Preventing peritoneal membrane fibrosis in peritoneal dialysis patients

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Long-term peritoneal dialysis causes morphologic and functional changes in the peritoneal membrane. Although mesothelial-mesenchymal transition of peritoneal mesothelial cells is a key process leading to peritoneal fibrosis, and bioincompatible peritoneal dialysis solutions (glucose, glucose degradation products, and advanced glycation end products or a combination) are responsible for altering mesothelial cell function and proliferation, mechanisms underlying these processes remain largely unclear. Peritoneal fibrosis has 2 cooperative parts, the fibrosis process itself and the inflammation. The link between these 2 processes is frequently bidirectional, with each one inducing the other. This review outlines our current understanding about the definition and pathophysiology of peritoneal fibrosis, recent studies on key fibrogenic molecular machinery in peritoneal fibrosis, such as the role of transforming growth factor-β/Smads, transforming growth factor-β β/Smad independent pathways, and noncoding RNAs. The diagnosis of peritoneal fibrosis, including effluent biomarkers and the histopathology of a peritoneal biopsy, which is the gold standard for demonstrating peritoneal fibrosis, is introduced in detail. Several interventions for peritoneal fibrosis based on biomarkers, cytology, histology, functional studies, and antagonists are presented in this review. Recent experimental trials in animal models, including pharmacology and gene therapy, which could offer novel insights into the treatment of peritoneal fibrosis in the near future, are also discussed in depth.

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Definition of peritoneal fibrosis

In 1994, Chaimovitz[1] described peritoneal fibrosis in a peritoneal dialysis (PD) patient at a nephrology forum. The most important feature that he noted was the universal absence of a mesothelial layer in the context of submesothelial thickening, representing tissue fibrosis, which had been previously described by Dobbie *et al.*[2,3] The definition of peritoneal fibrosis has been difficult because terms such as fibrosis, sclerosis, and encapsulation have not been clearly differentiated. Garosi *et al.*[4] stated that peritoneal sclerosis varied from clinically mild, silent sclerosis, which was almost always present, to the rare and fatal cases of encapsulating peritoneal sclerosis (EPS), a single disorder with variable manifestations and stages.[5] According to data from peritoneal biopsies performed during PD with bioincompatible solutions, the prevalence of peritoneal fibrosis is almost universal at midterm. In this review, we show a decrease in this prevalence using less bioincompatible solutions.

Pathophysiology

Peritoneal fibrosis has 2 cooperative parts, the fibrosis process itself and the inflammation promoted by the nonphysiologic content of solutions and infections.[6–11] The link between these 2 processes is frequently bidirectional, with each one inducing the other.

The fibrosis process itself. The loss of mesothelial cells (MCs) with thickening of extracellular matrix in submesothelial zones was reinterpreted by 2003 as also the consequence of their transformation into fibroblastoid cells, the mesothelial-to-mesenchymal transition (MMT). Transformed MCs are able to produce extracellular matrix and cause fibrosis, which is observed in submesothelial areas, demonstrating invasive capacity.[12] The system involved in this transition is transforming growth factor-β (TGF-β), being the high production of vascular endothelial growth factor (VEGF), an outstanding consequence. VEGF receptors and coreceptors have recently been characterized in human MCs and animal models.[13] The high production of VEGF causes vasodilation, increasing capillary wall permeability and inducing rapid solute transport and low ultrafiltration (UF) capacity, concurrent with fibrosis.[14] The MMT has been reviewed in depth,[15] and lineage tracing analyses in animal models question the transformation of MCs, suggesting distinctive fates for MCs and submesothelial fibroblasts during injury.[16] This aggressive model, however, does not represent PD in humans. Liu *et al.*[15] conceded the possibility

that both adult MCs and precursors can be the origin of transitional cells.

The most important agents in PD solutions responsible for altering MC function and proliferation are glucose and glucose-degradation products, which stimulate TGF-β and VEGF production by MCs and others.[17–21] Also endogenous systems such as Gremlin, an inhibitor of bone morphogenic proteins (inhibitor of TGF-β), has been found activated on PD and able to promote peritoneal injury *via* MMT with increased solute transport in humans.[22]

Peritoneal inflammation. Peritonitis remains a complication in PD patients, leading to MC damage and fibrosis.[23] Detrimental changes are correlated with the number, severity, and timing of episodes.[24–26]

Davies[27] proposed interleukin-6 (IL-6) as a determinant of solute transport increase related to inflammation, predisposing the membrane to fibrosis. Cho *et al.*[28] found significantly increased IL-6 effluent levels independent of the less bioincompatible of the PD solutions; however, an increase in solute transport occurred only under bioincompatible solutions over time. A synergistic interaction between peritonitis-induced cytokine and inflammation is proposed.[29] The origin of the inflammation begins with injury to the MCs,[30] involving the transcription nuclear factor of activated T cells whose upregulation is associated with nuclear factor κ light polypeptide gene enhancer in B cells (nuclear factor κB) induction, which results in macrophage recruitment.[31]

Macrophages are involved in peritoneal inflammation with direct fibrosing consequences. The alternatively activated macrophage system functions through the production of the fibrosing chemokine (C-C motif) ligand 18 (CCL18),[32] whose receptor was recently described.[33] In peritonitis, the macrophage phenotype varies over the follow-up according to the outcome. During the first days, macrophages are classically activated in response to infection; after resolution, the alternatively activated macrophage phenotype predominates to assist in the repair process. When the alternatively activated macrophage population persists, peritoneal fibrosis will appear. High CCL18 peritoneal effluent levels herald membrane failure and EPS, although contradictory results have been published.[34,35] In an experimental model, a monocyte chemoattractant protein-1/CCR2 system derived from activated macrophages has been involved in PD-related MMT activation and extracellular matrix synthesis *via* TGF-β.[36] By these pathways, inflammation and MMT have been linked as mechanisms promoting peritoneal fibrosis.

Other inflammatory systems implicated in human peritoneal inflammation-fibrosis (demonstrated by biopsy and effluent) are tumor necrosis factor–like weak inducer of apoptosis (TWEAK)/Fn14[37] and T helper 17 cell/IL-17.[38] During inflammation, MCs upregulate Fn14, and TWEAK activates MCs to express chemokines, which are dependent on nuclear factor κB activation. This attracts macrophages into the peritoneum, mediated by CCL21 chemokine. CCL21, one of whose targets are fibrocytes, may contribute to peritoneal fibrosis. The T helper 17 cell/IL-17 system of inflammation participates in immune-mediated and chronic inflammatory diseases.[38–41] IL-17A expression in peritoneal biopsy specimens from PD patients is associated with inflammatory infiltration. Elevated IL-17A concentrations were detected in effluents from long-term PD patients. The sequence of macrophage/lymphocyte systems promoting peritoneal fibrosis by different pathways suggests the possibility of peritoneal fibrosis prevention during and after peritonitis, not only by antibiotics. IL-17A inhibitors are currently available for use in ankylosing spondylitis.[42]

Key fibrogenic molecular machinery in peritoneal fibrosis

During the process of MMT and peritoneal fibrosis, key fibrogenic factors through their receptors on the cell membrane and specific downstream intracellular signal cascades to trigger the transcription factors that act on the promoter regions of the cell matrix genes to activate their transcription. Such signal transduction cascades, which include TGF-β and mitogen-activated protein kinase signal pathways are also regulated by a variety of noncoding RNAs (ncRNAs) (Figure 1).

TGF-β/Smad signaling. The TGF-β superfamily consists of a large variety of signaling proteins, including TGF-β isoforms, bone morphogenic proteins, activins, and related proteins.[43] Those proteins exert multiple biological functions in cell proliferation, apoptosis, embryonic development, and organ fibrosis.[43,44] Activation of TGF-β1 is an important early event that mediates fibrogenesis by glucose, glucose-degradation products, and advanced glycation end products in bioincompatible PD fluid.[19] As shown in Figure 1, TGF-β_1 can transduce signal through Smad-dependent and Smad-independent pathways, although most profibrotic actions of TGF-β_1 operate via Smad signaling. After injury, the activated Smad2/3 are released from the receptor complex to form a heterotrimeric complex of 2 R-Smads and a common Smad 4 and translocate into the nucleus to regulate the transcription of target genes in collaboration with various coactivators and corepressors.[45,46] A number of studies demonstrated that TGF-β_1 is the key mediator associated with progressive peritoneal fibrosis.[17,47]

Although the role of TGF-β/Smad3 in the development of renal, liver, and cardiac fibrosis has been well documented,[48] the distinctive role of Smad2 in organ fibrosis is still inconsistent.[49,50] In a recent study, TGF-β/Smad signaling was found to be highly activated in patients with increased collagen deposition and thickening of the peritoneal membrane who were receiving PD, but Smad2 and Smad3 have diverse roles in peritoneal fibrosis.[51] It was found that long-term exposure to high-glucose PD solution for 30 days induced significant peritoneal fibrosis with impaired peritoneal equilibrium, which was prevented in Smad3 knockout mice. In contrast, conditional *Smad2* gene deletion in the peritoneum exacerbated peritoneal fibrosis and dysfunction. These findings clearly suggested that Smad3 is critical for PD-induced peritoneal fibrosis, whereas Smad2 is protective.[51] Smad7, an inhibitory Smad, can be induced by a