Apabetalone, a Bromodomain and Extraterminal Protein Inhibitor, Decreases Key Factors in Vascular Calcification In Vitro and in Clinical Trials

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Abstract

INTRODUCTION AND AIMS:

Apabetalone, an orally active bromodomain and extraterminal (BET) protein inhibitor, reduced incidence of major adverse cardiac events (MACE) in patients with CVD and improved eGFR in a subpopulation with chronic kidney disease (CKD) in phase 2 trials. In CKD patients, vascular calcification (VC) increases CVD risk & is a predictor of all-cause mortality. The process of VC involves differentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells resulting in altered gene expression, loss of contractility & extracellular mineralization. Here we report clinical effects of apabetalone on circulating levels of factors involved in VC, including alkaline phosphatase (ALP), an enzyme that regulates pyrophosphate levels and contributes to calcium deposition. Circulating ALP is derived primarily from liver, & elevated ALP is associated with mortality in CKD or patients on dialysis. In vitro, cell systems demonstrate effects of apabetalone on expression of VC markers, differentiation of coronary artery VSMCs & pathological process of extracellular calcium deposition.

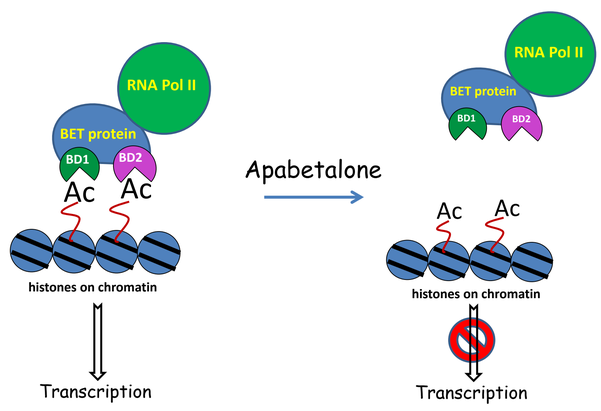
METHODS:

Effects of apabetalone on expression of osteogenic markers were investigated in primary human hepatocytes (PHH), human macrophages (U937), and primary human VSMCs. Extracellular calcium deposition induced by osteogenic culture conditions was measured in VSMCs. Proteomic assessment of plasma from a phase 1 trial in CKD patients receiving a single 100 mg oral dose of apabetalone was conducted using Ingenuity® Pathway Analysis. Proteins associated with VC were also assessed in plasma of CVD patients receiving apabetalone in 3 month (ASSERT) and 6 month (SUSTAIN & ASSURE) phase 2 trials.

RESULTS:

Factors involved in the process of VC are derived from multiple cell types. In PHH cells from multiple donors, ALP was downregulated 60-80% by apabetalone. Apabetalone also reduced expression of osteopontin, another established marker of VC, in PHH, VSMCs & U937 macrophages. Differentiation of primary VSMCs with osteogenic conditions induced expression of ALP, osteoprotegerin, RUNX2 & WNT5A, which was suppressed by apabetalone. Further, apabetalone dose dependently countered calcium deposition in VSMCs. Clinical trials support translational mechanisms investigated in vitro. Proteomic analysis of plasma from stage 4 CKD patients (n=8) demonstrated significant activation of pathways driving calcification including "BMP-2 signaling" and "RANK signaling in osteoclasts" versus age, gender & BMI matched individuals (n=8). Both pathways were downregulated by apabetalone 12 hours after a single dose. Apabetalone also significantly reduced circulating levels of proteins associated with VC in phase 2 trials in CVD patients, including ALP, osteopontin & osteoprotegerin.

Mechanism of Action

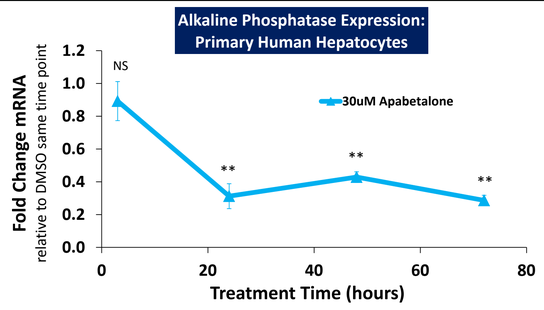


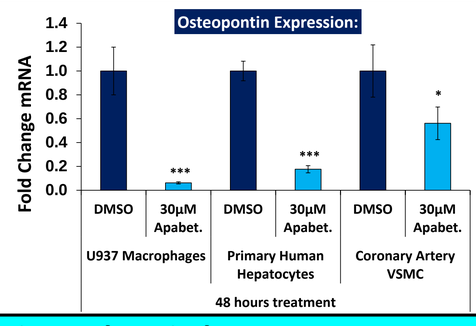
BET proteins, such as BRD4, bind acetylated lysine (Ac) on proteins such as histones via bromodomains (BD), and recruit transcriptional machinery to drive expression of BET sensitive genes. Apabetalone inhibits BET bromodomains, causing release from chromatin and downregulation of BET sensitive gene expression.

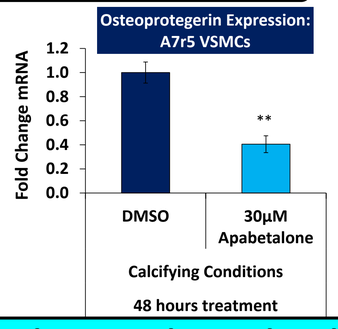
Acknowledgment: Figure originally developed by Dr. Olesya Kharenko

Results

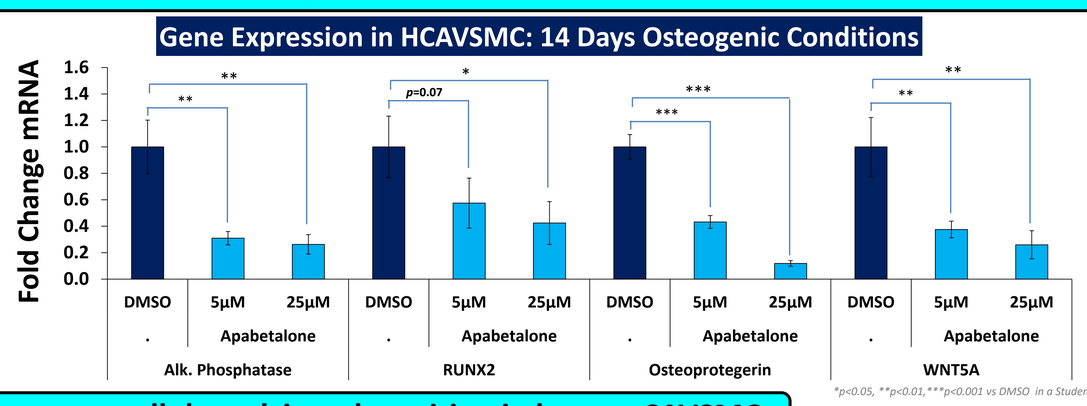
1. Apabetalone downregulates expression of genes related to vascular calcification in multiple cell types





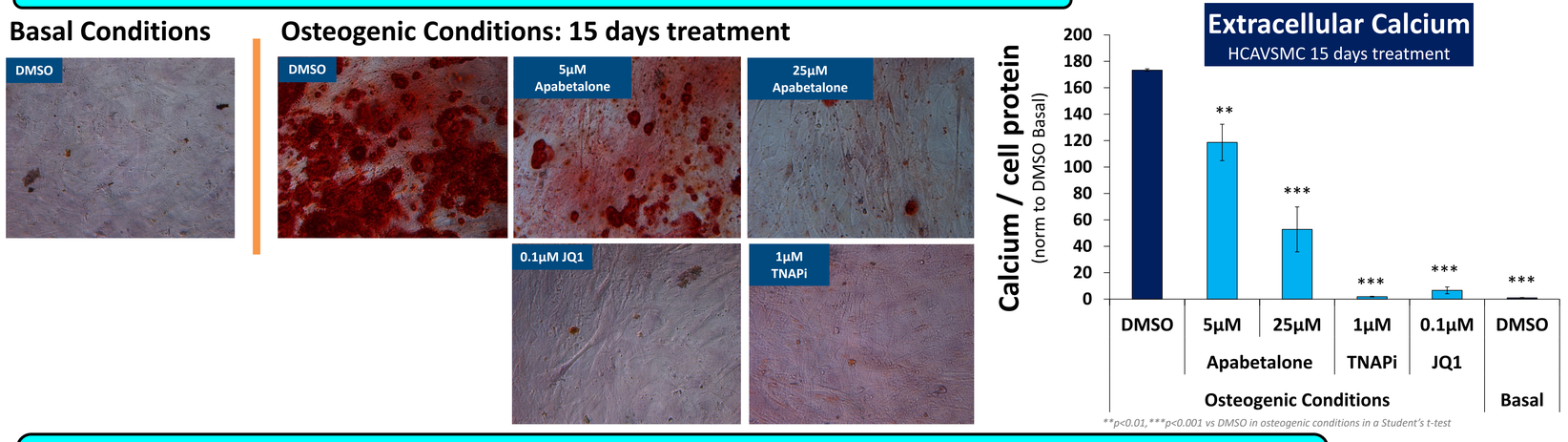


2. Apabetalone opposes induction of osteogenic markers in human coronary artery vascular smooth muscle cells



*p<0.05, **p<0.01,***p<0.001 vs DMSO in a Student's t-test

3. Apabetalone counters extracellular calcium deposition in human CAVSMCs



p<0.01,*p<0.001 vs DMSO in osteogenic conditions in a Student's t-test

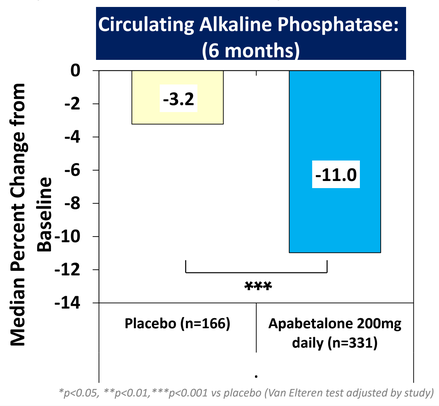
4. Plasma proteomics show pathways associated with VC are elevated in patients with stage 4 CKD versus matched controls. Apabetalone down regulates these pathways in CDK patients

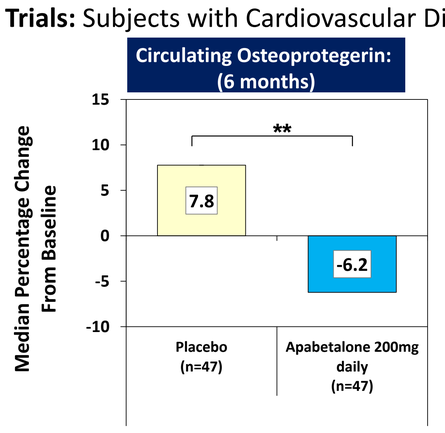
Phase I, Safety & PK Study: Subjects with Severe Renal Impairment (CKD); 12 hours post single 100mg dose (n=8)

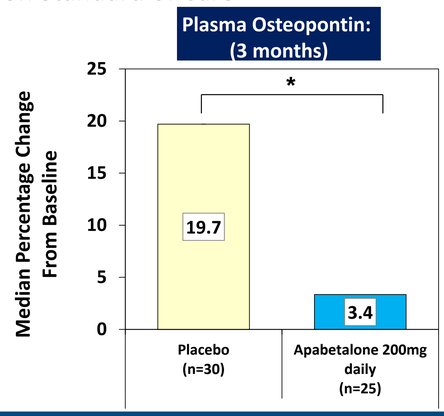
Bioinformatics (IPA) Analysis of the Plasma Proteome (SOMAscan™)				
Pathways associated with VC are elevated in CKD vs controls at baseline			Apabetalone downregulates pathways associated with VC in CKD patients 12 hr post-dose	
Pathway	**Regulation in CKD vs controls**	***P*-value**	**Response to apabetalone**	***P*-value**
BMP signaling pathway	IPA z-score: +2.12	0.0000062	IPA z-score: -2.67	0.000000102
RANK signaling in osteoclasts	IPA z-score: +1.89	0.00033	IPA z-score: -4.36	3.53×10^{-10}
z-score negative = downregulation ; z-score positive = upregulation			*no modulation of either pathway by Apabetalone in control subjects.	

5. Apabetalone decreases circulating levels of proteins associated with VC in patients with cardiovascular disease

Phase 2, Placebo Controlled, Double Blinded Trials: Subjects with Cardiovascular Disease on Standard of Care







*p<0.05, **p<0.01,***p<0.001 vs placebo (Van Elteren test adjusted by study)

Summary and Conclusions

- Apabetalone reduced expression of factors associated with vascular calcification (VC) in multiple cell types. Simultaneous effects on contributing cell types suggest apabetalone may decrease pathologic calcification.
- Apabetalone dose dependently countered calcium deposition in primary human coronary artery vascular smooth muscle cells, suggesting treatment can oppose this process in the vasculature.
- A single 100 mg dose downregulated pathways associated with VC in patients with chronic kidney disease (CKD).
- In phase 2 clinical trials in patients with cardiovascular disease (CVD), apabetalone reduced circulating factors associated with VC.
- Apabetalone may decrease VC in patients with CKD and contribute to reduction in coronary events in patients with high CVD risk, which Resverlogix is exploring in the phase 3 BETonMACE cardiovascular outcomes trial that enrolled patients with established CVD and diabetes mellitus on standard of care.