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Novel Ways to Preserve the Peritoneal Membrane

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The underlying pathophysiology that leads to morphologic and functional changes in the peritoneal membrane over time among patients on peritoneal dialysis is not well understood. Many studies have been conducted to try to abrogate those changes and so preserve peritoneal function. Conventional interventions that attempt to accomplish that end include prevention of peritonitis, timely removal of the peritoneal catheter in the face of non-resolution of peritonitis, use of biocompatible dialysate, and limitation of total glucose exposure by avoiding hypertonic dextrose solutions. Inhibition of the renin–angiotensin–aldosterone and vascular endothelial growth factor systems, peritoneal resting, combined peritoneal dialysis and hemodialysis, and N-acetylcysteine and gene therapy are more novel and experimental attempts to preserve the peritoneal membrane. We review the novel studies that have aimed to promote the health and function of this dialyzing membrane.

Key words
Peritoneal membrane, ACE inhibitors, peritoneal resting, N-acetylcysteine, adenovirus, gene transfer

Introduction
To maintain the long-term efficacy of peritoneal dialysis (PD) in patients with end-stage renal disease, the structural and functional integrity of the peritoneal membrane must be preserved. Some of the methods that have been used include limiting glucose exposure, using biocompatible solutions, preventing peritonitis, and putting a time limit on PD therapy. To date, none of those methods has proven unequivocally effective in preserving the integrity of the peritoneal membrane.

The present overview discusses novel and experimental ways to preserve the integrity of the peritoneal membrane in PD patients. These novel strategies range from the simple to the very complex.

Discussion
Inhibitors of the renin–angiotensin–aldosterone system
Many tissues are able to locally synthesize all of the components of the renin–angiotensin–aldosterone system (RAAS) [reviewed in Nessim et al. (1)]. Peritoneal mesothelial cells appear to have RAAS genes, and so they have their own ability to express those genes and their products. Studies have shown that cultured human peritoneal mesothelial cells are able to constitutively express angiotensinogen, angiotensin converting-enzyme (ACE), angiotensin II type 1 and 2 receptors, transforming growth factor β (TGF-β), and fibronectin [reviewed in Nessim et al. (1)].

One of the most important activators of the RAAS is glucose, as evidenced by the local RAAS activation that is seen in multiple tissues in patients with diabetes. In patients on PD using conventional solutions, the local peritoneal microenvironment harbors glucose concentrations ranging from 76 mmol/L (for a 1.5% dextrose solution) to 215 mmol/L (for a 4.25% dextrose solution). Because mesothelial cells have the capacity for glucose uptake, this glucose-rich environment leads to intracellular signaling that results in RAAS activation. It has been shown that exposing human peritoneal mesothelial cells to a glucose-rich environment leads to upregulation of angiotensinogen, ACE, and angiotensin II receptor type 1 expression. Exposure to glucose has also been shown to increase angiotensin II, a growth factor that regulates cell proliferation, apoptosis, and fibrosis, leading to upregulation of TGF-β and fibronectin expression and the stimulation of vascular endothelial growth factor (VEGF) and procollagen secretion (2,3). The increase in TGF-β associated with peritoneal glucose exposure may be further augmented in the presence of mesothelial cell stretch or fluid shear stress (4). Secretion of TGF-β is associated with submesothelial fibrosis and peritoneal membrane dysfunction.

Vascular endothelial growth factor is postulated to play an important role in the modification of
peritoneal membrane characteristics by increasing vascular permeability, inducing vasodilatation by stimulation of nitric oxide synthase, and stimulating angiogenesis. In mesothelial cells, VEGF expression can be also upregulated by proinflammatory cytokines such as interleukin (IL) 1 and tumor necrosis factor α, by thrombin, and by nonenzymatic glycation products such as glycated albumin, which is seen with treatment using PD fluids containing high concentrations of glucose (5).

In a study by Duman et al. (6), 21 albino Wistar rats were divided into three groups:

- A control group that received 10 mL isotonic saline intraperitoneally
- A dextrose group that received 10 mL 3.86% dextrose PD solution given intraperitoneally
- An enalapril-treated group that received 10 mL 3.86% dextrose PD solution intra-peritoneally, plus 100 mg/L enalapril in drinking water

After 4 weeks, a 1-hour peritoneal equilibration test was performed using 20 mL 2.27% dextrose PD solution. The parietal peritoneum was evaluated histologically by light microscopy. Administration of enalapril resulted in preserved ultrafiltration (UF), lower protein loss, and less peritoneal thickness. In the dextrose group, dialysate-to-plasma urea increased significantly. Higher levels of TGF-β1 and lower levels of cancer antigen 125 in dialysate effluent were both seen in the dextrose group (6).

In another study (7), human mesothelial cells were isolated from omental tissue and cultured. Incubation of those cells with captopril (100 – 1000 mmol/L) resulted in a concentration-dependent attenuation of VEGF synthesis. Incubation with captopril (500 – 1000 mmol/L), enalapril (100 – 1000 mmol/L), and losartan (1 – 100 mmol/L) significantly decreased the VEGF overproduction induced in mesothelial cells by inflammatory mediators (tumor necrosis factor α, IL-1α).

The first study to examine the effect of RAAS inhibition in humans retrospectively studied 66 patients who had been on PD for at least 2 years. Changes in transport status over time were assessed in those who had been on ACE inhibitors or angiotensin II receptor blockers (ARBs), as well as in those who had not been using any form of RAAS blockade. In that study, although small-solute transport increased with time on PD in the control group, no appreciable change in transport status was observed among the patients taking ACE inhibitors or ARBs (8). The same authors subsequently examined changes in solute transport and technique survival in a larger cohort of patients. Once again, the group of patients who were on ACE inhibitors or ARBs did not show the increased solute transport seen in the controls with time on therapy. However, no difference in PD technique survival was observed (9).

Peritoneal resting

Peritoneal resting that withholds exposure to dialysate for a period of time is thought to repair the dialysate-associated changes in membrane structure and function. On PD, the peritoneal membrane in some patients develops hyperpermeability to small solutes. That change leads to a more rapid dissipation of the glucose osmotic gradient and loss of UF (10). Loss of UF is one of the major causes of withdrawal from long-term PD.

Peritoneal rest through the use of temporary hemodialysis for a month has been reported to be of benefit for patients whose poor fluid removal is a result of excess absorption of glucose (11). Hemodialysis can be initiated during the rest period through a temporary venous line. During that time, 100 – 200 mL of 1.5% dialysate with 3500 U heparin is instilled and left in the peritoneal cavity for 2 weeks. Heparin is postulated to limit fibrin deposition onto the denuded peritoneal membrane and to remove advanced glycosylation end-products, which otherwise would promote the secretion of inflammatory cytokines and growth factors that stimulate angiogenesis (12). Angiogenesis increases vascular permeability, which enhances small-solute transport, resulting in faster reabsorption of glucose, early loss of the osmotic gradient, and ultimately UF failure (13,14). Furthermore, secreted growth factors and uremia itself are associated with the development of peritoneal fibrosis.

In 1993, de Alvaro and colleagues reported outcomes of peritoneal resting for 4 weeks (11). They showed a statistically significant improvement in mass transfer-area coefficient (MTAC) and UF capacity after the 4 weeks of peritoneal rest. That improvement was more notable in patients who were rapid transporters. In 1999, Kim et al. (15) tested the same method in an animal model, in which rats were given
3 weeks of PD followed by 4 weeks of rest. The rest period reduced peritoneal membrane permeability to glucose.

**Combined PD and hemodialysis**

Another novel method is combined PD and hemodialysis, which can be considered a variation of peritoneal resting. It is usually used in patients on long-term PD with failing residual kidney or peritoneal membrane function. This “bimodal” dialysis includes hemodialysis for 1 – 3 days and PD for 4 – 6 days per week. This method has the advantages of hemodialysis, which include maintaining euvolemia and increasing the weekly solute clearance of small molecules, with a reduction in membrane exposure to glucose. The drawbacks of using this method are difficulty in quantitating the total dialysis dose and managing the two accesses, with the associated increased risk of infection. Furthermore, the dual regimen may not prove convenient for some patients.

**N-Acetylcysteine**

The presence of increased oxidative stress and persistent inflammation has been well documented in dialysis patients (16). Reactive oxygen species have deleterious effects on various cells and may contribute to atherogenesis (17). A variety of antioxidant strategies have been evaluated with respect to reducing oxidative stress in patients with chronic kidney disease. Considering its safety, low cost, and possible benefits, N-acetylcysteine might be a potential anti-inflammatory and antioxidant drug in dialysis patients (18).

In 2009, PD patients in a study by Nascimento et al. received 600 mg N-acetylcysteine twice daily, and their plasma levels of inflammatory and oxidative stress markers were measured (18). The short-term oral N-acetylcysteine treatment resulted in a reduction of circulating IL-6, suggesting that such treatment could be a useful strategy in blunting the inflammatory response in PD patients (5). N-Acetylcysteine is also known to inhibit glucose-induced generation of Nε-(carboxymethyl)lysine, a product of glycation and oxidation that is a known independent mediator of peritoneal damage.

**Gene therapy**

The long-term changes in the peritoneal membrane suggest that both fibrogenic and angiogenic processes are active. The peritoneal concentration of VEGF and TGF-β—key angiogenic and fibrogenic growth factors—have been shown to correlate with peritoneal membrane function (19). The molecular mechanisms of membrane failure are multifactorial and complex in nature, and are a result not only of chronic exposure to PD solutions, but also the influences of uremia and other comorbid conditions.

Adenovirus (Ad)–mediated gene transfer has been shown to be a unique tool with which to dissect the molecular basis of peritoneal membrane pathophysiology. The Ad vector itself has a specific impact on peritoneal physiology, affecting well-being, peritoneal inflammation, cellular and humoral immunity, and peritoneal structure and function in recipient animals. Adenovirus-mediated gene transfer of TGF-β1 can create an experimental model of peritoneal fibrosis, a condition commonly found in peritoneal biopsies from PD patients and thought to be a factor in UF failure (20).

Several studies have implicated the involvement of TGF-β in the progressive changes found in the peritonea of PD patients (20,21). The TGF-β–mediated model of peritoneal fibrosis was used to examine the therapeutic potential of Flt1 (a soluble VEGF type 1 receptor) and the soluble receptor 2 for TGF-β in blocking inflammatory angiogenic and profibrotic pathways respectively. Intraperitoneal delivery of AdTGF-β1 in a mouse model resulted in increased serum levels of active TGF-β1 and marked thickening of the parietal peritoneum, with increased collagen deposition (22). An increased inflammatory response to the virus was noted, together with transient weight loss, elevated levels of serum amyloid P and IL-12, and increased gene expression of monocyte chemoattractant protein 1. Pretreatment of recipient animals by intramuscular gene transfer of 100 mg Flt1 complementary DNA 4 days before the induction of peritoneal fibrosis significantly attenuated the development of inflammation and fibrosis, as evidenced by an 81% decline in collagen deposition and decreased levels of intercellular adhesion molecule 1 and monocyte chemoattractant protein 1 messenger RNA (21).

Intervening in peritoneal fibrosis by blocking VEGF signaling may be of therapeutic value. It also confirms both the pro- and anti-inflammatory actions of TGF-β1. Adenovirus-mediated gene transfer can be used not only to establish a condition in the
peritoneal environment, but also to intervene in a pathogenic process.

Adenoviral vectors expressing decorin and angiostatin were delivered to rat peritoneum to investigate the potential of antifibrotic and antiangiogenic intervention in an acute fibroproliferative model of PD. Decorin is a proteoglycan that can bind and inactivate TGF-β, and angiostatin is a recognized inhibitor of angiogenesis (21). In the study, animals received an intraperitoneal injection of either the null virus AdDL70, AdDecorin, or AdAngiostatin 2 days before and 7 days after initiation of daily infusion with a high-glucose dialysate. After 4 weeks of infusion (coupled with administration of lipopolysaccharide), the result was a thickening of the submesothelial layer, increased collagen deposition with angiogenesis, and evidence of membrane dysfunction with a decrease in net UF and an increase in membrane permeability to small solutes (21). In rats that received the AdDecorin, the membrane showed a reduction in hydroxyproline content, but no effect on angiogenesis and little effect on membrane transport parameters. Intraperitoneal delivery of angiostatin led to a reduction in the number and density of peritoneal vessels, an improvement in net UF and small-solute transport, and a slight reduction in submesothelial thickness.

That study, while preliminary, has helped to define the individual contributions of peritoneal fibrosis and angiogenesis to peritoneal transport dysfunction, and demonstrates the value of Ad-mediated gene transfer not only in evaluating the therapeutic potential of anti-fibrogenic and anti-angiogenic molecules, but also in elucidating the role of those processes in membrane dysfunction (22).

**Summary**

In long-term PD, various pathophysiologic mechanisms play a role in the loss of peritoneal membrane function. The search for ways to prolong membrane viability and function in PD patients is still ongoing. Inhibition of the RAAS and the VEGF system are promising novel ways to preserve the peritoneal membrane. There are several reasons to keep patients on RAAS inhibitors, including preservation of residual kidney function. If membrane function is similarly preserved, that effect would present a strong argument for all patients to receive such agents. The other novel therapies remain to be proved effective and practicable.

**Disclosures**

The authors have no financial conflicts of interest to declare.

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