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Reactive Oxygen Species Mediates High Glucose-induced Fibronectin Synthesis in Human Peritoneal Mesothelial Cells

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We have recently demonstrated that high glucose (50 mM D-glucose) upregulates fibronectin mRNA expression and protein synthesis by human peritoneal mesothelial cells (HPMC) through activation of protein kinase C (PKC) and suggested that this may lead to progressive peritoneal fibrosis during a long-term peritoneal dialysis (PD) using glucose as an osmotic agent. High glucose-induced intracellular reactive oxygen species (ROS) have been proposed as important signaling molecules leading to extracellular matrix (ECM) accumulation in diabetic glomeruli. We, therefore, examined the role of ROS in high glucose-induced fibronectin synthesis by HPMC in the present study. D-glucose increased dichlorofluorescein (DCF)-sensitive intracellular ROS in synchronized confluent HPMC in a dose-dependent manner. 50 and 100 mM D-glucose increased intracellular ROS 1.9- and 3.7-fold, respectively, that of control (5.6 mM) glucose at 1 hour. 50 mM L-glucose did not induce ROS generation. Cytochalasin B, a noncompetitive glucose transporter inhibitor, at 0.2 μM completely blocked high glucose-induced ROS generation without discernable cytotoxic effects, suggesting that glucose uptake is required in ROS generation in HPMC. Both high glucose and 5.6 mM glucose plus 10 mU/ml glucose oxidase, an enzyme that continuously generates hydrogen peroxide from glucose, increased fibronectin secretion up to 1.7 fold that of control at 48 hours. Basal values for fibronectin secreted by HPMC for 48 hours was 433±140 μg/mg of cell protein. Both catalase at 500 units/ml and trolox, a water soluble vitamin E, at 500 μM effectively inhibited high glucose-induced ROS generation and firbronectin protein secretion by HPMC cultured under high glucose or glucose oxidase. Thus, the present data demonstrate that high glucose generates intracellular ROS which may in turn upregulate fibronectin synthesis by HPMC. This implies that ROS generated by high glucose in conventional PD solutions may constitute an important signal for activation of HPMC leading to progressive accumulation of ECM and eventual peritoneal fibrosis