Hepatic Expression of C-Reactive Protein is Epigenetically Regulated by BET Proteins and Inhibited by Apabetalone (RVX-208) in Vitro and in CVD Patients

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ABSTRACT

Chronic inflammation contributes to cardiovascular disease (CVD) and is characterized by elevated plasma levels of IL-6, IL-1β and C-reactive protein (CRP). CRP serves as a CVD stratification marker as it correlates with major adverse cardiac events (MACE). Here we show that hepatic induction of CRP in response to chronic cytokine signaling is regulated by epigenetic mechanisms in vitro and in patients.

Apabetalone is a small molecule inhibitor of epigenetic readers called bromodomain and extraterminal (BET) proteins that bind to acetylated DNA-associated proteins to regulate inflammatory gene transcription. In vitro, apabetalone attenuated CRP gene and protein expression under basal conditions in cultured primary human hepatocytes (PHH). Moreover, IL-6 and IL-1β mediated induction of CRP expression was also suppressed by apabetalone in both PHH and the HepaRG hepatic cell line. In HepaRG, PROTAC M2-1 targeted degradation of BET proteins also reduced cytokine mediated CRP expression, demonstrating that inflammatory expression of CRP is BET-dependent. Short-term cytokine treatment increased occupancy of the BET family member BRD4 on the CRP promoter, which was countered by either apabetalone or a structurally unrelated BET inhibitor JQ1, as shown by chromatin immunoprecipitation (ChIP). These data directly link BRD4 to CRP transcription.

In a pooled analysis of phase 2 trials ASSET, SUSTAIN and ASSURE, treatment with apabetalone resulted in a 62% relative risk reduction in MACE in patients with coronary artery disease (CAD) and elevated CRP (>2mg/L). In both ASSET (12 weeks; n=55) and ASSURE (26 weeks; n=94), a comparison of baseline and end-of-study plasma proteome (SOMAscan 1.3K platform) detected a downregulation of inflammatory mediators, including CRP, in apabetalone treated patients versus placebo. Consequently, bioinformatics analysis of the proteomics data predicted an apabetalone-driven downregulation of inflammatory pathways.

MECHANISM OF ACTION

Apabetalone targets bromodomains in BET proteins, causing release from chromatin and downregulation of BET sensitive gene expression.

Apabetalone suppresses CRP expression in vitro

Primary human hepatocytes: Following 72h treatment with DMSO (vehicle) or 30 µM apabetalone ± 10 ng/ml IL-6, mRNA was analyzed by nPCR; protein secretion in the final 24h of the experiment was examined by ELISA. 1-way ANOVA (Tukey’s), *** p<0.001.

Apabetalone downregulates inflammatory pathways in CVD patients

Primary human hepatocytes: Following 72h treatment with DMSO (vehicle) or 30 µM apabetalone ± 10 ng/ml IL-6, mRNA was analyzed by nPCR; protein secretion in the final 24h of the experiment was examined by ELISA. 1-way ANOVA (Tukey’s), *** p<0.001.

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