Morphological and functional changes in the dialysed peritoneal cavity: impact of more biocompatible solutions

Olivier Devuyst, Nicholas Topley and John D. Williams

Division of Nephrology, St Luc Academic Hospital, Université Catholique de Louvain Medical School, Brussels, Belgium and 1Institute of Nephrology, University of Wales College of Medicine, Cardiff, UK

Abstract
Loss of peritoneal function is a major factor leading to treatment failure in peritoneal dialysis (PD). To date, however, the relationship between the observed functional changes (reduction in ultrafiltration and changes in solute transport) and the structural alterations in the membrane have not been fully defined. Here we present data from the Peritoneal Biopsy Registry identifying and characterizing both changes in parietal peritoneal membrane thickness (degree of fibrosis) and vascular alterations (blood vessel degenerative changes) and relate these to the duration of dialysis. The genesis of functional changes in the membrane may be related to these vascular alterations. This issue is discussed in relation to the importance of nitric oxide and its synthetic enzymes in this process and its potential interaction with endothelial cell aquaporin function. It is widely believed that conventional acidic, lactate-buffered glucose-containing dialysis solutions contribute to both the structural and functional changes in the dialysed peritoneal membrane. The introduction of new more biocompatible solutions potentially allows us to reverse or attenuate these negative changes. This will be discussed in the context of our current understanding of peritoneal pathology in PD.

Keywords: aquaporin; membrane morphology; NO; peritoneal dialysis; vasculopathy

Introduction
Loss of peritoneal function is a major factor leading to treatment failure in peritoneal dialysis (PD) [1–3]. Although the precise biological mechanisms responsible for these changes have not been defined, it is widely assumed that alterations in peritoneal function [ultrafiltration (UF) loss and increased solute clearance] are related to structural changes in the peritoneal membrane. In addition, there is accumulating indirect evidence that continuous exposure to bio-incompatible dialysis solution components as well as repeated episodes of bacterial peritonitis play a major role in the long-term changes seen in peritoneal function [2,4–7]. To date, however, the relationship between structure and function has not been fully defined. Likewise, although a number of studies have identified various mesothelial, vascular and interstitial changes in peritoneal morphology during PD, neither the factors responsible for these changes nor the time scale over which they develop have been identified [8–10]. The changes observed include loss or degeneration of mesothelium and submesothelial thickening (variously described as fibrosis or sclerosis) [11–17].

More recently, investigators have focused on changes within the peritoneal vascular bed since it is presumed that changes in vessel morphology or density might impact directly on membrane function [18]. Honda and co-workers have, in a small number of samples, observed structural changes to the venular walls and compared these with UF changes in these same patients [8,9]. There was a correlation between decreased UF and the appearance of vasculopathy, and with the development of submesothelial fibrosis. In patients with peritoneal ‘sclerosis’, Mateijesen et al demonstrated an increase in blood vessel numbers in the submesothelial zone compared with controls. There was also thickening of vessel walls and dilatation of capillaries [10]. More recently, an increased expression of nitric oxide synthase (NOS; a putative marker of blood vessel number) has been observed to correlate directly with PD therapy duration [19].

The Peritoneal Biopsy Registry
The aim of the Peritoneal Biopsy Registry was to establish a system where peritoneal biopsies could
be collected in a standard and reproducible manner. In addition, contemporaneous peritoneal function data were collected. Analysis of these samples would allow the precise structural changes in the dialysed peritoneal membrane to be characterized as well as allowing an initial evaluation of some of the potential factors responsible for the observed structural alterations.

Interim analysis examined the changes in the morphology (fibrosis and vascular changes) of the parietal peritoneal membrane in 130 patients on PD and compared these with the peritoneal membranes of normal individuals, uraemic pre-dialysis patients and patients on haemodialysis.

The median thickness of the submesothelial compact collagenous zone in biopsies obtained from PD patients increased significantly with duration of therapy from 180 μm (n = 58) (0–24 months) to 300 μm (n = 13) (49–72 months) and to 700 μm (n = 19) in patients on therapy for > 97 months [20]. If these same samples were analysed according to their surgical origin, compact zone thickness in patients with membrane failure or undergoing surgery for PD-related problems was significantly greater than that seen in those patients whose samples were obtained at transplantation or at unrelated abdominal surgery (P = 0.0001).

Vascular changes comprised progressive subendothelial hyalinization with luminal narrowing or obliteration. These changes were absent in controls, but present in 28% of uraemic samples and in 56% of biopsies from patients on PD. In the PD group, the prevalence of vasculopathy increased significantly with therapy duration (P = 0.00001). With duration of PD, there was no significant increase in the number of blood vessels per unit length of sample, although vessel numbers were significantly higher in patients with membrane failure and correlated with the degree of fibrosis. The data also demonstrate that whilst some of the morphological changes pre-date PD therapy, in a large cohort of patients (dialysed for periods up to 5 years without apparent problems) no increase in thickening of the submesothelial compact zone nor significant vascular changes develop. In contrast, those patients who exhibited clinical problems with PD had significantly greater fibrotic change and vasculopathy. Analysis also suggests that vasculopathy may lead to the development of fibrosis.

Functional changes in the peritoneal membrane

UF failure has been documented in up to 50% of PD patients treated for > 6 years and is second to recurrent peritonitis as the most frequent cause for technical dropout [2]. The pathophysiology of UF failure has been attributed to an increase in the effective peritoneal surface area (EPSA), with increased absorption of glucose and dissipation of the osmotic gradient, and/or an alteration in the ultra-small pores located in the capillary endothelium, with impaired free water permeability [21]. Recent studies focusing on the nitric oxide (NO) pathway in the peritoneum have provided new insights in the molecular mechanisms involved in the pathophysiology of UF failure in PD.

NO is synthesized from L-arginine by three NOS isoforms—the neuronal NOS (nNOS), the endothelial NOS (eNOS) and the inducible NOS (iNOS)—that are expressed in numerous tissues including the peritoneum [22]. Among countless biological functions, NO plays a role in controlling vascular tone and reactivity, and interacts with growth factors to regulate angiogenesis. The increase in EPSA and peritoneal permeability observed after addition of NO donors to the dialysate suggested a role for NO in the regulation of peritoneal transport during PD [23]. Studies in human and rat peritoneum have shown that eNOS is markedly up-regulated in peritoneal inflammation [24] or acute peritonitis [22]. In the latter case, increased NOS activity is negatively correlated with sodium sieving and UF capacity [22].

The hypothesis that NO might play a role in the increased EPSA observed in long-term PD patients was tested recently in a large series of peritoneal biopsies from control subjects and uraemic patients treated or not treated with PD [19]. This study demonstrated that PD is associated with a progressive increase of NOS activity within the peritoneum. This increase (> 5-fold for patients on PD > 18 months) is mediated by an up-regulation of eNOS, paralleled by an increase in vascular density and endothelial area. Vascular proliferation within the peritoneum of long-term PD patients was shown to be associated with an increased expression of vascular endothelial growth factor (VEGF), which might be triggered by the accumulation of advanced glycation end-products (AGEs) in the peritoneum during PD [19]. NO and related species are able to induce post-translational modifications of proteins. In particular, S-nitrosylation and subsequent functional modifications of cysteine-containing proteins, such as receptors, enzymes, ion channels and transcription factors, have been reported [25]. These biochemical modifications induced by NO might be relevant in the peritoneum, provided they affect a critical cysteine residue within a functionally important protein.

Morphological and functional studies have provided compelling arguments suggesting that the water channel, aquaporin-1 (AQP1), is the ultrasmall pore located in the endothelium lining peritoneal capillaries. The expression of AQP1 apparently is not modified in situations associated with UF failure and abolition of sodium sieving (i.e. disappearance of transcellular water permeability) [22, 26]. Rather, these conditions are associated with increased NOS activity in the peritoneum and an increased reactivity for nitrotyrosine and nitrosocysteine in endothelial cells. The potential interaction between NO and AQP1 at the molecular level has been supported by in vitro data showing that a critical cysteine residue (C189) located in the water pore of AQP1 mediates a significant inhibition of water permeability in the presence of NO donors [27].
The data summarized above provide a structural and molecular basis to propose that a NO-mediated increase in EPA, followed by a faster than normal glucose absorption and dissipation of the osmotic gradient, is a major mechanism accounting for the loss of UF in PD. Other consequences of increased NO levels in the peritoneum might include initiation of angiogenesis and post-translational modification of functionally important proteins such as aquaporin.

**Peritoneal dialysis solution biocompatibility**

Consideration of the possible predisposing factors to such changes in the peritoneal membrane leads to the hypothesis that solution bio-incompatibility may both reduce host defence within the peritoneal cavity and increase cell activation [28,29]. The result of this might be an overall chronic peritoneal inflammation which would include activation of interstitial fibroblasts and the production of collagen. In addition, there might be endothelial activation within the blood vessels resulting both in vasculopathy and in new vessel formation. One might even speculate that the activation of endothelial cells, leading to vasculopathy, might itself lead secondarily to fibrosis through ischaemia. The possible contributions of conventional PD solutions have been well defined. These include low pH (5.2–5.5), the presence of lactate to provide a buffer (35–40 mmol), the hyperosmolality produced by this glucose concentration (250–550 mOsm/kg) as well as the glucose degradation products generated during sterilization of the fluid [29,30].

Finally, there is the production of AGEs which may result from the high glucose exposure coupled with the presence of glucose degradation products such as pentosidine [31,32]. Thus the information to date indicating a potential for intraperitoneal damage by bio-incompatible fluid components is circumstantially strong and is supported by recent clinical data [7].

Over the past few years, there has been accumulating evidence for solution-induced changes in vivo mainly based on ex vivo studies. Ex vivo evaluation of PMØ function was first identified in 1993 when phagocytes exposed to intraperitoneal solution at pH 5 had a significantly decreased phagocytic index for pathogenic bacteria compared with cells isolated from fluids which had only been exposed to solutions of pH 7 [33]. There is also significant evidence that function of macrophages is depressed early during the dialysis cycle but recovers over the subsequent 4 h of dialysis [34].

Most recently, in a comparative study of bicarbonate/lactate-based dialysis fluid vs lactate-based dialysis fluid, peritoneal macrophages were collected at 3-monthly intervals from patients exposed to either fluid [35,36]. With increasing time of exposure to bicarbonate-based fluid, there was an overall recovery of cell function in response to stimulation, with an increased ability of cells to generate tumour necrosis factor, suggesting a normalization of the function of cells isolated from the peritoneal cavity [36]. In the same study, an examination of effluent showed that patients who were exposed to bicarbonate/lactate-based fluid had a significant increase in the quantity of CA125 present in the fluid over a 6-month period and a decrease in hyaluronic acid, interleukin-6 (IL-6) and VEGF levels [35,37]. Taken together, these data suggest that whilst host defence against infection is more competent in patients continuously exposed to more biocompatible solutions, markers of the ongoing inflammatory process [IL-6 and haemagglutinin (HA)] are significantly reduced, indicative of a reduction in overall inflammation. These data concur and extend previous studies using a low glucose degradation product solution [38].

**Conclusions**

There is accumulating evidence that in some patients there are long-term changes taking place in the peritoneal membrane in terms of both structure and function which may be caused by lengthy exposure to bio-incompatible components within dialysis fluids [7,20]. There is also some evidence that solution components have an impact in vivo on parameters related to host defence and inflammation [34–36,38–40]. These data suggest that as well as direct effects of solution components on parameters of membrane structure, indirect effects related to reduced host defence and exacerbated inflammation contribute to these processes. Conversion to more biocompatible PD solutions with physiological pH, more biocompatible buffer systems and reduced glucose degradation product levels should have a positive impact on membrane structure as well as on long-term function and help us to achieve the goal of longer and more successful therapy in PD.

**References**

Membrane structure–function relationships in PD


33. de Fijter CWH, Verbrugh HA, Peters EDJ et al. *In vivo* exposure to the currently available peritoneal dialysis fluids decreases the function of peritoneal macrophages in CAPD. *Clin Nephrol* 1993; 39: 75–80


