**SGLT2 expression and cardioprotective mechanisms of SGLT2 inhibitors in preclinical human models of diabetogenic stress**

Introduction. While sodium-glucose co-transporter-2 (SGLT2) inhibitors exhibit notable cardioprotective effects, their mechanisms of action, which result in improved heart failure outcomes, remain unclear.

Aims. To investigate SGLT2 expression in human induced pluripotent stem cell (iPSC)-derived cardiac cells and evaluate the therapeutic effects of SGLT2 inhibitors under diabetogenic conditions using in vitro cardiac cellular models.

Methods. SGLT2 expression and therapeutic effects were assessed in iPSC-derived cardiomyocytes, endothelial cells, and cardiac fibroblasts, and in a multicellular cardiac organoid model under diabetogenic conditions using protein, RNA, and cardiac functional assays.

Results. Human iPSC-derived cardiac cells expressed SGLT2 with nuclear and perinuclear localisation, no membrane-bound SGLT2 localisation was observed. SGLT2 mRNA and protein were detected under both control and diabetogenic conditions, with endothelial cells showing elevated expression in response to diabetogenic stress (p<0.05). In 2D cell culture, diabetogenic stress significantly reduced the viability of cardiomyocytes and endothelial cells both by ~40% (p<0.0001), and while empagliflozin rescued endothelial cell viability by 11% (p<0.0001), cardiomyocyte viability was unaffected. Furthermore, empagliflozin reversed diabetogenic stress-induced activation of cardiac fibroblasts almost to the level of the control (p<0.05). 3D multicellular cardiac organoids exposed to diabetogenic conditions successfully modelled prolonged diastole (p<0.05), reduced beat rate variability (p<0.01), and reduced viability (p<0.001), representative of early-stage diabetic heart disease. However, no therapeutic effects of SGLT2 inhibitors were observed in this 3D model.

Conclusion. SGLT2 was detected in iPSC-derived cardiac cells. The responsiveness of non-myocytes (endothelial cells and cardiac fibroblasts) to SGLT2 inhibitors under diabetogenic stress suggests that crosstalk between non-myocytes and cardiomyocytes may contribute to the cardioprotective effects of SGLT2 inhibitors. Overall, 2D and 3D human iPSC cardiac models exhibit an excellent potential for studying the cardio-protective mechanisms of SGLT2 inhibitors and for the screening of other novel cardio-protective agents.