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

Laura Tsujikawa 1, Emily Daze 2, Li Fu 3, Sylvia Wasiak 1, Chris D. Sarsons 1, Chris Halliday 1, Stephanie C. Stotz 1, Deborah Studer 1, Kristina D. Rinker 1, Ravi Jahagirdar 1, Michael Sweeney 1, Jan O. Johannson 1, Norman C. Wong 1 and Ewelina Kulikowski 1

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 transcription 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 inflammation and adhesion molecule expression (CCR1, CCR2, IL-1β, MCP-1, MYD88, ILR4, 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 monochytic THP-1 cell adhesion to inflamed endothelial cells under both static (HUVECs) and flow (HACEC) conditions. Acute endothelial expressions 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)

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

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

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

5. Apabetalone suppressed monocyctic THP-1 cell adhesion to inflamed endothelial cells under static (HUVECs) and flow (HACEC) conditions.

Graphical Summary

Apabetalone downregulates the inflammatory response in endothelial and monocyte cells.

Downregulation of vascular inflammation may contribute to the reduction in MACE, a hypothesis currently being evaluated 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.