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Effects of hyperglycemia on adipose-derived mesenchymal stromal cells: a study on their proangiogenic and immunomodulatory potential

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Background. Hyperglycemia and poor glycemic control are two main features of diabetes mellitus. People affected by this disease are prone in developing serious secondary complications, which actually affect their life resulting in impaired life quality and even increased mortality. These are microvascular complications such as diabetic retinopathy (DR), diabetic nephropathy and diabetic neuropathy. The pathophysiological features of these complications are very similar and concern a progressive microvascular degeneration, which impairs the normal microvascular functions and vascular permeability. Hyperglycemia represents a connection point between all these secondary complications since high glucose has been identified as one of the causes mediating cell damage. Through years, clear evidences on hyperglycemia-mediated cell damage have been produced, leading to the elaboration of the "unifying hypothesis". Here, glucose-mediated cell damage is explained as the result of a deleterious increase of oxidative stress in cells, which induces cell dysfunction and death. This mechanism has been demonstrated in several cell types from endothelial to neuronal cells, however, some aspects of hyperglycemia-mediated cell damages still need to be elucidated.
The application of cell/stem cell-mediated therapies in diabetes and diabetic complications already gave some positive outcomes. In particular, mesenchymal stromal cells (MSC)-based interventions appeared to be safe and efficient. Thanks to their differentiating potential, proangiogenic features and immunomodulatory properties, pre-clinical studies of MSC application in DR gave very promising results. For instance, in animal models of DR, MSC applications often resulted in positive outcomes, delaying the progression of the disease, reducing vascular permeability and restoring functionality. However, the majority of studies used an allogenic approach proposing the application of MSC from healthy donors. Indeed, MSC from diabetic donors often revealed to be impaired by the disease, which caused reduction of their potency and even affecting their survival. In this context, specific investigations focused on possible effects of hyperglycemia on MSC are lacking.
Aim. The main goal of this study was to evaluate whether adipose-derived MSC were impaired by hyperglycemia in their phenotype and functions through *in vitro* experimentations.
Methods. Our study proposes a large-spectrum of investigations of possible hyperglycemia-mediated cell damage on adipose derived MSC (ASC) through *in vitro* experimentations. In particular, we assessed effects of hyperglycemia on (1) cellular phenotype, (2) ASC proangiogenic potential, (3) ASC pericyte-like function and (4) immunomodulatory potential.

1. ASC were cultured in normal glucose (1g/L, NG) and high glucose (4.5g/L, HG) culture media. These two counterparts were compared in terms of phenotype, cell growth, differentiation potential and cell surface markers. Oxidative stress and glucose uptake were also measured. Similarly, hyperglycemia-mediated effects were also assessed on microvascular retinal endothelial cells (HRMVEC).
2. The proangiogenic potential of ASC was evaluated establishing cocultures with endothelial cells (EC) and analyzing their supernatants for angiogenic growth factor. These supernatants were used as conditioned media to rescue HRMVEC angiogenic capacity in presence of HG.
3. Immunofluorescence was used to investigate ASC for pericyte-like marker when cocultured with HRMVEC. Anti-α-smooth muscle (SMA) and anti-NG-2 antibodies were tested.
4. ASC and CD4 T cell interactions were characterized through ASC and peripheral blood mononuclear cells (PBMC) direct and indirect (transwell) cocultures. Two distinct conditions were evaluated: (a) stimulated coculture, where PBMC were stimulated with CD3/CD28 beads and (b) unstimulated (or not-stimulated) cocultures, where resting PBMC were used. For both

(a) and (b) CD4 T cell proliferation, quantification of CD4+CD25+ and regulatory T cell (Treg) fractions and analysis of coculture supernatants were performed.

Results. Overall, we observed that hyperglycemia did not affect any of the evaluated aspects.
Indeed, ASC demonstrated a strong refractory behavior towards HG exposure resulting only in a transient increase of intracellular oxidative stress. On the contrary, HRMVEC appeared sensitive to HG, which increased the level of oxidative stress and affected their angiogenic potential suggesting a possible connection between the two aspects.
ASC proangiogenic potential was not affected by glucose and ASC sustained HRMVEC angiogenesis giving structural support and secreting proangiogenic growth factors. ASC conditioned media rescued angiogenic potential of HG HRMVEC.
ASC pericyte-like function was detected independently of glucose, showing a strong α-SMA expression in ASC in contact with endothelial cells. Moreover, pericytes did not support the angiogenesis of EC, confirming their role in support homeostasis in the vascular department.
Finally, we found that glucose did not affect the immunomodulatory potential of ASC. Actually, the major discriminant in the outcomes of the experiments was the presence of CD3/CD28-stimulated or not stimulated (resting) PBMC. In stimulated cocultures, ASC strongly inhibited CD4 proliferation via the IDO-kynurenine pathway, caused an increased CD25 expression on CD4 cells and did not induce Treg formation. In resting cocultures, IDO and kynurenine were also measured at low levels but ASC-inhibiting effect of CD4 was not detectable. Here, Treg highly proliferated in cocultures. Cytokine analysis confirmed Treg induction finding high concentrations of CCL-18 and TGF-β, two well-known factors involved in ASC-mediated Treg generation. Moreover, cocultures caused a long-lasting priming of PBMC, which secreted high concentration of IL-10 and CCL-18, denoting a switch towards an anti-inflammatory phenotype.
Conclusions. In relation to DR and the eventual application of MSC-based cell therapy in diabetic patients our study provides the important evidence that hyperglycemia does not affect ASC in their basic characteristics, proangiogenic and immunomodulatory potential. Having demonstrated the strong immunomodulatory effect of ASC *in vitro* and because of recent publication on Treg involvement in the retina, we auspicate that *in vivo* experimentations may clarify and characterize this mechanism, which may represent a real novelty in DR treatment.