Epigenetic BET Reader Inhibitor Apabetalone (RVX-208) Counters Proinflammatory Aortic Gene Expression in a DIO Mouse Model

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BACKGROUND

Obesity increases the risk of type 2 diabetes (DM2) and cardiovascular disease (CVD), due to associated insulin resistance, dyslipidemia, high blood pressure and chronic inflammation. Bromodomain and extraterminal (BET) proteins such as BRD4 enhance the expression of proinflammatory genes by recruiting transcription factors to promoters and enhancers. Inhibition of BET binding to chromatin leads to anti-inflammatory and anti-atherogenic effects in models of DM2 and CVD. ***Here we evaluate the effects of apabetalone (Apa) - a clinical stage small molecule with potential to reduce CV events - on vascular inflammation in a DIO mouse model.***

METHODS

C57 BL/6J mice were manipulated as indicated (**FIG 1**). Gene expression was analyzed in the aorta by PCR and nCounter® Inflammation Panel (**FIG 2 and 4A**). Bioinformatics were performed with GSEA **(FIG 2)** and IPA® **(FIG 4B)**. Primary human aortic endothelial cells (HAECs) from T2D patients were treated with combinations of Apa and TNFα, followed by BRD4 ChIP-sequencing (1+1h), RNA-sequencing (1+1h) or real-time PCR (4+2h) (**FIG 5**).

RESULTS

Metabolic dysregulation in the DIO model: HFD induced weight gain, visceral obesity, high fasting blood glucose, glucose intolerance and insulin resistance compared to LFD controls, confirming metabolic dysregulation. Apabetalone did not impact metabolic parameters measured (not shown).

Gene profiling in the aorta: Profiling of 254 genes in the aorta showed an upregulation of 27 inflammatory genes in HFD mice as compared to LFD, including transcription factors *Rela*, *Hif1a* and *Tcf4,* involved in endothelial inflammation, hypoxia and growth. Other upregulated genes mapped to cytokine, cytoskeleton, coagulation and complement pathways (**FIG 2**).

Effects of apabetalone treatment: Apabetalone reduced aortic mRNA expression of transcription factors, chemokines, leukocyte and endothelial cell adhesion receptors (**FIG 4A**). Bioinformatics predicted enhanced signaling by cytokines in the HFD vs. LFD aorta, which was countered by apabetalone (**FIG 4B**). In HAECs, apabetalone lowered BRD4 binding to genes associated with endothelial activation and remodeling (**FIG 5**).

CONCLUSIONS

HFD induces vascular inflammation in mice. Apabetalone treatment diminishes this proinflammatory phenotype, providing mechanistic insight into how apabetalone reduces CVD risk in DM2 patients in clinical trials.

FIG 1: THE DIET INDUCED OBESITY (DIO) MOUSE MODELS HUMAN CHARACTERISTICS OF OBESITY, PRE-DIABETES AND DYSLIPIDEMIA

Pharmacological intervention with APABETALONE (clinical-stage BET inhibitor)

DIO Mouse

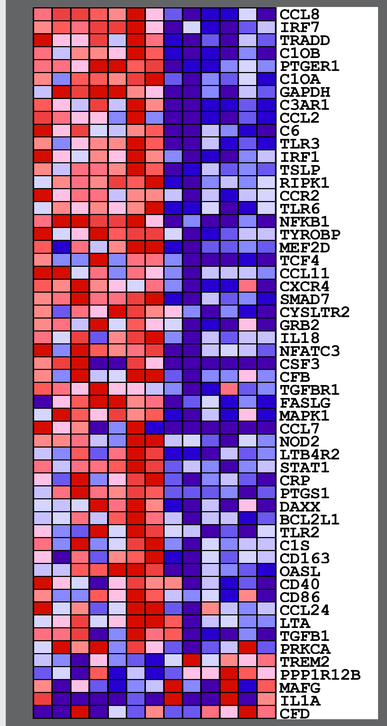
Age:

8 weeks	14 weeks	20-21 weeks	30 weeks
Low-fat (LFD) or High-fat (HFD) Diet	**Vehicle or APABETALONE 150 mg/kg b.i.d.**	Tests: Fasting glucose Glucose tolerance Insulin resistance	Aorta dissection Gene expression with Nanostring Inflammation Panel (254 genes)

FIG 2: HFD INDUCES PROINFLAMMATORY AORTIC GENE EXPRESSION

Gene expression (mouse aorta)

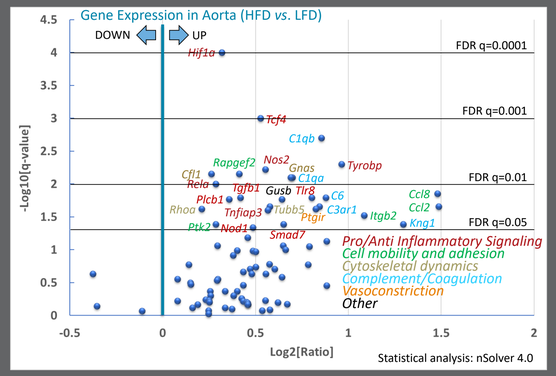
HFD (n=7) LFD (n=6) Gene



HFD enhances the expression of proinflammatory gene sets in the mouse aorta

GENE SETS (GSEA) Upregulated in HFD-fed mice	NES	Nominal p-value	FDR q-value
HALLMARK_INTERFERON_GAMMA_RESPONSE	1.43	0.019	0.364
HALLMARK_INTERFERON_ALPHA_RESPONSE	1.41	0.038	0.210
HALLMARK_COMPLEMENT	1.39	0.042	0.167
HALLMARK_ALLOGRAFT_REJECTION	1.38	0.033	0.135
HALLMARK_INFLAMMATORY_RESPONSE	1.34	0.117	0.170
HALLMARK_APOPTOSIS	1.34	0.089	0.142
HALLMARK_PI3K_AKT_MTOR_SIGNALING	1.28	0.200	0.182
HALLMARK_IL6_JAK_STAT3_SIGNALING	1.25	0.160	0.189

HFD enhances the expression of proinflammatory genes in the mouse aorta



Gene Set Enrichment Analysis (GSEA) Red: Upregulated; Blue: Downregulated

FIG 3: APABETALONE INHIBITS THE EPIGENETIC ACTIVITY OF BET PROTEINS

Clinical stage bromodomain inhibitor apabetalone binds competitively to BET histone acetylation "readers", causing release from chromatin, and downregulation of BET-sensitive genes expressed during chronic vascular inflammation.

Apabetalone's Epigenetic Mechanism of Action

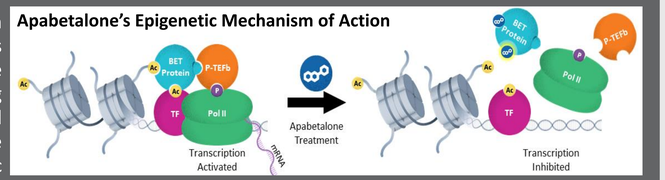


FIG 4: APABETALONE DOWNREGULATES PROINFLAMMATORY GENES AND CYTOKINE PATHWAYS ELEVATED IN THE DIO MOUSE AORTA

A. Aortic Gene Expression

NFk-B Signaling					Chemokines				
Gene	HFD *vs* LFD	*p*-value	Apa *vs* HFD	*p*-value	Gene	HFD *vs* LFD	*p*-value	Apa *vs* HFD	*p*-value
Tnfaip3	1.48	**	0.57	***	*Ccl2*	2.80	**	0.53	*
Nod1	1.40	***	0.78	**	*Ccl8*	2.79	****	0.27	****
Ly96	1.37	*	0.66	**	*Ccl7*	1.84	*	0.57	*
Nfkb1	1.23	*	0.78	**	*Ccr2*	1.58	**	0.32	****
Rela	1.22	****	0.88	**	*Cxcl1*	1.56	**	0.69	*
Map3k7	1.06	*	0.92	**					
Cell Adhesion Receptors					**Growth and Hypoxia**				
Gene	HFD *vs* LFD	*p*-value	Apa *vs* HFD	*p*-value	Gene	HFD *vs* LFD	*p*-value	Apa *vs* HFD	*p*-value
Sele	2.76	**	0.98	**	*Tcf4*	1.44	****	0.85	*
Itgam	1.23	ns	0.88	*	*Hif1a*	1.25	****	0.96	ns
Icam1	1.01	ns	0.64	****					

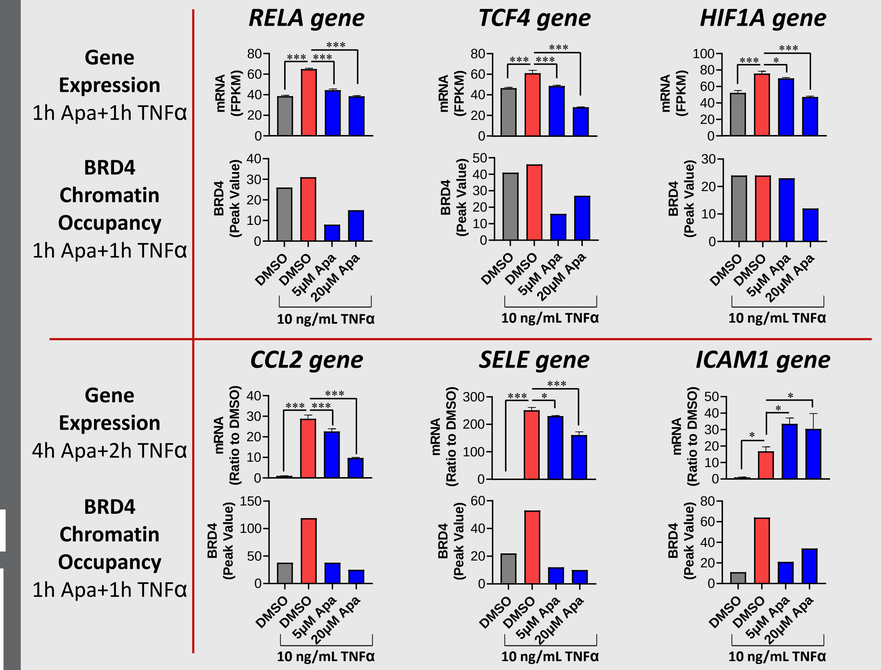
Ratio between groups indicate gene upregulation (yellow) or downregulation (blue); 3-group statistical analysis with 1-way ANOVA followed by Dunnett's multiple comparisons test where *, p<0.05; **, p<0.01; ***, p<0.001, ****p<0.0001; ns, non-significant

B. IPA® Pathway Analysis: Upstream Regulators

	HFD *vs* LFD		Apa *vs* HFD	
	z-score	*p*-value	z-score	*p*-value
TNFα	3.8	<0.0001	-2.3	0.003
IFNγ	2.6	<0.0001	-2.2	0.0002
IL-1β	3.1	<0.0001	-2.1	0.0004
TGF β1	3.3	<0.0001	-2.0	0.03
GM-CSF	3.7	<0.0001	-2.0	<0.0001

z-score>2 indicates pathway activation; z-score<-2 indicates pathway suppression; *p*-value adjusted with Benjamini-Hochberg method

FIG 5: APABETALONE DOWNREGULATES BRD4 ASSOCIATION WITH GENES INVOLVED IN ENDOTHELIAL ACTIVATION AND REMODELING



Statistical Analysis: 1-way ANOVA with Dunnett's test where *, p<0.05; **, p<0.01; ***, p<0.001

DISCLOSURE

SW, ED, LMT, DG, CS, SCS, BDR, RJ, MS, JOJ, NCW and EK receive(d) a salary from Resverlogix and own stock and stock options. SA has no competing interests.