P129

Protein Kinase C and Reactive Oxygen Species Mediate PD Solution-induced Growth Factors and Procollagen Secretion by Peritoneal Mesothelial Cells

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Background: We have previously demonstrated that human peritoneal mesothelial cells (HPMC) secrete vascular endothelial growth factor (VEGF), transforming growth factor β1 (TGF-β1), and procollagen III N-terminal peptide (PIIINP) in response to conventional glucose-based PD solution. We have also shown that high glucose upregulates TGF-β1 and fibronectin mRNA expression and protein secretion by HPMC through activation of protein kinase C (PKC) and reactive oxygen species (ROS). We, therefore, examined the role of PKC and ROS in PD solution-induced VEGF, TGF-β1, and PIIINP secretion by HPMC in the present study.

Methods: Using M199 culture medium as control, conventional PD solutions containing 1.5% (G1.5) or 4.25% (G4.25) glucose and 40 mM lactate were tested. Growth arrested and synchronized HPMC were stimulated by PD solutions diluted 2-fold with M199, 80 nM phorbol 12-myristate 13-acetate (PMA), and 100 μM H2O2 for 48 hours in the presence or absence of a PKC inhibitor (100 nM calphostin C) or an antioxidant (5 mM N-acetylcystein: NAC). VEGF and TGF-β1 were analyzed by ELISA, PIIINP by radioimmunoassay, and dichlorofluorescein (DCF)-sensitive intracellular ROS by FACS.

Results: G4.25-induced VEGF, TGF-β1, and PIIINP secretion was significantly higher than those by G1.5 and M199. PMA significantly upregulated VEGF and TGF-β1 secretion compared to M199. H2O2 significantly increased VEGF secretion compared to M199. Calphostin C effectively inhibited G4.25-induced, but not basal, VEGF, TGF-β1, and PIIINP secretion. NAC also effectively inhibited G4.25-induced VEGF, TGF-β1, and PIIINP secretion and basal TGF-β1 secretion. Both PD solutions and PMA increased DCF-sensitive intracellular ROS 1.7- and 1.8-fold, respectively, compared to M199 in HPMC.

Conclusion: The present data demonstrate that both PKC and ROS mediate PD solution-induced VEGF, TGF-β1, and PIIINP secretion by HPMC. This implies that PKC activation and ROS generation by conventional PD solutions may constitute important signals for activation of HPMC leading to progressive membrane hyperpermeability and accumulation of extracellular matrix and eventual peritoneal fibrosis.