SIRT1-DEPENDENT RESPONSE IS ESSENTIAL FOR RESVERATROL TO UPREGULATE ANTIOXIDANT AND ANTIGLYCATIVE DEFENCES IN HIGH GLUCOSE-CHALLENGED HUVECS

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Abstract

Reactive oxygen species (ROS) and methlyglyoxal (MG) are partly responsible for the endothelial dysfunction observed in diabetes-related vascular complications. Endothelial function in high glucose (HG) is improved by resveratrol (RSV), a natural phytoalexin, however the mechanisms underlying RSV-dependent protection of endothelial cells upon HG are still debated. More importantly, the role of sirtuin 1 (SIRT1) in such cytoprotection remains to be elucidated.

Our work aimed to: a) provide details about the redox- and MG-related mechanisms underlying the protective effects of RSV in endothelial cells upon HG; b) establish whether SIRT1 is essential for RSV to protect endothelial cells against HG-dependent cytotoxicity; c) demonstrate whether SIRT1 is required for RSV to regulate ROS- and MG-targeting enzymatic systems in endothelial cells.

Human umbilical vein endothelial cells (HUVECs) were kept in 5.55 mM glucose (CTR) or 30.55 mM glucose (HG), and co-incubated with either RSV (5 μM) or RSV+EX527 (SIRT1 inhibitor) (5 μM+13.4 μM), on the basis of concentration-response curves. Cell viability and apoptosis were assessed by Trypan blue and Annexin V/PI staining, respectively. Morphological assessment was performed by scanning electron microscopy. Expression and function of SIRT1, SOD1, SOD2, CAT, and GLO1 were studied by quantitative relative real time RT-PCR, Western blotting (WB), and spectrophotometric enzyme assays. ROS- and MG-dependent damage was evaluated by TBARS assay and anti-argpyrimidine-based WB, respectively.

We revealed that: a) HG-challenged HUVECs showed increased oxidative/glycative damage, most likely due to impaired ROS and MG scavenging; b) RSV rescued the HG-induced impairment of ROS/MG scavenging, as well as prevented the pro-oxidant and pro-glycation effects of HG; c) the up-regulation of SIRT1 was essential for RSV to protect HUVECs from HG cytotoxicity, and to elicit antioxidant/antiglycative effects on HG-challenged HUVECs.

Keywords

Diabetes, redox homeostasis, glycation