OXIDATIVE STRESS IN RESPONSE TO HIGH GLUCOSE LEVELS IN ENDOTHELIAL CELLS AND IN ENDOTHELIAL PROGENITOR CELLS. EVIDENCE FOR DIFFERENTIAL RESPONSES

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Background: Endothelial cells and endothelial progenitor cells (EPCs) play a key role in the pathogenesis of vascular disease. Both cell types can be affected by the oxidative stress generated by hyperglycemia but their susceptibility to oxidative damage may be different. Thus, the effects of high glucose on intracellular ROS levels, cell viability, and expression/activity of anti-oxidant enzymes have been evaluated on HUVECs and EPCs isolated from healthy individuals.

Methods: EPCs were isolated and characterized by specific surface antigens (CD31, vWF, KDR, VE-Cad, CD14); HUVECs were obtained from fresh human umbilical cord. Both cell types were incubated in the presence of normal (5 mM) and high constant (25 mM) D-glucose, as well as 5 mM D-glucose plus 20 mM L-glucose as osmotic control. After 48- and 96-hr, intracellular ROS accumulation was determined by ROS-sensitive fluorescent probe CM-H2DCFDA, cell viability by WST-1 assay, and mRNA expression of catalase, superoxide dismutase 2, and glutathione peroxidase-1 (GPx-1) by RT-PCR and TaqMan real-time PCR.

Results: In HUVECs, ROS increased after 48-hr high glucose incubation and remained high at 96-hr. ROS increased in EPCs as well though to a less extent than in HUVECs at 48-hr incubation. However, ROS levels declined at 96-hr. In spite of ROS increase no change in mRNA expression of antioxidant enzymes occurred in HUVECs, while mRNA expression (and activity) of GPx-1 increased (p< 0.01) in EPCs after 96-hr incubation at 25 mM glucose. Cells viability was reduced after 48-hr incubation at 25 mM glucose both in EPCs (p<0.01) and HUVECs (p< 0.05). After 96-hr, there was no difference in viability between EPCs grown at 5 or 25 mM glucose. On the contrary, HUVECs viability was halved (p< 0.01) at 25 mM glucose.

Conclusions: EPCs may be more resistant than mature endothelial cells to the oxidative stress associated to constant high glucose exposure. This resistance is due to increased expression and activity of GPx-1 allowing better cell survival. Unfortunately, in the hyperglycemic states, the final balance may still favour the development of atherosclerosis.