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High Ambient Glucose Stimulates Reactive Oxygen Species Generation through Protein Kinase C-dependent Pathway in Cultured Podocytes

순천향대학 천안병원 내과 및 임상의학연구소

이은영, 양종오, 홍세용

Background and Aims: The increased reactive oxygen species (ROS) production may be involved in the onset or development of diabetic vascular complications. The podocyte plays a crucial role in maintaining the permselectivity function of the glomerular capillary wall. The release of ROS from podocyte may play a role in the pathogenesis of glomerular damage and proteinuria. Although it is assumed that the podocyte injury play an important role in diabetic renal injury, the mechanism is still unknown.

We examined whether high ambient glucose increased ROS in cultured podocyte, whether it was restored by various antioxidants, and whether the protein kinase C (PKC) pathway was involved in this process.

Materials and Methods: To examine the effect of high glucose on ROS generation by podocytes in vitro, differentiated murine podocytes were stimulated for 5, 12, 24, 48, 72 hours with 30mM glucose (5.6mM glucose as a control). Dichlorofluorescein(DCF)-sensitive intracellular ROS was measured by a laser scanning confocal microscope.

Results: High glucose (30mM for 24 hr) raised ROS generation 3.8 fold than control (5.6mM) ($p<0.05$) in cultured podocytes. This high glucose-induced ROS generation were increased time-dependent manner at 5, 12, and 24 hours and the ROS level was higher in high glucose medium up to 72 hours than control.

The increase of high glucose-induced ROS generation by podocyte was effectively inhibited by pretreatment with catalase (300 U/ml) (2.3 fold, $p<0.05$), superoxide dismutase (30 U/ml) (1.9 fold, $p<0.05$), 2- mercaptopropionyl glycine (5 mM) (1.8 fold, $p<0.05$), and glutathione (5mM) (2.0 fold, $p<0.05$). We also observed the increase of free radical production by high glucose medium which was completely restored by PKC depletion by pretreatment of the cells with phorbol myristate acetate (80mM).

Conclusions: In this study, we showed that cultured murine podocytes produce ROS in response to high glucose, and identified PKC was involved in this process. These results suggest that increased oxidative stress in podocytes may play a role in the pathogenesis of podocyte injury and proteinuria in diabetic nephropathy.

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