BS38 SRT1: A NOVEL REGULATOR OF INTEGRIN αIIbβ3 AND ACTIN CYTOSKELETON DYNAMICS IN PLATELETS

Maria Blanco, Amanda Unsworth, Sarah Jones. Manchester Metropolitan University, John Dalton Building Chester Street Manchester, G15 M15GD United Kingdom, UK

10.1136/heartjnl-2023-BCS.251

Abstract BS38 Figure 1  SRT1720 decreases platelet aggregation induced by collagen and TRAP-6. Washed platelets (A, B) and PRP (C, D) were incubated with SRT1720 for 10 (A, B) and 60 min (C, D) at 37°C. Platelet aggregation was induced with collagen and TRAP-6. Absorbance was measured and the aggregation percentage calculated. Dose-response curves were constructed (A, C) and aggregation was compared to vehicle (V) by Two-way ANOVA. SRT1720 reduced aggregation after TRAP-6 (0.3 μM, B) and collagen (0.3 μM, D) stimulation. Error bars represent ± SEM *p<0.05 n=6.
Sirtuin 1 (SIRT1) is an NAD+ dependent deacetylase with vasculoprotective properties, which maintains cardiometabolic homeostasis under oxidative stress and prevents ischaemia-reperfusion injury. Patients at high risk of atherothrombosis, such as diabetics and obese, display reduced SIRT1 levels, which were associated with enhanced thrombus formation in a murine model of arterial thrombosis. However, the importance of SIRT1 in regulating platelet function has not been evaluated. The aim of this study was to investigate the effect of the SIRT1 activators on platelet function and assess their potential use as a novel antithrombotic therapy.

Unless stated otherwise, all experiments were performed with platelets from healthy donors and SIRT1 was activated with SRT1720 10μM. The expression of SIRT1 was confirmed in platelets by Western blotting. Using PBA, it was demonstrated that SIRT1 activation with SRT1720 attenuates platelet aggregation induced with collagen and TRAP-6. Further evaluation of the role of SIRT1 in platelet activation showed that SRT1720 causes a 50% decrease in fibrinogen binding and a 30% reduction in α-granule release. The effect of SIRT1 activation in cytoskeletal rearrangement during platelet activation was also explored. Incubation of platelets with SRT1720 significantly reduced actin polymerization after platelet stimulation. Activation of SIRT1 with SRT1720 inhibited platelet adhesion and spreading on collagen and fibrinogen, but it did not alter agonist-induced coiling of the tubulin ring, indicating that SIRT1 is involved in platelet actin cytoskeleton reorganization. This was further supported by clot retraction assays, which demonstrated that SIRT1 inhibition with EX 527 caused a dramatic reduction in clot retraction, represented by an increase in the clot weight and the area occupied by the thrombus at different time points.

These results suggest that SIRT1 regulates platelet integrin αIIbβ3 activation and the subsequent cytoskeletal rearrangement. SIRT1 may therefore provide a novel therapeutic target to limit platelet activation and accelerate reperfusion after a thrombotic obstruction in a vessel while protecting against oxidative damage.

**Conflict of Interest None**

BS38 Figure 2  SIRT1 activation reduces platelet adhesion and spreading on collagen (A) and fibrinogen (B). Washed platelets were incubated with SRT1720 (10 μM) or vehicle for 10 min and exposed to fibrinogen 45 min at 37°C. Platelets were labeled with FITC-phalloidin. Representative images are shown. Error bars represent ± SEM. *p<0.05, **p<0.01 n=5.

---

TARGETING BACE1 TO IMPROVE ANGIOGENESIS IN TYPE 2 DIABETES

Eva Clavane, University of Leeds, Light Laboratories University of Leeds Leeds, WYK LS2 3AA United Kingdom, UK

10.1136/heartjnl-2023-BCS.252

**Background** β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) is an aspartyl protease that is known for its role in the formation of amyloid plaques in Alzheimer’s disease. Recent research has shown that BACE1 also plays a role in vascular homeostasis and proteolytically cleaves various angiogenic signaling factors including VEGF receptor 1 (VEGFR1), NOTCH ligands, the insulin receptor, and occludin. BACE1 activity is elevated in models of type 2 diabetes, suggesting a potential role for its contribution to abnormal vessel growth characteristic of diabetes-related complications.

**Methods** Retinal staining and the fibrin gel angiogenesis assay were used to identify a role for BACE1 in vessel growth in vivo and in vitro, respectively. Endothelium of the developing retinal vasculature in BACE1 deficient (KO) and wild type (WT) mice was stained with IsolectinB4-Alexa488 and imaged using confocal microscopy. Sprout formation was further analysed using the fibrin gel angiogenesis assay with human umbilical vein endothelial cells (HUVECs) treated with or without a highly specific BACE1 inhibitor or transfected to over-express BACE1. Primary isolated pulmonary endothelial cells (PECs) were isolated from BACE1 KO and WT control mice prior to Western blots.

**Results** BACE1 KO retinas had decreased radial outgrowth (16.82% ± 69.99, P=0.05), but increased branch points, vascular area, and quantity of filopodia compared to WT mice. Moreover, BACE1 KO PECs had reduced NOTCH1 signalling (26.73% ± 14.15, P=0.05) and Jagged-1 proteolysis (28.48% ± 14.61, P=0.05) compared to WT PECs. Also, when treated with a highly specific BACE1 inhibitor, WT PECs had increased phosphorylation of eNOS (51% ± 13.8% P=0.01).

HUVECs treated with a BACE1 inhibitor had increased sprouting (18.70%± 5.92, P=0.05) as well as increased