Sulodexide prevents high glucose damage in human retinal endothelial cells through inhibition of AGE/RAGE signaling

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Diabetic retinopathy is characterized by the breakdown of the inner endothelial blood-retinal barrier. We tested the hypothesis that sulodexide (SDX) protects human retinal endothelial cells (HREC) from high glucose (HG) -induced damage through the suppression of inflammatory cPLA2-COX-2 pathway by blocking the effect of advanced glycation end-products (AGEs). HREC were treated with HG (25 mM) or AGEs (2 mg/ml) for 48h with or without SDX (60 µg/ml) or Aflibercept (AFL, 40 µg/ml), an anti-VEGF agent. After treatments cells were analyzed with MTT and LDH assays, Trans Endothelial Electrical Resistance (TEER) measurement, Immunohistochemistry, Tube Formation Assay, Western Blot and Real-time PCR. SDX prevented cell viability reduction induced by HG or AGE (by about 70% and 60% respectively, $p<0.05$) and reduced LDH release by about 75% ($p<0.05$). Furthermore, SDX reduced the HG-induced decreases of TEER, tube formation and Claudin-5 expression. Phospho-cPLA2 and prostaglandin E2 (PGE2) levels increased by HG (2.4 and 3.1 fold vs control) were rescued by SDX (by 40% and 60% respectively, $p<0.05$ vs control). AFL lowered PGE2 release (by about 75% $p<0.05$) in VEGF-treated cells (80 ng/ml) but was ineffective in HG treated cells, whereas SDX reduced PGE2 release induced by either HG or VEGF. SDX dumped VEGF mRNA and release in HG-challenged HREC and reduced phospho-ERK1/2, which was increased (about 2.8 fold vs control $p<0.01$) by AGE-treatment. Analysis of the NFκB activity revealed that HG increased the abundance of p65 in the nuclear fraction (nuclear translocation), an effect entirely reverted by SDX, but only partially by AFL. These data indicate that SDX protects HREC from HG damage counteracting cPLA2/COX-2/PGE2 pathway, reducing VEGF up-regulation and blocking the effect of AGEs and NFκB activation.