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(Article begins on next page)

Hypoxia and hyperglycaemia-induced pericyte apoptosis: identification of pro-apoptotic markers

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Design of study: Loss of pericytes is the key-event in the pathogenesis of microvascular diabetic retinopathy. We have previously demonstrated that human retinal pericytes (HRP) are more vulnerable to intermittent than stable high glucose concentrations, with an increase in their apoptosis.

Purpose: The aim of the present study was to investigate the effects of hypoxia combined with high glucose on HRP and explore the expression of molecules involved in the pro-apoptotic and survival pathways, in order to clarify the mechanisms of action of these diabetic-like stress stimuli.

Methods: HRP were exposed intermittently at 48 hr-intervals to high (28 mM)/physiological (5.6 mM) glucose for 8 days (intHG) and/or hypoxia for the last 48 hrs, with or without the addition of somatostatin. Cell proliferation was assessed by cell counting and BrdU incorporation and apoptosis as DNA fragmentation (ELISA). The expression of pro-apoptotic (FasL, pro-caspase-8, active-caspase-8, Bid, t-Bid, Bax, calpain-2) and anti-apoptotic (PCNA, p-Akt) molecules were evaluated by Western blot.

Results: Hypoxia, alone and combined with intHG, is able to increase HRP apoptosis (+27 and +34%, respectively, $p < 0.05$ vs ctrl) and decrease their proliferation (-28 and -31% vs ctrl, $p < 0.005$). Pro-apoptotic molecules (FasL, active caspase-8, t-Bid, Bax) were significantly increased in HRP cultured in hypoxic conditions, both in physiological and intHG conditions, while no significant differences, but a trend towards decrease, were observed in the expression of survival markers (PCNA, p-Akt). Addition of somatostatin did not determine any variation.

Conclusions: Diabetic-like conditions (intHG and hypoxia) are able to stimulate pericyte apoptosis through activation of pro-apoptotic molecules, thus leading to an imbalance between pro-apoptotic and survival signaling pathways. Our identification of such intermediates could help finding new therapeutic approaches for the prevention of DR.

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