The Epigenetic BET Protein Inhibitor Apabetalone Counters Brain Endothelial Activation and Monocyte Adhesion

Sylwia Wasiak[1], Li Fu[1], Emily Daze[1], Dean Gilham[1], Brooke D. Rakai[1], Stephanie C. Stotz[1], Laura M. Tsujikawa[1], Chris D. Sarsons[1], Deborah Studer[3], Kristina D. Rinker[3], Ravi Jahagirdar[1], Norman C. W. Wong[1], Michael Sweeney[2], Jan O. Johansson[2] and Ewelina Kulikowski[1]

[1]Resverlogix Corp. Calgary, AB Canada, [2]Resverlogix Inc. San Francisco, CA USA, [3]Department of Chemical and Petroleum Engineering, Calgary, AB Canada

Background

Increased immune activity at the blood brain barrier (BBB) can exacerbate neuroinflammation. With aging, overexpression of tissue nonspecific alkaline phosphatase (ALPL) abundance in the cerebrovasculature, can reduce BBB function. Bromodomain and extraterminal (BET) proteins are histone acetylation readers that activate cytokine-dependent transcription in monocytes and endothelial cells and promote ALPL expression. Targeting BETs with epigenetic therapies may reduce BBB dysfunction due to immune-brain signaling and ALPL expression.

Methods

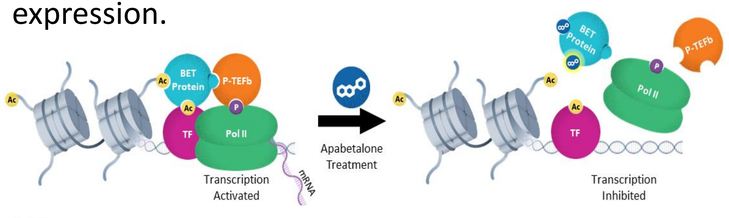
- **Polarized hCMEC/D3 cell monolayers** grown on suspended inserts: cytokine secretion (Multianalyte Profiling) was assessed in response to 25 μM apabetalone or 0.025% DMSO + 100 ng/mL IL-1β or TNFα+IFNγ (24h).
- **Primary human brain microvascular endothelial cells (HBMVECs)**: effect of apabetalone on gene expression and adhesion protein surface levels ±TNFα+IFNγ stimulation (4h) was assessed by PCR and FACS.
- **THP-1 monocyte cell line**: THP-1 adhesion to HBMVEC monolayers was measured under laminar flow conditions. Surface receptor expression was assessed by PCR and FACS.

Results

- **Stimulated hCMEC/D3 cells**: In response to TNFα+IFNγ or IL-1β, cells had polarized secretion profiles across the luminal and abluminal membranes. Apabetalone treatment (25μM) reduced gene expression and protein secretion of key inflammatory cytokines. BET dependency was confirmed with MZ-1 treatment, which degrades BET proteins.
- **HBMVEC-THP-1 interactions**: During TNFα+IFNγ stimulation, apabetalone inhibited surface expression of cell adhesion proteins VCAM-1 (5 and 25μM) and E-selectin (25μM) in HBMVECs. In THP-1 cells, chemokine receptors CCR1, CCR2 and CX3CR1 were also suppressed by apabetalone. Upon cytokine activation of the monolayer, HBMVEC-THP-1 interactions were reduced by both concentrations of apabetalone under flow conditions.
- **Non-stimulated HBMVECs**: Apabetalone treatment decreased *ALPL* gene expression in a dose dependent manner by up to 70%.

Drug Mechanism of Action

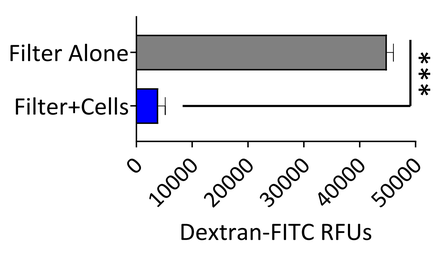
Apabetalone binds competitively to bromo-domains in histone acetylation "readers" termed BET proteins, causing their release from chromatin and downregulation of BET sensitive gene expression.

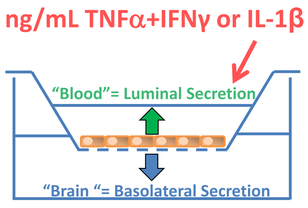


BET: bromodomain and extraterminal proteins; ac: acetylated lysine residue on DNA associated proteins; BD: bromodomain; TF: transcription factor

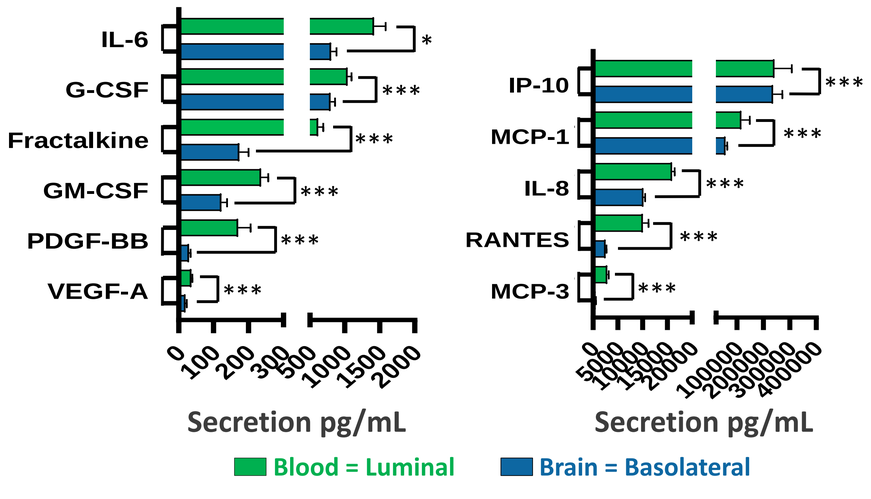
Apabetalone Counters Proinflammatory Cytokine Secretion of Brain Endothelial Cells

hCMEC/D3 Monolayer is Impermeable to Macromolecules





Protein Secretion is Polarized in hCMEC/D3 Monolayers (+TFNα/IFNγ, 24h)



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

Apabetalone Decreases Pro-inflammatory Secretion

Stimulation	Secretion	Fold Induction "Blood"/Luminal	% Reduction "Blood"/Luminal	Fold Induction "Brain"/Basolateral	% Reduction "Brain"/Basolateral
IL-1β	TNFα	**47**	**45**	**37**	**49**
TNFα+IFNγ	MCP-3	**305**	**93**	**58**	**83**
	Fractalkine	**101**	**89**	**16**	**87**
	GM-CSF	**11**	**85**	6	82
	IL-1RA	**7**	**52**	**11**	**47**
	G-CSF	**8**	**52**	**7**	52
	IL-6	**49**	52	**15**	46
	IL-8	**16**	**41**	**8**	**39**
	MCP-1	**16**	**41**	**29**	**74**
	RANTES	**21**	21	**6**	**44**
	IP-10	**4450**	30	**3435**	**26**

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

Apabetalone Reduces Cell Adhesion Receptors in Endothelial Cells and Monocytes

HBMVECs were stimulated for 4h with 10 ng/mL TNFα+IFNγ ± DMSO or apabetalone, followed by gene expression analysis (PCR) and surface protein quantification (FACS).

	mRNA Expression TNFα+IFNγ Fold Induction	mRNA Expression 5μM Apa % Reduction	mRNA Expression 25μM Apa % Reduction	Surface Protein Level TNFα+IFNγ Fold Induction	Surface Protein Level 5μM Apa % Reduction	Surface Protein Level 25μM Apa % 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

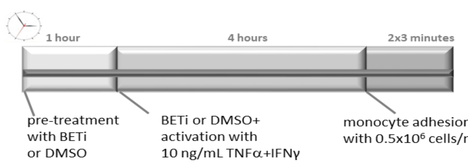
Unstimulated THP-1 cells were treated with 25μM apabetalone, followed by gene expression analysis (24h; PCR) and surface protein quantification (48h; FACS).

	mRNA Expression 5μM Apa Fold Induction	mRNA Expression 25μM Apa % Reduction	Surface Protein Level 5μM Apa % Reduction	Surface Protein Level 25μM Apa % Reduction
CCR1	**-33**	**-69**	**-50**	**-59**
CCR2	**-28**	**-74**	**-54**	**-79**
CX3CR1	No effect*	**-28***	**-44**	**-66**

Statistics: One-Way ANOVA with Tukey's multiple comparisons test; bold: p<0.05
* mRNA levels were measured at 48h post-dose.

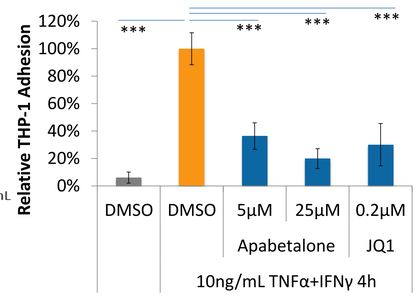
Apabetalone Reduces Monocyte Adhesion to Activated Brain Endothelial Cells

THP-1 cell adhesion to cytokine-treated HBMVECs in laminar flow conditions.



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

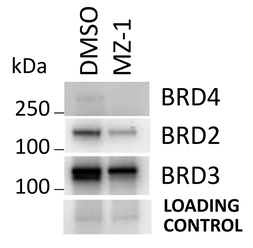
Monocyte – HBMVEC Adhesion



Cytokine Gene Expression in Brain Endothelial Cells Is BET Protein Dependent

hCMEC/D3 cells were treated with 10 ng/mL TNFα+IFNγ ± 0.2μM MZ-1 (BET degrading compound). BET protein knock down was analyzed by Western blot.

BET knockdown by MZ-1, 24h



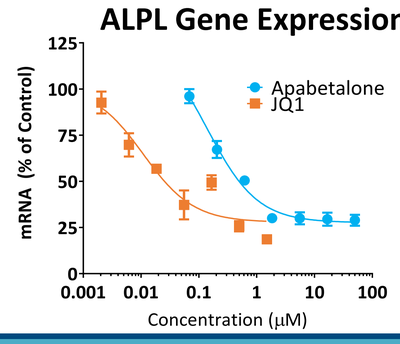
Cytokine Gene	TNFα+IFNγ Fold Induction	MZ-1 % Reduction
MCP-3	**25**	**-91**
Fractalkine	**124**	**-86**
MCP-1	**6**	**-48**
RANTES	**24**	**-43**
IL-6	**13**	**-42**
IL-8	**5**	**-38**
G-CSF	**4**	No effect
IP-10	**608**	**-23**
GM-CSF	**2**	No effect

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

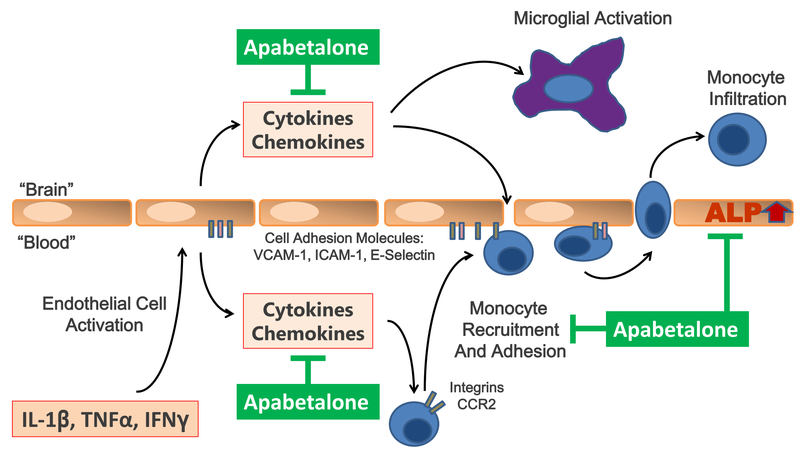
Alkaline Phosphatase Gene Expression in Brain Endothelial Cells is Suppressed by BETi

HBMVECs were treated with apabetalone or JQ1 for 4h, followed by gene expression analysis (PCR).

ALPL Gene Expression



Summary and Conclusions



- Apabetalone decreases endothelial chemokine secretion and endothelium-monocyte adhesion in a BBB model.
- This may reduce immune cell transmigration into the brain during neurovascular inflammation and neurodegeneration.
- Apabetalone may improve BBB function during aging due to alkaline phosphatase reduction (Haarhaus 2019 , Curr Opin Nephol Hyperten).
- These effects may contribute to the favourable effect of apabetalone on cognition in patients from the phase 3 cardiovascular outcomes trial (BETonMACE).

Disclaimer: Authors are Resverlogix' employees or contractors.