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TREHALOSE ALLEVIATES HUMAN PODOCYTE INJURY VIA THE INDUCTION OF AUTOPHAGY IN AN MTOR INDEPENDENT MANNER

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OBJECTIVES: Podocytes are highly differentiated cells which play an important role in guarding the permeability of the tripartite renal filtration barrier. Many glomerular diseases are attributed to podocyte damage. Strategies for alleviating podocyte injury remain insufficient. Autophagy has been regarded as a vital cytoprotective mechanism in podocyte homeostasis. Trehalose, a natural disaccharide, is an mTOR independent autophagy inducer. It is unclear whether trehalose alleviates podocyte injury. Therefore, we investigated the efficacy of trehalose in puromycin aminonucleoside (PAN)-induced podocyte injury model. METHODS: Human conditional immortalized podocytes were treated with PAN or (and) trehalose. Autophagy and its signaling pathways were investigated by immunofluorescence staining for LC3 puncta and western blotting for LC3, Atg5, p-mTOR, p-p70S6K, p-4E-BP-1 and p-AMPK. Reactive oxygen species (ROS) was measured by H2DCFDA assay. The outcome measurement includes the evaluation of apoptosis, necrosis, and actin cytoskeleton and podocyte motility. Podocyte apoptosis was analyzed by flow cytometry (YO-PRO-1/PI assay and active caspase-3 assay), while necrosis was evaluated by measuring lactate dehydrogenase activity. F-actin was stained for studying the stability of actin cytoskeleton. In addition, we performed migration assay to examine podocyte motility. To verify the role of trehalose-induced autophagy in alleviating podocyte injury, chloroquine (CQ) and wortmannin (WT) were used to block the autophagic flux. RESULTS: It was shown that trehalose induced podocyte autophagy in an mTOR independent manner without ROS involvement. Podocyte apoptosis significantly decreased after trehalose treatment, while inhibition of trehalose-induced autophagy abolished its protective effect. No significant changes were found in podocyte necrosis after PAN with or without trehalose treatment. The disrupted actin cytoskeleton was partially reversed by trehalose, accompanying with less lamellipodias and diminished mobility. CONCLUSIONS: Trehalose induced podocyte autophagy in an mTOR independentmanner and showed cytoprotective effects in PAN-treated human podocytes.