

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Identification of pro-apoptotic markers responsible for hypoxia and hyperglycaemia-induced pericyte apoptosis.

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1527651> since 2015-11-04T16:41:25Z

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

Identification of pro-apoptotic markers responsible for hypoxia and hyperglycaemia-induced pericyte apoptosis**E. Beltramo**¹, A.I. Arroba², A. Mazzeo¹, R. Simò^{3,4}, M. Porta¹, A.M. Valverde²;¹Dept Medical Sciences, University of Turin, Italy, ²Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, ³Vall d'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona, ⁴CIBERDEM, Barcelona, Spain.

Background and aims: 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. Somatostatin, a well-known neuroprotective drug, may protect retinal cells, both microvascular and neuronal, from stress conditions. 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. This research will further clarify the mechanisms of action of these diabetic-like stress stimuli.

Materials and 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).

Conclusion: 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.

Supported by: EU-FP7-EUROCONDOR