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Molecular and Peritoneal Microvascular Changes Cause Peritoneal Membrane Dysfunction by Uremia-Related Mechanisms

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Background

Long-term peritoneal dialysis (PD) is associated with distinct peritoneal structural changes characterized by thickening of the sub-mesothelial cell layer, fibrosis and angiogenesis. These changes were assumed to be the cause for peritoneal membrane dysfunction and technique failure that was observed in some long-term PD patients. However, this assumption was refuted by the findings from animal models of chronic PD that showed the exact structural phenotype of the long-term PD, and yet a normal peritoneal function. This study was set to determine that the peritoneal microvascular and interstitial changes associated with long-term PD in rats produce peritoneal dysfunction by uremia related mechanisms. Our studies have demonstrated that acute exposure of the peritoneum to glucose-based PD solutions produces rapid and sustained visceral peritoneal microvascular vasodilation via the nitric oxide (NO) pathway. At present, there is no literature data on the reactivity of these peritoneal microvessels after long-term exposure of the peritoneum to PD solutions. NO as the mediator of the peritoneal microvascular reactivity to PD solutions is also involved in angiogenesis. Angiogenesis is initiated by proliferation of endothelial cells, which penetrate into the surrounding tissue, and is tightly regulated by growth factors and inhibitors. Matrix metalloproteinases (MMPs) regulate angiogenesis, on the one hand by facilitating extracellular matrix (ECM) degradation to allow new vessel expansion, and on the other hand, by interfering with angiogenesis through

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the production of angiostatin. Angiostatin is generated by the proteolytic cleavage of plasminogen by MMP-2, -7, -9, and -12. Angiogenesis was observed in patient undergoing PD as proven by peritoneal biopsy studies and in animal models of chronic PD. As a possible mechanistic explanation, angiogenesis and vasodilation increase the peritoneal surface area available for exchange, for rapid dissipation of the osmotic gradient, and hence, peritoneal membrane dysfunction.

Methodology

Rat studies were done on old animals as typical in chronic PD models because of the time required to develop the animal model. Three animal groups were used: Group - I: Chronic PD solution infusion: Interventions included indwelling peritoneal catheter placement and daily infusion of a glucose-based clinical PD solution for one month. Group – II: Interventions included renal injury and indwelling peritoneal catheter placement: Renal injury was induced by unilateral nephrectomy and ipsilateral kidney injury by cryosurgery. Group – III: Interventions included induction of uremia and indwelling peritoneal catheter placement: Uremia was induced by 5/6 nephrectomy. In all groups, procedures for animal model development including surgery were conducted under anesthesia and according to standard aseptic techniques. Studies on each animal were conducted after complete healing of the surgical incisions. These include: 1) Peritoneal equilibration test (PET-test) to assess the peritoneal transport properties; 2) Intravital microscopy to assess the visceral peritoneal microvascular reactivity to a clinical glucose-based PD solution, and endothelial functions of these visceral peritoneal micro vessels; 3) Assess the NO pathway via immunoblotting. Endothelial nitric oxide synthase (eNOS) which releases NO was measured via western blot. eNOS is activated by serine/threonine protein kinase Akt protein and itself activates matrix metalloprotease 2 (MMP2). Akt is activated by shear stress through activation of PI3K. Protein expression of eNOS, MMP-2 and Akt was assessed.

Results

Uremia caused a remarkable increase in the reactivity of the visceral peritoneal microvasculature to the dialysis solution as compared with the renal injury and the chronic infusion groups. Inversely, Uremia markedly decreased the sensitivity and reactivity of the visceral peritoneal microvasculature to the exogenous endothelium-dependent receptor-dependent Acetylcholine to indicate marked endothelial cell dysfunction. A subset of uremic animals exhibited very high net ultrafiltration which seems to correlate with a phenotype of extensive angiogenesis and uremia-induced hypertension. However, this high net ultrafiltration was not seen in any of our normotensive non-uremic animals that were subjected to indwelling peritoneal catheter placement and daily infusion of a dialysis solution for one month. Arterioles from the renal injury group reacted similarly to the arterioles from the chronic infusion animals. On the animals that showed prolific angiogenesis, expression of Akt and eNOS was elevated as compared with the animals that had kidney injury or control animals. MMP-2 showed elevated expression in animals with angiogenesis compared to animals with kidney injury. That is confirmation in molecular level that angiogenesis occurs in animals that display uremic phenotype. Control animals exhibited a significantly reduced MMP levels, indicating impaired angiogenesis process. Unlike in the naïve control animals, experimental groups demonstrated significant activation of MMP-2. In addition, Enos was found to be upregulated in the experimental groups, as compared with the naïve controls.

Conclusions

Four major conclusions were drawn from this study. 1) Aging is an independent risk factor for peritoneal microvascular endothelial cell dysfunction; 2) indwelling peritoneal catheter placement compounds a pre-existing age-related endothelial cell dysfunction; 3) Uremia unlike reduction in renal mass and injury causes marked peritoneal microvascular endothelial cell dysfunction as proven by the measurement of proteins involved in the vasodilation and angiogenesis pathways; 4) Uremia unlike aging or indwelling peritoneal catheter enhances the basic permeably of the peritoneal membrane for small solute transport.