Anti-VEGF agents inhibit activation of PLA_2/COX-2 and expression of VEGF-A induced by high glucose, in human retinal pericytes in vitro

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Diabetic retinopathy, a major cause of vision loss, is currently treated with anti-VEGF agents, including aflibercept, bevacizumab and ranibizumab, to counteract the detrimental effects of VEGF on retinal vasculature. Because these agents are administered intravitreally, when diffusing to the retinal vessel wall, they reach first, and in a larger proportion the external layer (pericytes), before reaching the inner layer (endothelial cells). In the present *in vitro* study we tested two hypotheses: i) high glucose damages retinal pericytes, *via* VEGF induction, which may be counteracted by anti-VEGFs and ii) activation of PLA_2/COX-2 pathway by high glucose might be upstream and/or downstream of VEGF in pericytes, as previously observed in endothelial cells (Lupo et al., 2013).

Human retinal pericytes were treated with high glucose (25 mM) for 48 h and/or anti-VEGFs (40 μg/ml aflibercept, 25 μg/ml bevacizumab, 10 μg/ml ranibizumab). All anti-VEGFs significantly reduced high glucose-induced cell damage (assessed by LDH release) and improved cell viability (assessed by MTT and Evans blue). High glucose increased VEGF-A expression, as detected both at mRNA (qPCR) and protein (ELISA) level, while receptor ($VEGFR_1$ and $VEGFR_2$) expression was unchanged. High glucose also activated the PLA_2/COX-2 pathway, as revealed by increased phosphorylation of $cPLA_2$, COX-2 protein expression and PGE_2 release. Treatment with $cPLA_2$ (50 μM AACOCF3) and COX-2 (5 μM NS-392) inhibitors prevented both cell damage and VEGF-A induced by high glucose. Finally, challenge with exogenous VEGF-A (10 ng/ml) induced VEGF-A expression, while anti-VEGFs reduced VEGF-A expression following either high glucose- or VEGF-A-treatment.

These data indicate that high glucose directly damages pericytes through activation of PLA_2/COX-2/VEGF-A pathway. Furthermore, a kind of feed-forward loop between $cPLA_2$/COX-2/PG axis and VEGF appears to operate in this system. Thus, anti-VEGFs afford protection of pericytes from high glucose by inhibiting this loop.

Lupo et al. (2013). Biochem Pharmacol. 86, 1603-13.