Apabetalone (RVX-208) Suppresses Expression of Key Vascular Inflammation Markers in Monocytes, Endothelial Cells and LPS-Challenged Mouse Liver and Monocyte Adhesiveness to Activated Endothelial Cells

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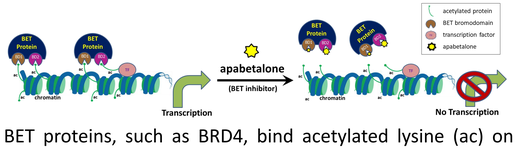
Abstract

Apabetalone (RVX-208) is a small molecule bromodomain & extraterminal (BET) protein inhibitor that selectively targets the second bromodomain (BD2). A phase 3 trial (BETonMACE) is being conducted to evaluate apabetalone's ability to prevent major adverse cardiac events in post-acute coronary syndrome patients with type 2 diabetes mellitus (DM) and low-HDL-C. In phase 2b trials, cardiovascular disease (CVD) patients treated with apabetalone demonstrated a 44% relative risk reduction in CVD events (Nicholls 2017). In CVD and DM, elevated cytokines drive vascular inflammation (VI). TNFα mediated activation of the transcription factor NF-κB is linked to the induction of inflammatory and adhesion marker expression in vascular endothelial cells and monocytes (Pierce 1988, Baltimore 2011). In human umbilical vein endothelial cells (HUVECs), apabetalone treatment did not prevent TNFα-induced translocation of NF-κB subunit RelA to the nucleus but did inhibit the transcription of genes regulated by RelA. These include cell adhesion molecules (CD44, E-selectin, VCAM1 and MCP-1) and inflammatory cytokines (IL-6, IL-8, IL-1β, and CSF2). At the protein level VCAM1, MCP-1, and E-selectin expression was also suppressed. In TNFα-stimulated monocytes (THP-1 cells), apabetalone also reduced the upregulation of inflammatory and adhesion molecule expression (CCR1, CCR2, IL-1β, MCP-1, MYD88, TLR4, TNFα, and VLA-4). *In vivo*, leukocytes adhere to an inflamed endothelium where they extravasate into arterial walls and initiate atherosclerotic plaque formation. In our *in vitro* assays, apabetalone suppressed monocytic THP-1 cell adhesion to inflamed endothelial cells under both static (HUVEC) and flow (HAEC) conditions. Acute endotoxemia is associated with activation of liver macrophages and endothelial cells and infiltration of immune cells. In mice exposed to 50 μg of LPS for 24h, apabetalone reduced liver mRNA marker expression for infiltrating monocytes, activated macrophages, and cellular adhesion (CD14, CCR2, ICAM and P-selectin). Our data indicate that apabetalone attenuates VI through the regulation of transcription.

Mechanism of Action



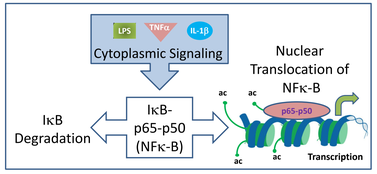
BET proteins, such as BRD4, bind acetylated lysine (ac) on histones or transcription factors (TF) via bromodomains (BD), and recruit transcriptional machinery to drive expression of BET sensitive genes. Apabetalone targets bromodomains in BET proteins, causing release from chromatin and downregulation of BET sensitive gene expression.

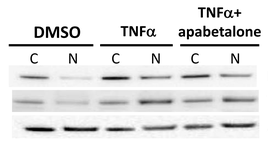
Yellow star size indicates selectivity of apabetalone for BD2.

Results

1. Apabetalone does not prevent the translocation of NF-κB subunit p65 to the nucleus in TNFα-stimulated endothelial cells (HUVECs)

NF-κB p65 subunit and its phosphorylated form (phospho-p65) are found almost exclusively in the cytoplasm (C) under non-stimulated conditions (DMSO). TNFα stimulation causes p65 translocation to the nucleus (N). Apabetalone (20 μM) pretreatment (4h) prior to TNFα stimulation does not alter p65 translocation.





Legend: C: Cytoplasm, N: Nucleus

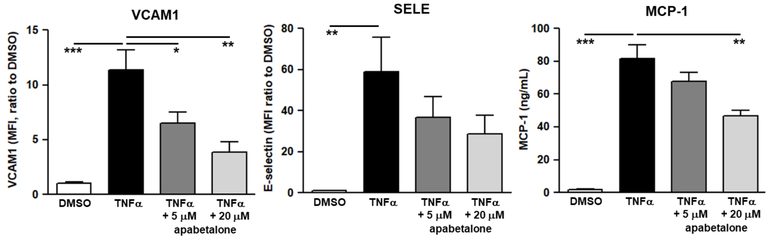
2. Apabetalone inhibits the transcription of HUVEC genes induced by TNFα, IL-1β and LPS stimulation.

mRNA transcripts of key HUVEC cytokine, chemokine, TLR Signaling, and adhesion molecules are induced by TNFα, IL-1β and LPS (1h pre-treatment), and reduced by apabetalone (4h treatment).

Simulation:		TNFα			IL-1β			LPS		
		Fold induction	% reduction		Fold induction	% reduction		Fold induction	% reduction	
			Apabetalone			Apabetalone			Apabetalone	
	Gene	Control	5 μM	20 μM	Control	5 μM	20 μM	Control	5 μM	20 μM
Cytokines	COX2	4	NS	86	19	46	85	1	42	83
	CSF2	945	82	98	8096	59	91	9	64	85
	IL-1β	1685	90	99	ND	ND	ND	ND	ND	ND
	IL-6	9	51	91	191	54	84	1.6	67	69
	IL-8	26	ND	48	ND	ND	ND	ND	ND	ND
	OPG	43	95	99	142	96	99	1.4	71	84
Chemokine	MCP-1	4	21	71	44	35	62	4	50	82
TLR signaling	MYD88	1	NS	56	1	30	66	1.6	44	38
Adhesion molecules	CD44	2	NS	34	3	NS	NS	1	33	34
	SELE	1164	NS	54	368	17	40	11	51	76
	VCAM1	196	59	83	96	72	91	6	73	96

3. Apabetalone suppresses protein expression of VCAM1 and MCP-1, but not E-selectin in endothelial cells.

4 hour TNFα stimulation induced HUVEC VCAM1 and SELE surface expression (FACS) and MCP-1 secretion (BD™ cytometric bead array). Co-treatment with apabetalone reduced VCAM1 and MCP-1 protein levels. Statistical analysis: 1-way ANOVA, Dunnett's Multiple Comparison Test



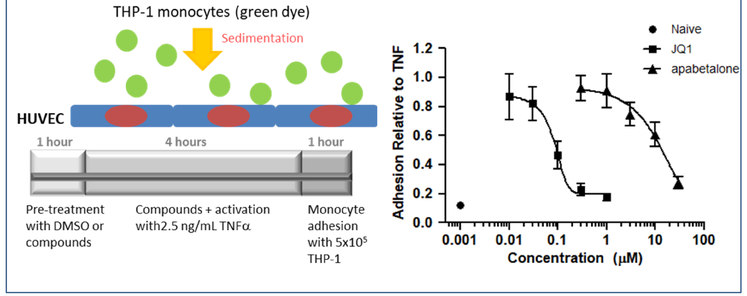
4. Apabetalone inhibits the transcription of THP-1 monocyte genes induced by TNFα stimulation.

mRNA transcripts of key THP-1 cytokine, chemokine, TLR Signaling, and adhesion molecules are induced by TNFα (4h) and reduced by apabetalone.

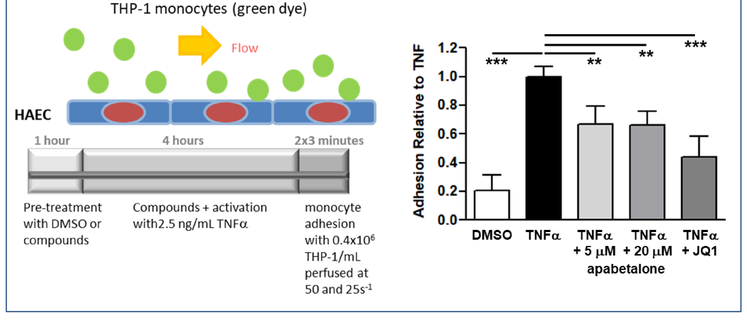
TNFα		Fold induction	% reduction	
		Control	Apabetalone	
	Gene		5 μM	20 μM
Cytokines	IL-1β	3.5	75	84
	TNFα	3.8	NS	54
Chemokines	CCR1	1.4	51	85
	CCR2	0.5	50	92
	MCP-1	3.7	77	91
TLR signaling	MYD88	2.6	39	71
	TLR4	0.7	NS	51
Adhesion molecules	CD44	1.8	26	39
	VLA-4	0.9	35	61

5. Apabetalone suppressed monocytic THP-1 cell adhesion to inflamed endothelial cells under both static (HUVEC) and flow (HAEC) conditions.

Static Cell Adhesion Assay

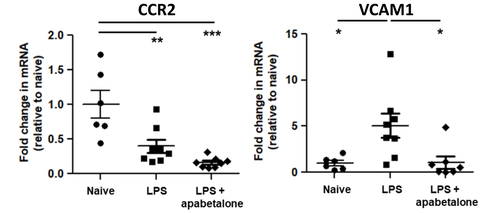


Flow Chamber Cell Adhesion Assay



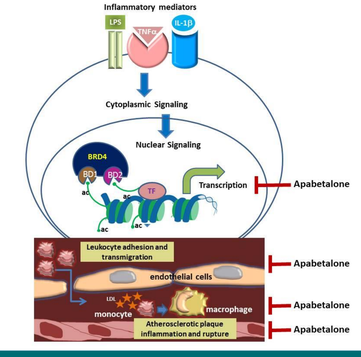
6. In the mouse endotoxemia model apabetalone reduces gene expression associated with monocyte infiltration, macrophage activation, and cellular adhesion.

Mice received apabetalone (gavage, 150 mg/kg, BID) for 7 days prior to an IP injection of LPS (50 μg per animal). Animals were sacrificed and tissue harvested 24h later.



Graphical Summary

Apabetalone downregulates the inflammatory response in endothelial and monocytic cells.



Downregulation of vascular inflammation may contribute to the reduction in MACE, a hypothesis currently being tested in the phase 3 cardiovascular outcomes trial BETonMACE in patients with CVD, diabetes mellitus and low HDL-c.

†**Disclosure:** Resverlogix employees received salaries & stock options from RVX.