VEGF EXACERBATES DAMAGE INDUCED BY HIGH-GLUCOSE IN HUMAN RETINAL ENDOTHELIAL CELLS

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Diabetic retinopathy (DR) is characterized by high levels of VEGF in the back of the eye. Here we assessed the effect of VEGF in presence of high glucose (HG) in human retinal endothelial cells (HREC), an in vitro condition that mimic DR. HREC were treated with normal concentration of glucose (NG, 5 mM), with HG (25 mM) or with HG plus VEGF (40 or 80 ng/ml), for 48h. In some experiments, aflibercept (40 μg/ml), bevacizumab (25 μg/ml) or ranibizumab (10 μg/ml) were added to the HG medium. Effects of treatments were analyzed by MTT and LDH assays, Tube Formation Assay and Western Blot. Cell viability was reduced by about 35% ($p<0.05$) in HG-treated cells, and by about 55% ($p<0.05$) in cells treated with both VEGF and HG. Moreover, LDH release (cytotoxicity marker) was induced by HG treatment (about 2.2 fold, $p<0.05$) and by co-treatment with VEGF plus HG (3.2 fold, $p<0.05$). VEGF strongly reduced tube formation properties in presence of HG (about 65%, $p<0.05$) after 24h of treatment while, as expected, triggered about 40% increase of tube-like structures in absence of HG. Co-treatment with aflibercept, bevacizumab and ranibizumab partially restored cell viability reduction HG-induced by about 60, 40 and 55% ($p<0.05$) respectively, as well as prevented the increase of phospho-cPLA2 level prompted by HG (assessed by western blot). Furthermore, aflibercept, bevacizumab and ranibizumab prevented HG-induced reduction of tube-like formation of about 80, 60 and 75% ($p<0.05$) respectively. These data suggest that VEGF participates to HG-induced damage in human retinal endothelial cells through the induction of pro-inflammatory cPLA2.

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