Thiamine transporter-2 is involved in high glucose-induced damage and altered thiamine metabolism in cell models of diabetic retinopathy

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Background and aims: High glucose-induced damage in microvascular cells *in vitro* and progression of retinopathy and nephropathy in diabetic animals are prevented by thiamine supplementation. Impaired thiamine availability facilitates metabolic damage, and renal loss of this vitamin is described in diabetic patients. Two SNPs located in the SLC19A3 gene encoding for the thiamine transporter-2 (ThTR-2) are associated with resistance to development of proliferative diabetic retinopathy and end-stage renal disease in type 1 diabetic subjects, but the mechanisms of these protective effects remain to be understood. We previously showed that diabetic-like conditions modulate ThTR-2 expression in human retinal cells. Our aim was to further investigate the involvement of the two thiamine transporters ThTR-1 and ThTR-2 and their transcription factor Sp1 in high glucose-induced damage and altered thiamine metabolism in cell models mimicking the diabetic retinopathy microenvironment.
Materials and methods: Human retinal pericytes (HRP), human microvascular endothelial cells (HMEC) and human Müller cells (MIO-M1) were cultured for 8 days in physiological glucose (NG), stable high glucose (HG) or intermittent high glucose (intHG). Cells were also cultivated in thiamine-deficient medium (noT) or high thiamine conditions (HT), to evaluate substrate influence. Transketolase (TK) activity and intracellular thiamine concentration were studied through metabolic assays, and cellular localization of the transporters by immunofluorescence staining (IF). To better mimic the retinal microenvironment and the complex intercellular exchanges, triple co-cultures were established. ThTR-1, ThTR-2 and Sp1 mRNA and protein expression were checked by RT-PCR and Western blotting.
Results: TK activity and intracellular thiamine were markedly decreased in HRP and HMEC cultured in noT, as expected, and increased in HRP cultured in HT. Increased intracellular thiamine and TK activity were found in MIO-M1 cultured in HG and intHG and, surprisingly, in HG and intHG without thiamine. IF staining evidenced cytoplasmic localization of ThTR-1 and Sp1 in all cell types. ThTR-2 showed a characteristic nuclear speckle distribution in HRP and MIO-M1, while in HMEC it was uniformly distributed in the cytoplasm. As regards co-culture models, ThTR-1 and Sp1 expression were unchanged in all cell types regardless of treatment, whereas ThTR-2 mRNA and protein expression were decreased in HRP, HMEC and MIO-M1 cultured in HG and intHG conditions, showing differences from data in single cultures.
Conclusion: Down-regulation of ThTR-2 in co-culture models mimicking the diabetic retinal microenvironment suggests its major role in thiamine transport in retinal cells and its involvement in high glucose-induced damage and impaired thiamine metabolism. As expected, altered thiamine supplementation influences TK activity. The increased intracellular thiamine and TK activity in Müller cells following deficient thiamine supplementation may be interpreted as an adaptive mechanism of these cells to counteract lack of substrate.
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