SIRT1-Dependent Cytoprotective Effect of Resveratrol in HG-Challenged Human Endothelial Cells Involves Antiglycative and Antioxidant Responses

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Abstract

Overproduction of methylglyoxal (MG) and reactive oxygen species (ROS) occurs in hyperglycemia-induced endothelial dysfunction associated with diabetes. Resveratrol (RSV) has been proposed as an effective treatment for diabetic complications, that is likely associated with activation of SIRT1, a NAD^+ dependent enzyme, which deacetylates histones and regulates redox sensitive transcription factors. Although some preclinical studies attempt to investigate the role of RSV as an antioxidant and antiglycating agent on MG-induced toxicity, the precise effects of RSV against hyperglycemia in human endothelium environment are unknown. In particular, the interplay between protective effects of RSV and ROS/MG targeting defense mechanisms in the endothelium context remain to be elucidated. Alternatively, whether SIRT1 activation by RSV treatment participates in the modulation of MG-directed antiglycative system combating high glucose (HG) cytotoxicity. Here we identified key molecular players involved in the glycative/oxidative perturbations occurring in endothelial cells exposed to HG, we further attempted to determine whether RSV requires SIRT1 to trigger adaptive responses in HG-challenged endothelial cells.

Human umbilical vein endothelial cells (HUVECs) underwent a 24-h treatment with HG (25 mM), and co-incubated with either RSV (5 μM) or RSV+EX527 (SIRT1 inhibitor) (5 μM+13.4 μM, respectively). We evaluated: cell viability, apoptosis along with cellular morphology, expression of SIRT1, CAT (catalase), SOD1/2 (superoxide dismutase), glyoxalase 1 (GLO1), ROS- and MG-dependent damages, and redox balance of glutathione. The mitochondrial biomarkers were evaluated by investigating the expression of SIRT3 and acetylated SOD2.

We found that HG-induced (glycative stress) GS and (oxidative stress) OS, by reducing SIRT1 activity, diminishing the efficiency of MG- and ROS-targeting protection. RSV abolished the HG-dependent cytotoxicity, and this was associated with SIRT1 upregulation, together with increased expression of GLO1, improved ROS-scavenging efficiency, and total suppression of HG-related GS and OS. Moreover, we found that mitochondrial biomarkers positively responded to RSV treatment, as seen by both SIRT3 and acetyl SOD2 expression. Finally, we discovered that RSV failed to exert protective response against HG cytotoxicity when SIRT1 was inhibited, suggesting that the upregulation of SIRT1 is essential for RSV to activate the major antiglycative and antioxidative defense and avoid MG- and ROS-dependent molecular damages in HG environment.

Keywords: oxidative stress; glycative stress; dicarbonyl stress; antioxidant defense; superoxide dismutase; catalase; methylglyoxal; glyoxalase; SIRT1; SIRT3; EX527.