Oxidative Stress and Peritoneal Dialysis

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〈Abstract〉

Oxidative stress plays an important role in tissue injury. Evidence suggests that oxidative stress increases with the progression of chronic renal failure and is exacerbated by peritoneal dialysis (PD). High glucose increases cellular reactive oxygen species (ROS) in human peritoneal mesothelial cells (HPMC) through increased mitochondrial metabolism and activation of protein kinase C (PKC) and NADPH oxidase. ROS thus generated activate signal transduction cascade and transcription factors and upregulate profibrotic genes and proteins. We have demonstrated that high glucose increases fibronectin secretion and induces epithelial-mesenchymal transition (EMT) in HPMC through ROS. Antioxidants effectively inhibit fibronectin upregulation and EMT in HPMC cultured under high glucose or H_2O_2 and in rats treated with conventional glucose-based PD solution. These observations suggest that ROS generated by high glucose in PD solution may be responsible for peritoneal fibrosis observed during long-term PD. In addition to high glucose, glucose degradation products (GDP) may also play a role in oxidative stress in PD through a direct cellular action or indirectly through AGE formation and AGE-AGE receptor binding. Thus, strategies to inhibit high glucose-induced ROS generation and the use of non-glucose based and/or new PD solutions containing low GDP may reduce oxidative stress and allow better preservation of the structural and functional integrity of the peritoneal membrane during long-term PD.

Introduction

Oxidative stress is defined as a relative increase in reactive oxygen species (ROS) that outbalances the existing antioxidative defense mechanisms and is known to play an important role in the development and progression of many diseases including atherosclerosis, ishchemia/reperfusion injury, aging, cancer, and chronic inflammatory disease. ROS can induce tissue injury through oxidation of macromolecules including lipids, proteins, carbohydrates, and nucleic acids leading to abnormal structure and function. ROS activate signal transduction cascade [protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), janus kinase (JAK)] and transcription factors [nuclear factor-κB (NF-κB), activated protein-1 (AP-1), specificity

protein 1 (Sp1), and signal transducers and activators of transcription (STAT)] leading to alte rations in gene transcription and protein synthesis[1]. In this review, we will focus on the role of locally generated oxidative stress during peritoneal dialysis (PD) in the structural and functional alterations in the peritoneum during long-term PD and the mechanisms involved.

Oxidative stress, chronic renal failure, and peritoneal dialysis

Chronic renal failure (CRF) is now viewed as a state of chronic inflammation[2, 3] and the prevalence of atherosclerosis is strikingly higher in CRF than in normal population[4]. It is, therefore, reasonable to speculate that oxidative stress is increased in CRF. Indeed, surrogate markers of oxidative stress were found increased and antioxidative defense mechanisms decreased in CRF[5]. Recent experimental studies also suggest that CRF plays a role in inducing oxidative stress. Vaziri et al[6] demonstrated that superoxide dismutase expression was decreased and NADPH oxidase expression increased in the kidneys of experimental CRF rats. Buzello et al[7] reported that nitrotyrosine expression in atherosclerotic plaque was increased in uninephrectomized Apo E knock-out mice and further increased with subtotal nephrectomy.

Oxidative stress in CRF appears to be further increased by PD. Tarng et al[8] demonstrated stepwise increase in serum 8-hydroxy 2'-deoxyguanosine (8-OHdG) and decrease in glutathione in nondialyzed uremic and chronic ambulatory PD (CAPD) patients. Both basal and phorbol ester-induced cellular ROS in leukocytes increased in nondialyzed uremic patients and further increased in CAPD patients. We[9] found that local oxidative stress in the peritoneum is increased in CAPD patients. Lipid peroxide (LPO) level in overnight dialysate and peritoneal creatinine transport rate increased with time on CAPD. Recent studies including our own[10-14] suggest that this local oxidative stress may play a central role in peritoneal fibrosis and ultrafiltration failure in long-term PD as summarized below. Clinical trials are required to verify the role of oxidative stress and the therapeutic effect of antioxidants on the structural and functional changes in long-term PD patients.

Role of glucose in PD-induced oxidative stress

Bioincompatible constituents of conventional PD solution may participate in increased oxidative stress in the peritoneum and our recent study[13] suggests that high glucose plays a central role in increased ROS generation in human peritoneal mesothelial cells (HPMC).

High glucose increases cellular ROS in HPMC in a dose- and time-dependent manner. The observations that high glucose-induced cellular ROS is effectively inhibited by cytochalasin B, an inhibitor of glucose transporter, and that L-glucose does not increase cellular ROS at the same osmolality (50 mM) as D-glucose suggest that high glucose-induced cellular ROS depends on cellular uptake of glucose. Data from diabetes research suggest that PKC[15], NADPH oxidase [16], and mitochondrial electron transfer chain complexes[17] all participate in high glucose-induced ROS generation. We found that inhibition of PKC (calphostin C and PKC depletion), NADPH oxidase (DPI and apocynin), or mitochondrial electron transfer chain subunit I (rotenone) effectively inhibit high glucose-induced ROS in HPMC[13]. Ishibashi et al[18] reported increased rhodamine 123 uptake and mitochondrial 8-OHdG immunostaining in HPMC cultured under high glucose compared to mannitol at the same osmolality suggesting that mitochondrial metabolism plays a role in high glucose-induced ROS

generation in HPMC.

Role of oxidative stress in high glucose-induced fibronectin expression and epithelial-mesenchymal transition (EMT) in the peritoneum during PD

High glucose increases TGF-β1 and fibronectin mRNA and protein expression by HPMC through activation of PKC[19] and ROS amplify PKC signal in high glucose-induced fibronectin upregulation in HPMC[13]. The observation that depletion of endogenous PKC by preincubating cells with phorbol ester for 24 hours or a PKC inhibitor, calphostin C, effectively inhibits high glucose- and H_2O_2-induced fibronectin secretion suggest that PKC is a down-stream signaling molecule of ROS in high glucose-induced fibronectin secretion by HPMC[13]. On the other hand, high glucose induces de novo synthesis of diacylglycerol and activates PKC in HPMC[19]. Phorbol ester-induced PKC activation increases cellular ROS in HPMC and antioxidants (trolox and catalase) effectively inhibits high glucose- and PKC-induced fibronectin mRNA expression and protein secretion suggesting that PKC is also an upstream signaling molecule to ROS[13]. PKC-induced ROS in turn activate PKC thus amplifying PKC signaling in high glucose-induced fibronectin upregulation in HPMC[13].

ROS-regulated signaling pathways in HPMC leading to fibronectin expression are not completely understood. However, diabetes studies have demonstrated that PKC, MAPKs, and transcription factors (NF-κB, AP-1, and Sp1) are activated under high glucose through ROS. To this end, high glucose-induced fibronectin secretion by HPMC was found to be associated with activation of MAPK and cyclic AMP-responsive element binding protein pathway[20]. In long-term PD, thickening of submesothelial compact zone and vasculopathy increase with time on PD and glucose exposure[21]. These observations suggest that high glucose leads to alterations in gene and protein expression involved in accumulation of extracellular matrix (ECM) in the peritoneal tissue in long-term PD through ROS generation and activation of down-stream signaling cascade.

Epithelial-mesenchymal transition (EMT) of epithelial cells, characterized by loss of epithelial cell characteristics and gain of ECM producing myofibroblast characteristics, is an important mechanism involved in tissue fibrosis[22-25]. Recent data[26-28] suggest that HPMC undergo EMT during PD and may play a major role in the development and progression of peritoneal fibrosis leading to failure of peritoneal membrane function. We[29] recently reported that i) high glucose, H_2O_2, and glucose-based PD solution upregulate α-SMA and downregluate E-cadherin in HPMC, ii) antioxidants, N-acetylcystein (NAC) and catalase, effectively reverse high glucose-induced α-SMA and E-cadherin expression in HPMC, and iii) prolonged exposure of rat peritoneum to glucose-based PD solution upregulates α-SMA expression in the peritoneum, which is effectively inhibited by NAC. All these data suggest that ROS play a major role in peritoneal EMT induced by high glucose contained in conventional PD solution. This is consistent with the observation that ROS is involved in TGF-β1-induced EMT in renal tubular epithelial cells[30].

Role of glucose degradation products (GDP) in PD-induced oxidative stress

Conventional glucose-based PD solution contains high concentrations of GDP. GDP are formed during heat sterilization process and during storage on shelf. GDP can increase oxidative stress during PD through direct cellular action or indirectly through AGE formation and AGE-AGE receptor binding. Methylglyoxal and 3-deoxyglucosone were shown to upregulate mRNA and

protein expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF), a potent mitogen for smooth muscle cells, through cellular ROS generation in rat aortic smooth muscle cells[31]. It is well known that some GDPs promote AGE formation[32] and ROS are generated in the process of AGE formation[33] and through AGE-AGE receptor interaction[34].

The observations that methylglyoxal and acetaldehyde significantly increase secretion of vascular endothelial growth factor (VEGF) by HPMC, that NAC effectively inhibits methylglyoxal-induced VEGF secretion by HPMC, and that exogenous H_2O_2 increases VEGF secretion suggest that ROS mediate methylglyoxal-induced VEGF secretion by HPMC[35]. In contrast, 3,4-dideoxyglucosone-3-ene (3,4-DGE) significantly decreased VEGF secretion[35]. None of GDP tested (methylglyoxal, acetaldehyde, and 3,4-DGE), however, affected TGF-β1 and fibronectin secretion by HPMC[35]. It is not known if AGE are formed in the media during 48-hour culture but receptor for AGE (RAGE) is expressed in cultured HPMC[36].

Role of PD solution in PD-induced oxidative stress

Conventional PD solution containing high concentrations of glucose and GDP was found to upregulate VEGF, TGF-β1, and procollagen III N-terminal peptide (PIIINP) secretion by HPMC and this upregulation was effectively inhibited by NAC[37] suggesting that conventional PD solution can activate HPMC through ROS generation. In fact, rats treated with conventional glucose-based PD solution intraperitoneally for 12 weeks had significantly lower drain volume and D_4/D_0 glucose but higher D_4/P_4 creatinine and increased membrane thickness and endothelial NOS (eNOS) expression compared to control rats[14]. Omental TGF-β1, VEGF, collagen I, and heat shock protein (hsp) 47 expression and LPO levels and dialysate VEGF concentrations were significantly increased in rats treated with PD solution compared to control[14]. All of these changes were prevented by NAC, suggesting that ROS generated by conventional PD solution are, in large part, responsible for peritoneal fibrosis and membrane hyperpermeability.

Conclusion

Data from cell culture studies and experimental animals studies demonstrate that high glucose and conventional glucose-based PD solution increase cellular ROS leading to upregulation of profibrotic gene transcription and EMT of HPMC resulting in peritoneal fibrosis and membrane hyperpermeability. These observations suggest that strategies to inhibit high glucose-induced ROS generation and the use of non-glucose based and/or new PD solutions containing low GDP may reduce oxidative stress and allow better preservation of structural and functional integrity of the peritoneal membrane during long-term PD.

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