proliferation in response to flow were identified. Future work will validate the role of these genes in endothelial proliferation and atherosclerosis using mammalian models, and human cells and tissues.

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Conflict of Interest none

BS16 THE ROLE OF PARASYMPATHETIC NERVOUS SYSTEM IN THE INFARCT-LIMITING EFFECT OF SGLT2 INHIBITORS

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Introduction As long-term outcome in patients with acute myocardial infarction (MI) is predicted by final infarct size (IS), reducing IS is of paramount importance. Recent experimental studies have demonstrated a strong infarct-sparing effect of SGLT2 inhibitors – a class of drugs which have proved to be safe and beneficial in patients with heart failure. Repurposing SGLT2 inhibitors for the benefit of patients presenting with an acute MI should be preceded by investigation of the underlying mechanisms of infarct limitation. Experimental and clinical data indicate a potential role for autonomic modulation in these mechanisms, specifically sympatho-inhibition. The aim of this study was to evaluate the role of parasympathetic tone in the infarct-sparing effect of SGLT2 inhibitors.

Methods Twenty seven Sprague Dawley rats were fed with the diet containing the SGLT2 inhibitor Ertugliflozin or vehicle

Abstract BS16 Figure 1 The effect of parasympathetic denervation on acute cardioprotection by SGLT2 inhibitor Ertugliflozin (Ertu). IS – infarct size, AAR – area at risk. ** - $p < 0.01$, *** - $p < 0.001$.

for 3 days. Myocardial ischaemia/reperfusion injury was caused by a 40-min left anterior descending coronary artery occlusion followed by 2 hours of reperfusion under isoflurane anaesthesia (4% for induction and 1.5–2% for maintenance). Two groups of animals, pre-treated with Ertugliflozin, were subjected to parasympathetic denervation prior to myocardial ischaemia, either with the muscarinic receptor antagonist, atropine sulfate i.v. (2 mg/kg bolus, then 1 mg/kg/h), or bilateral cervical vagotomy (figure 1).

Results Pre-treatment with Ertugliflozin reduced IS by 63% ($p < 0.001$). Blocking muscarinic receptors with atropine abolished the infarct-limiting effect of Ertugliflozin ($IS = 45 \pm 2\%$, $p > 0.05$ vs. vehicle, $p < 0.001$ vs. ertugliflozin), whereas bilateral mechanical vagotomy only attenuated cardioprotection ($IS = 32 \pm 5\%$, $p < 0.01$ vs vehicle and Ertugliflozin).

Conclusion These results suggest that the Infarct-limiting effect of SGLT2 inhibitor Ertugliflozin may be mediated via M-cholinoreceptors.

Conflict of Interest No

BS17 RNA BINDING PROTEIN MULTIPLE SPLICING (RBPMS) DRIVES A CONTRACTILE SPLICING NETWORK IN HUMAN EMBRYONIC STEM CELL DERIVED VASCULAR SMOOTH MUSCLE CELLS

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Introduction Contractile Vascular Smooth Muscle cells (VSMCs) lining the walls of major arteries express a unique network of alternative splicing (AS) patterns in critical VSMC regulatory and functional genes (SM-AS). The regulation and impact of SM-AS on VSMC phenotype and behaviour, however, remain poorly understood. Previously, we uncovered a master splicing regulator, RNA Binding Protein Multiple Splicing (RBPMS), a factor highly expressed in tissue VSMCs. RBPMS contributes to about 20% of SM-AS seen in contractile VSMCs.^{1 2}

Aim To understand the contribution of RBPMS and the SM-AS splicing network to VSMC function.

Basic Methods We have used human embryonic stem cell derived VSMCs (hES-VSMC)³ – a system compatible with stable genetic manipulation and facile molecular and phenotypic assessment. We generated hESC clones housing Doxycycline-inducible⁴ RBPMS alongside GFP at the pUCAVS genomic safe harbour. Notably, induction from this locus was heterogeneous with GFP intensity acting as an indicator of RBPMS expression. This uniquely lends itself to downstream SM-AS and phenotypic comparison of cells with varying levels of RBPMS expression within the same population. For SM-AS assessment, mRNA-seq was performed on flow assisted cytometric sorted hES-VSMCs based on GFP expression followed by splicing network analyses using rMATS⁵, Matt⁶ and gene ontology (stringDB). For phenotype assessment, mitogen-induced proliferation of GFP high and low cells was monitored by estimating a cell-tracer dye dilution using flow cytometry (figure 2A). Motility was examined using live cell imaging followed by cell tracking analysis over 24 hours (ibidi) (figure 2B).

Results and Conclusions We observe that RBPMS-high cells possessed distinct splicing profiles from RBPMS-low cells. Statistical comparison of the two groups using rMATS5, revealed