abluminal membranes. Apabetalone treatment (25μM) reduced gene expression and protein secretion of key inflammatory cytokines, including GM-CSF, fractalkine, MCP-3, IP-10, IL-6, IL-8, MCP-1 and RANTES (40% to 90% reduction, $p<0.05$). BET dependency was confirmed with MZ-1 treatment, which degrades BET proteins.
- **HBMVECs**: During TNFα+IFNγ stimulation, apabetalone inhibited surface expression of cell adhesion proteins VCAM-1 (5 and 25μM) and E-selectin (25μM) in HBMVECs. Consequently, HBMVECs' interactions with THP-1 cells were reduced by both concentrations of apabetalone under flow conditions.

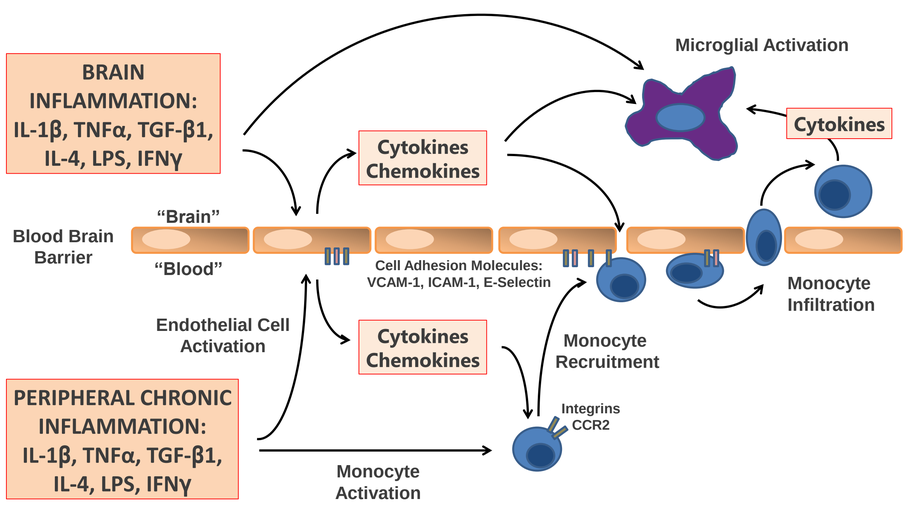
Drug Mechanism of Action

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



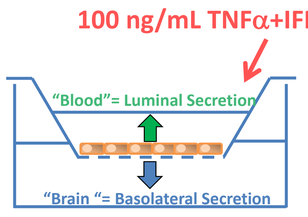
BET: bromodomain and extraterminal proteins; ac: acetylated lysine residue on DNA associated proteins; BD: bromodomain; TF: transcription factor

Monocyte Infiltration into the Central Nervous System Contributes to Neuroinflammation

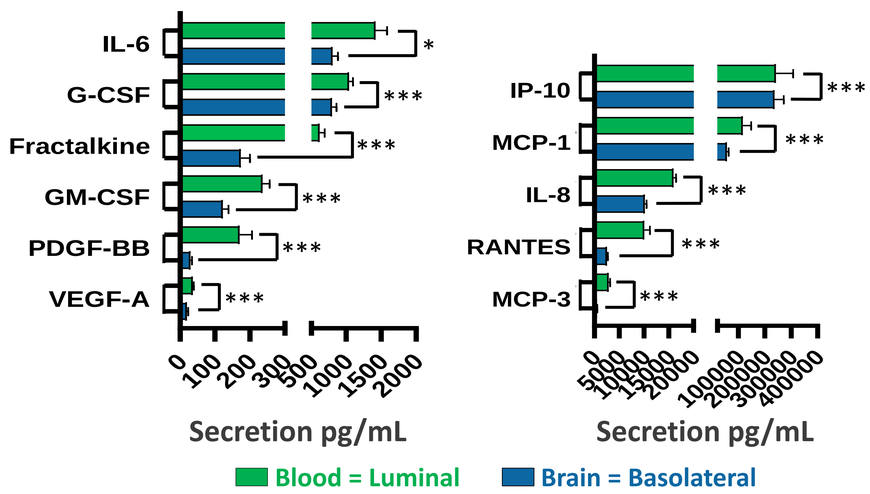


Polarized Secretion of Proinflammatory Cytokines by Brain Endothelial Cells

hCMEC/D3 cell monolayer was cultured on suspended inserts. Cytokines and compounds were added to the luminal chamber, followed by secretion profiling.



Luminal vs. Basolateral Secretion (TNFα+IFNγ, 24h)



Statistics: Two-Way ANOVA with Bonferroni's multiple comparisons test; * $p<0.05$; *** $p<0.001$

Apabetalone Counters Proinflammatory Cytokine Secretion of Brain Endothelial Cells

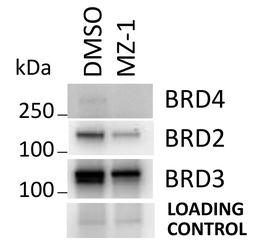
Protein Name	TNFα+IFNγ Fold Induction vs. DMSO	Apabetalone % Reduction	IL-1β Fold Induction vs. DMSO	Apabetalone % Reduction
"Blood"/Luminal Cytokines				
MCP-3	**305**	**93**	**101**	**81**
Fractalkine	**101**	**89**	5	47
GM-CSF	**11**	**85**	**64**	**42**
TNFα	No effect	No effect	**47**	**45**
IL-1RA	**7**	**52**	**5**	**28**
G-CSF	**8**	**52**	**21**	**9**
IL-6	**49**	52	**236**	**45**
IL-8	**16**	**41**	**31**	**39**
MCP-1	**16**	**41**	31	39
RANTES	**21**	21	6.0	54
IP-10	**4450**	30	23	55
"Brain"/Basolateral Cytokines				
Fractalkine	**16**	**87**	3	43
MCP-3	**58**	**83**	**43**	**78**
GM-CSF	6	82	**62**	**58**
MCP-1	**29**	**74**	**8**	**58**
TNFα	No effect	No effect	**37**	**49**
G-CSF	**7**	52	**48**	**12**
IL-1RA	**11**	**47**	**9**	37
IL-6	**15**	46	**92**	**50**
RANTES	**6**	**44**	1	7
IL-8	**8**	**39**	**20**	**37**
IP-10	**3435**	**26**	25	45

Statistics: Bold values represent a statistically significant change ($p<0.05$). One-Way ANOVA with Tukey's test.

Cytokine Expression in Brain Endothelial Cells Is BET Protein Dependent

hCMEC/D3 endothelial cell line was co-treated for 24h with 10 ng/mL TNFα+IFNγ and DMSO or 0.2 μM MZ-1 (BET degrading compound). BET expression was analyzed by Western blot. Gene expression was analyzed by PCR.

BET knockdown by MZ-1



Cytokine Gene	TNFα+IFNγ Fold Induction	0.2μM MZ-1 % Reduction
MCP-3	**25**	**-91**
Fractalkine	**124**	**-86**
MCP-1	**6**	**-48**
RANTES	**24**	**-43**
IL-6	**13**	**-42**
IL-8	**5**	**-38**
G-CSF	**4**	No effect
IP-10	**608**	**-23**
GM-CSF	**2**	No effect

Statistics: One-Way ANOVA with Tukey's test; bold: $p<0.05$

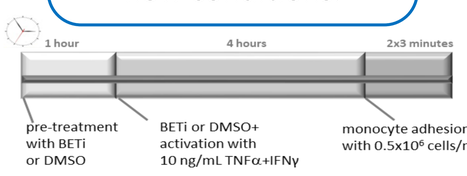
Apabetalone Reduces Monocyte Adhesion to Activated Brain Endothelial Cells

Primary human brain microvascular endothelial cells were stimulated for 4h with 10 ng/mL TNFα+IFNγ ± DMSO or apabetalone, followed by gene expression analysis (real-time PCR) and surface protein quantification (FACS).

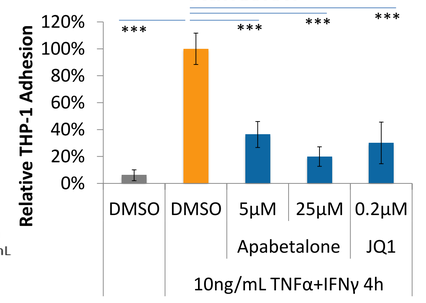
	mRNA Expression			Surface Protein Level		
	TNFα+IFNγ	5μM Apa	25μM Apa	TNFα+IFNγ	5μM Apa	25μM Apa
	Fold Induction	% Reduction		Fold Induction	% Reduction	
VCAM-1	355	-45	-89	12	-53	-81
E-selectin	32	-16	-43	372	No effect	-53

Statistics: One-Way ANOVA with Tukey's multiple comparisons test; bold: $p<0.05$

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

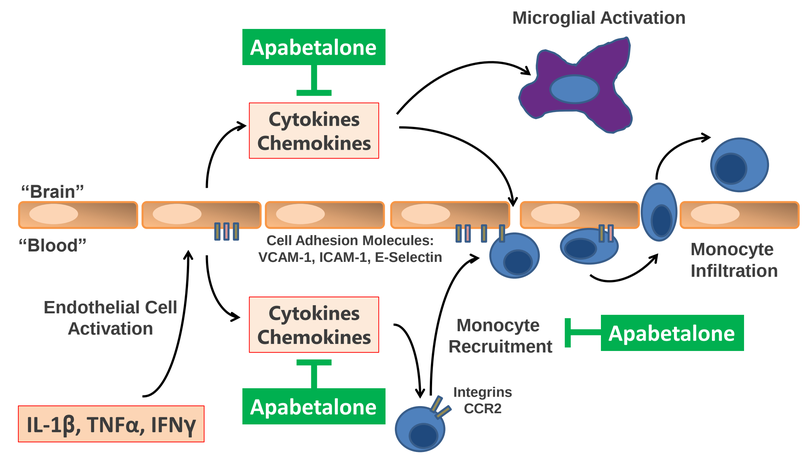


Monocyte – HBMVEC Adhesion



Statistics: ANOVA with Tukey's multiple comparisons test; *** $p<0.001$

Summary and Conclusions



- **Apabetalone decreases endothelial chemokine secretion and endothelium-monocyte adhesion in an *in vitro* BBB model.**
- **This may reduce immune cell transmigration into the brain during neurovascular inflammation and neurodegeneration.**
- **These anti-inflammatory effects may contribute to the favourable effect of apabetalone on cognition in patients with MoCA scores XX-XX from the phase 3 cardiovascular outcomes trial (BETonMACE).**

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