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PERSPECTIVES IN RENAL MEDICINE

Toward better dialysis compatibility: Advances in the biochemistry and pathophysiology of the peritoneal membranes

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Toward better dialysis compatibility: Advances in the biochemistry and pathophysiology of the peritoneal membranes. Peritoneal dialysis (PD) has modified our concept of the peritoneal membrane, which is now a topic of active research. Peritoneal solute transport progressively increases with time on PD, enhances the dissipation of the osmotic gradient and, eventually, reduces ultrafiltration capacity. The causes of peritoneal membrane failure remain elusive. Recurrent episodes of peritonitis are not a prerequisite for the development of ultrafiltration failure. Functionally, the changes of the failing peritoneal membrane are best described as an increased functional area of exchange for small solutes between blood and dialysate. Histologically, these events are associated with vascular proliferation and structural changes of pre-existing vessels. Gathered evidence, including information on the composition of peritoneal cavity fluids and its dependence on the uremic environment, have cast a new light on the molecular mechanisms of decline in peritoneal membrane function. Chronic uremia per se modifies the peritoneal membrane and increases the functional area of exchange for small solutes. Biochemical alterations in the peritoneum inherent to uremia might be, at least in part, accounted for by severe reactive carbonyl compounds overload originating both from uremic circulation and PD fluid ("peritoneal carbonyl stress"). The molecular events associated with long-term PD are similar but more severe than those present in chronic uremia without PD, including modifications of nitric oxide synthase (NOS) and angiogenic growth factors expression, and advanced glycation and lipoxidation of the peritoneal proteins. This review focuses on reactive carbonyls and their association with a number of molecular changes observed in peritoneal tissues. This hypothetical approach will require further testing. Nevertheless, the insights gained on the peritoneal membrane offer a new paradigm to assess the effect of uremic toxins on serosal membranes. Furthermore, the progresses made in the dissection of the molecular events leading to peritoneal membrane failure open new avenues to develop safe, more biocompatible peritoneal dialysis technologies.

Key words: ultrafiltration failure, effective peritoneal surface area, angiogenesis, nitric oxide, vascular endothelial growth factor, carbonyl stress, advanced glycation end products, glucose degradation products.

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Peritoneal dialysis (PD) is now an established alternative in the treatment of end-stage renal failure. It is utilized in approximately 15% of the dialysis patients in the developed world [1]. It has proven to be better than hemodialysis, especially in its protection of residual renal function [2] and in the lower overall cost to society [3]. Its use has been limited by the morbidity associated with acute peritonitis episodes, a problem recently overcome by newer technical devices and better antibiotic management. The major remaining problem is the progressive deterioration of the peritoneal membrane structure and function [4], which curtails its use in approximately 50% of PD patients within five years [5]. Cross-sectional and longitudinal studies have shown that peritoneal solute transport progressively increases with time on PD, enhances the dissipation of the osmotic gradient, and eventually reduces ultrafiltration capacity [6–8]. This increased small solute transport rate mainly reflects the peritoneal vascular surface area [4]. A raised peritoneal membrane permeability is associated not only with technique failure, but also with increased mortality and morbidity in PD patients [9].

The causes of peritoneal membrane failure remain elusive. Although recurrent episodes of peritonitis with associate inflammatory changes damage the peritoneal membrane over time [10], they are by no means a prerequisite for the development of ultrafiltration failure [4, 11]. Recent studies have cast a new light on the molecular mechanisms of decline in peritoneal membrane function during PD therapy. This review summarizes a few of them, delineates the causal role of reactive carbonyl compounds (RCOs) within the peritoneal cavity whether they originate from conventional heat-sterilized glucose PD fluid or from the uremic circulation, provides a hy-

pothesis on the molecular events leading to alterations of the peritoneal membrane transport and, finally, discusses therapeutic perspectives toward more effective and biomedically more suitable PD technologies.

THE PERITONEAL MEMBRANE: A UNIQUE AND EFFICIENT EXCHANGE SURFACE BETWEEN BLOOD AND DIALYSATE

The histology of the peritoneal membrane has been clearly described to include the mesothelium, interstitial space, and blood microvessels. Under normal physiological conditions, the mesothelial layer [4, 12, 13] and interstitial tissue [14, 15] are not thought to be important barriers at least for small solute transport. By contrast, the peritoneal vascular walls, mainly through their endothelium, are the main obstacles to small solute transport during PD [12, 13].

In standard PD using glucose as the osmotic agent, the peritoneal membrane exchange potential has been evaluated by clinical tools such as the peritoneal equilibrium test (PET) and sodium sieving. Its efficiency relies on the osmotic gradient between peritoneal fluid and plasma as it allows water influx, the diffusion of small uremic solutes evaluated by urea and creatinine, and the counter diffusion of glucose.

The results of the PET and sodium sieving have been modeled and a series of pores have been described [16]: ultrasmall pores ($<$0.3 nm) for water, small pores (4 to 5 nm) for water and smaller solutes such as urea and glucose, and large pores ($>$15 nm) for all the former constituents and large solutes. The fact that 50% of the ultrafiltration during 2.27 or 3.86% dwell occurs through the ultrasmall pores illustrates their major clinical importance in PD patients [17].

Recent studies have identified a number of molecules that functionally mediate the peritoneal membrane characteristics. The water channel aquaporin-1 (AQP-1) is located in the apical and basolateral membranes of endothelial cells lining non-fenestrated capillaries in numerous tissues including the peritoneum [18]. Several lines of evidence demonstrate that AQP-1 is the molecular counterpart of the ultrasmall pore of the peritoneal membrane [19, 20]. The small and large pores are not yet specifically identified. Additional molecules include nitric oxide (NO)/nitric oxide synthase (NOS) [21, 22], vascular endothelial growth factor (VEGF) [23], and basic fibroblast growth factor (FGF2) [24]. Both VEGF and FGF2 are instrumental in vascular proliferation and neo-angiogenesis. In addition, FGF2 mediates fibrotic changes of the interstitium and smooth muscle cell proliferation [25].

DOES UREMIA MODIFY THE PERITONEAL MEMBRANE REGARDLESS OF PD?

Until very recently it was generally accepted that the peritoneal membrane was unaffected by uremia, although an increased permeability had been already documented [26]. In a rat model of chronic uremia, Combet et al observed structural modifications including submesothelial and perivascular fibrosis, and angiogenesis [27]. These changes were matched by functional modifications characterized by an augmented permeability for small solutes. Several potential mediators identified thus far include VEGF and FGF2 expression, followed by endothelial NOS (eNOS) and neuronal NOS (nNOS), as they were transiently up-regulated after subtotal nephrectomy. The severity of these various uremia-induced alterations correlated with the degree of renal failure. Of note, a similar increase in FGF2 expression has been observed in the peritoneum of uremic patients, prior to the initiation of dialysis (Ferrier ML and Devuyst O, unpublished observation).

In addition to changes in some factors already present in the normal rat peritoneum, uremia is associated with vascular deposits of N^{ε}-carboxymethyllysine (CML) and pentosidine [27], two well-known epitopes generated by advanced glycation, that is, advanced glycation end products (AGEs). Their localization coincides with that of overexpressed VEGF and FGF2. In contrast to the rat model, in humans AGE modification of peritoneal proteins appears, at best, faint [28, 29].

The peritoneal membrane structural alterations detected in the uremic rat model have been supported in humans [30, 31]. Even in non-dialyzed uremic patients, submesothelial fibrosis is present and the density of vessels increased, as compared to non-uremic controls. Altogether, these data suggest that biochemical alterations inherent to uremia *per se* modify the peritoneum prior to the use of PD, and thus provide a paradigm to better understand the modification of the several serosal membranes in uremia.

CHANGES IN THE PERITONEAL MEMBRANE DURING PD

The permeability characteristics of the peritoneal membrane are progressively modified during PD, imposing occasional switching of the patient from PD to hemodialysis.

Functional analysis

The most striking functional characteristics of peritoneal membrane failure are detected in patients on chronic PD by the PET test and a measure of sodium sieving [4, 6–8]. These characteristics include a loss of free water permeability identified by the disappearance of sodium sieving, an increased transport rate of small solutes (urea, creatinine and glucose), with an attendant

enhanced dissipation of the glucose-dependent osmotic gradient across the peritoneal membrane and the loss of ultrafiltration capacity.

The changes in the functional characteristics of the failing peritoneal membrane are best described as an increased functional area of exchange for small solutes between blood and dialysate, the so-called "effective peritoneal surface area" (EPSA) [4, 12]. They rely on enhanced angiogenesis (described below) and, possibly, vasodilation of peritoneal vessels. The increased transport of small solutes stands in contrast with the impaired water transport, witnessed by the fading sodium sieving.

Histological analysis

Histopathologic studies have revealed that prolonged PD is associated with profound modifications of the peritoneal membrane. First, an increased density of blood vessels, documented in a rat model of long-term PD [32], has been recently confirmed in humans [31, 33, 34]. Quantification of factor VIII immunoreactivity in peritoneal sections from long-term PD patients, showed a 2.5-fold increase in the density of stained vessels in long-term PD patients as compared to non-uremic controls [33]. The endothelial area also was increased in the PD patients [33]. Thus, PD progressively increases the EPSA as the result of vascular proliferation and, possibly, of a vasodilation of pre-existing vessels. Interstitial fibrosis and mesothelial alterations have been reported in addition to the vascular changes [30, 31, 34].

Molecular biological analysis

In humans, the molecular events associated with long-term PD and peritoneal membrane failure are similar but more severe than those present in chronic uremia without PD. They include up-regulation of eNOS, overexpression of VEGF and FGF2, and advanced glycation of peritoneal proteins [33]. In contrast, the expression of AQP1, the pore involved in water transport, remains apparently unaffected [35].

The up-regulation of VEGF and FGF2 fits with an enhanced angiogenesis and the interstitial fibrosis observed on histological analysis: its determinants are discussed later in this article. The increased NOS activity is also of considerable interest as NO has been shown to be clinically relevant [36–38]. Under some circumstances, the NO donor, nitroprusside, enhances both the EPSA and the intrinsic permeability of the membrane both in animal models and in PD patients, whereas NOS inhibitors such as L-NAME have the reverse effect. These effects of NO are in good agreement with its previously described activities in other systems [39–42]: NO augments vascular permeability and modulates the expression and activity of VEGF. Furthermore, there is accumulating evidence indicating that under physiological conditions, NO targets cysteine thiols by *S*-nitrosylation and that these covalent modifications modulate the function of several proteins [43]. Interestingly, preliminary data suggest that *S*-nitrosylation of a single cysteine within the functional pore of AQP-1 is associated with a significant loss of water permeability [43, 44]. This finding might help understand the mechanisms of the decreased water permeability of the peritoneal membrane despite a normal expression of AQP1 [35, 45].

It is interesting that in an acute peritonitis rat model, ultrafiltration failure is associated with a major, tenfold increase in peritoneal NOS activity due to the up-regulation of both eNOS and inducible NOS (iNOS) [45]. In this model, up-regulated eNOS is paralleled by a significant increase in vascular density, whereas inflammatory derived cytokines activate the transcription of iNOS.

In humans on long-term PD, regulation of NOS also might be involved in the increased EPSA [33]. Peritoneal NOS activity increases fivefold above control levels and is positively correlated with the duration of PD. Increased NOS activity is solely mediated by calcium-dependent eNOS. Its biological relevance is further demonstrated by concomitant, enhanced immunoreactivity for nitrotyrosine, which points to oxidative stress and increased peroxynitrite formation.

Several factors might contribute to the increased expression of eNOS in long-term PD patients. eNOS up-regulation reflects, at least in part, vascular proliferation and thus increased endothelial surface area. In addition, it may be modulated by changes in immune defense systems induced by long-term PD, such as, local cytokine production [46]. Observations that eNOS is up-regulated in the inflammatory peritoneum [18] or upon virus-induced activation of interferon-γ and tumor necrosis factor-α support the latter hypothesis [47].

In long-term PD patients, advanced glycation and advanced lipoxidation of peritoneal proteins becomes a striking feature [28, 29]. Prior to the onset of PD, in contrast with uremic rats, the AGEs and advanced lipoxidation end products (ALEs) in humans are only faintly detectable. They increase subsequently in parallel with PD duration. Of interest, AGEs colocalize with VEGF along the endothelium lining peritoneal blood vessels [33, 48], and several studies have hypothesized that AGE modification of the peritoneum plays a critical role in its functional alteration [27–29, 33, 49].

PERITONEAL CARBONYL STRESS: THE CAUSE OF AGE MODIFICATION OF THE PERITONEAL MEMBRANE

Advanced glycation of proteins is induced by reactive carbonyl compounds (RCOs) [50]. Several RCOs leading to the formation of AGE epitopes such as pentosidine [51] or CML [52] have been identified, for example, glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone

(3-DG), glycolaldehyde, and arabinose [53–56]. While many others are present and can be estimated by the 2,4-dinitrophenylhydrazine (DNPH) method, their identity remains elusive [29]. In PD, RCOs precursors of AGEs may originate from two sources: the peritoneal dialysate and the uremic circulation [29].

RCOs originating from glucose containing PD fluid

Heat sterilization of PD fluids degrades glucose into products including RCOs, such as GO, MGO, and 3-DG. Research on the role of AGEs in the peritoneum has focused mostly on these RCO precursors [57–59].

RCOs originating from uremic circulation

Plasma RCO precursors of AGEs also have to be taken into consideration as they invade the peritoneal cavity during the dwell time [29]. RCOs, derived not only from carbohydrates but also from lipids, accumulate in uremic plasma ("carbonyl stress") and contribute to the production of AGEs as well as of ALEs in the body [50]. Their accumulation in uremic plasma has been demonstrated, for example, in in vitro incubation experiments during which pentosidine, an AGE moiety, increases over time [60]. Addition of inhibitors of the carbonyl amine reaction, such as aminoguanidine [61] or ±2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195) [62, 63], represses the production of pentosidine, pointing to the RCO nature of the pentosidine precursors. Notably, plasma pentosidine levels are correlated with the level of in vitro pentosidine generated after incubation [60], suggesting that they mirror the level of RCO precursors and therefore might be used as a marker of their accumulation [64].

Several mechanisms might account for RCO accumulation in renal failure [65]. Production of RCOs might be enhanced by the oxidative stress associated with uremia [66]. This hypothesis is supported by the positive correlations demonstrated in uremic serum between the levels of pentosidine and markers of oxidative stress such as advanced oxidation protein products (AOPP) [67], dehydroascorbate [68] and superoxide dismutase [69], as well as by the negative correlation reported between serum pentosidine and glutathione peroxidase levels [69]. Alternatively, the rise in RCOs in uremia could be accounted for by a decreased removal by the failing kidneys [64, 70] and/or through enzymatic pathways of detoxification such as the glyoxalase pathway [54]. In the latter pathway, RCOs such as MGO and GO react reversibly with glutathione and are subsequently detoxified by glyoxalases I and II into D-lactate and glutathione. Decreased levels of glutathione therefore can augment the levels of a wide range of RCOs. Glutathione concentration in red blood cells and the serum activity of glutathione-dependent enzymes are significantly reduced in uremic patients [69, 71, 72]. Other, as yet unexplored enzymatic mechanisms also might contribute to a decreased removal of RCOs and thus to the uremic carbonyl stress.

Peritoneal carbonyl stress

We recently demonstrated that not only PD fluid, but also serum-derived RCOs contribute to the genesis of peritoneal AGEs [29]. During the peritoneal dwell time, the RCO content of commercial heat-sterilized glucose PD fluids, that is, GO, MGO and 3-DG, as well as glucose levels fall markedly. In contrast, during the same time interval, total RCO levels (assessed by the DNPH method) increase progressively in the dialysate toward concentrations similar to those observed in plasma. This pattern is compatible with an outward diffusion of GO, MGO, 3-DG from the peritoneal cavity and an inward diffusion of plasma RCOs within the peritoneal fluid.

Plasma derived RCOs are instrumental in the genesis of AGEs, as the generation of pentosidine and CML after incubation of PD effluent increases progressively with dwell time. Further evidence for their role in the modification of peritoneal membrane proteins is provided by the demonstration that ALEs, such as malondialdehyde (MDA)-lysine and 4-hydroxynonenal (HNE)-protein adduct, appear together with AGEs in the peritoneum of long-term PD patients [29]. As already mentioned, uremic plasma RCOs are derived not only from carbohydrates, but also from lipids. The former produce AGEs and the latter produce ALEs. During their diffusion within the peritoneal cavity, uremic RCOs are thus expected to generate not only AGEs, but also ALEs. The presence of the latter adducts, which cannot proceed from glucose degradation products present in commercial heat-sterilized glucose PD fluids, confirm the role played by circulating RCOs crossing the peritoneal membrane during PD.

Advanced glycation end products and ALEs are only faintly present in the peritoneum of uremic patients prior to the onset of PD [28, 29]. Their marked rise after the onset of PD suggests that their formation is enhanced by the augmented mass transfer of plasma RCOs across the peritoneal membrane into the peritoneal cavity, likely as a result of the dialysis procedure itself.

The major RCOs that diffuse from uremic circulation into the peritoneal cavity have yet to be identified. They are not GO, MGO, or 3-DG [29].

Pathological significance of carbonyl stress

The peritoneal cavity of PD patients is thus in a state of severe overload of RCOs derived both from uremic circulation and from conventional glucose-based PD fluids. Which role do these RCOs play in the pathogenesis of peritoneal membrane deterioration?

As stated earlier, RCOs promote the AGE and ALE modification of proteins. In vitro, AGE and ALE modi-

fied proteins initiate a range of cellular responses [73–78], including stimulation of monocyte chemotaxis and apoptosis, secretion of inflammatory cytokines from macrophages, proliferation of vascular smooth muscle cells, stimulation of platelet aggregation, and of VEGF production from endothelial cells. Independently of their AGE- and ALE-mediated effects, RCOs also interfere with various cellular functions and induce both structural and functional alterations of proteins. For example, exposure in vitro of cultured mesothelial and endothelial cells to MGO increases mRNA and protein synthesis of VEGF [48]. Repeated intraperitoneal loads of MGO, given to rats, also increase the peritoneal membrane expression of VEGF in vivo [48]. Noteworthy in this context is the demonstration that, both in long-term PD patients and in the chronic uremic rat model [33, 48], an increasing staining for AGEs, CML and pentosidine is detected in peritoneal arterial walls together with an augmented VEGF and FGF2 expression.

There are two major pathways (direct and indirect) through which carbonyl stress is sensed by cells and triggers a cascade of intracellular signal transduction. In the indirect pathway, the RCOs first interact with proteins or lipids in the physiological environment surrounding the cells, which then undergo nonenzymatic glycation and lipoxidation resulting in the production of AGEs and ALEs. They bind with the receptor(s) on cell surfaces, such as RAGE, thereby initiating intracellular signal transduction [79]. By contrast, the direct pathway works before generation of AGEs and ALEs. The RCOs directly attack target molecules on cell surfaces or inside the cells, which initiate the subsequent signal transduction [80–83]. For example, GO and MGO possess two reactive carbonyl groups to make protein aggregates by cross-linking, which may amplify the signals for tyrosine phosphorylation of cellular proteins [80]. The binding of 4-hydroxynonenal (HNE), a major RCO generated during membrane lipid peroxidation, with epidermal growth factor receptor (EGFR) induces its clustering on the cell surface, thereby activating the mitogen-activated protein (MAP) family kinases [81].

HYPOTHESIS

These new data cast some light on putative mechanisms of peritoneal membrane deterioration in long-term PD patients. They include neoangiogenesis, enhanced growth factor expression, elevated RCO content in the peritoneal cavity, advanced glycation and lipoxidation of peritoneal membrane proteins, and up-regulation of NOS expression with an increased bioactive NO release. In this section, we provide a hypothetical framework to integrate them with the intent to provide clues for future investigations and, possibly, for innovative therapeutic strategies (Fig. 1). Although each step has been documented in vitro, the entire hypothesis remains to be validated in vivo, that is, by testing its various therapeutic implications.

Chronic uremia *per se* modifies the peritoneal membrane and increases the EPSA. Biochemical alterations in the peritoneum inherent to uremia, at least in part, might be accounted for by severe RCO overload originating both from uremic circulation and PD fluid ("peritoneal carbonyl stress"). Plasma RCOs derived from both carbohydrates and lipids accumulate in the uremic circulation and slowly diffuse into the peritoneal cavity. They initiate a faintly detectable AGE as well as ALE protein modifications. During PD, RCOs resulting from heat-sterilization of glucose PD fluid enter the peritoneum, and are complemented by an increased mass transfer of serum RCOs due to the dialysis procedure itself.

The peritoneal carbonyl stress accelerates the AGE and ALE modification of proteins. AGEs as well as their RCO precursors are known to initiate a number of cellular responses. We hypothesize that they stimulate cytokine and growth factors (including VEGF) production and regulate NOS expression in peritoneal cells. The combination of VEGF and NO stimulates angiogenesis, increases permeability, and vasodilates peritoneal capillaries. These combined modifications increase EPSA, which results in faster than normal dissipation of the osmotic and an eventual loss of ultrafiltration. The up-regulation of FGF2 and transforming growth factor-β (TGF-β) also stimulates interstitial fibrosis of the peritoneum [84].

A closer analysis of the interrelationship between RCOs, AGEs, VEGF, eNOS and NO discloses the complexity of the possible pathogenic pathways leading to angiogenesis in the peritoneum. Accumulation of RCOs and AGEs in the peritoneum activates endothelial cells, which promotes VEGF expression. In turn, VEGF further stimulates endothelial cells with an attendant up-regulation of eNOS and the release of NO. The latter contributes, at least in part, to the angiogenic effect of growth factors such as VEGF. Vascular proliferation and increased endothelial area lead to a further up-regulation of eNOS in the peritoneum, and, in some conditions, to sustained release of NO. The latter has a threefold effect. First, it may act as a negative feedback and down-regulate the expression of VEGF [85]. Second, it might target critical cysteine residues and regulate proteins by *S*-nitrosylation [43, 44]. Third, it might decrease AGE production and quench its consequences [86]. Finally, in case of oxidative stress, NO might combine with superoxide and generate peroxynitrite, a labile, cytotoxic reactive oxidant species [87].

These hypothetical mechanisms open a number of therapeutic approaches that might protect the peritoneal membrane against the consequences of long-term PD.

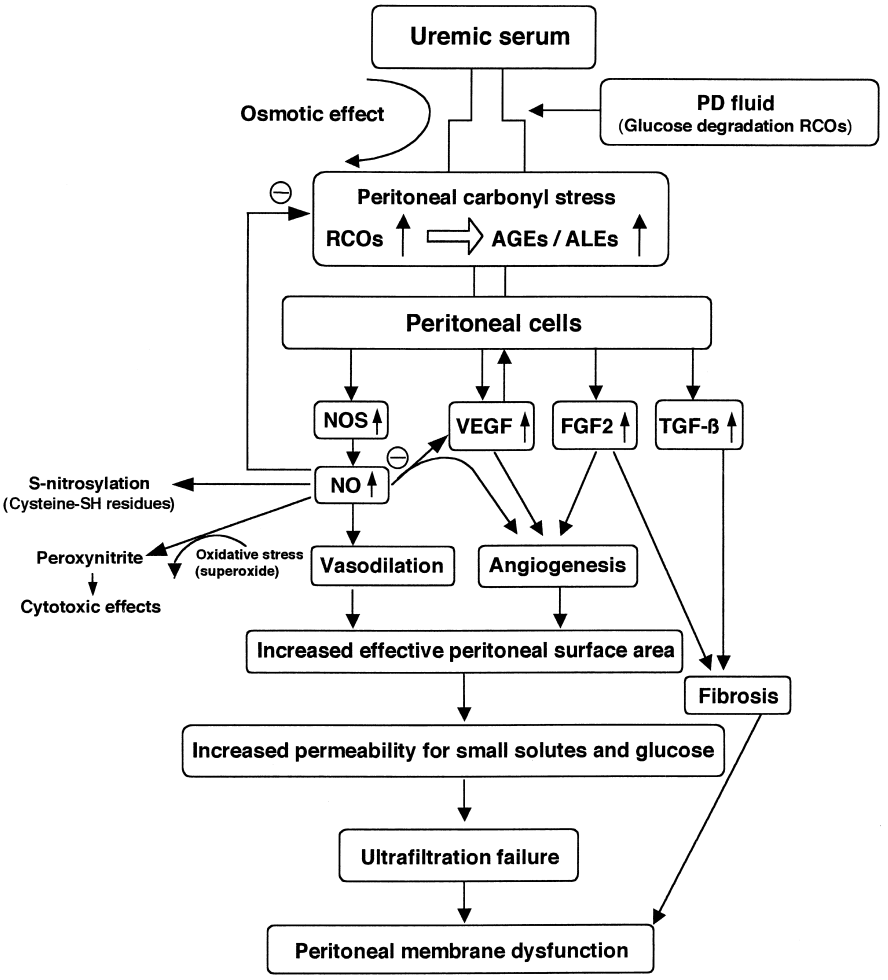

Fig. 1. Hypothesized structural and functional alterations of the peritoneal membrane in long-term peritoneal dialysis (PD). In chronic uremia, plasma reactive carbonyl compounds (RCOs) derived from both carbohydrates and lipids accumulate in the circulation and slowly diffuse into the peritoneal cavity. Biochemical alterations in the peritoneum inherent to uremia are exacerbated by the PD procedure itself. During PD, RCOs resulting from heat-sterilization of glucose PD fluid enter the peritoneum and are complemented by an increased mass transfer of serum RCOs (osmotic effect). The "peritoneal carbonyl stress" accelerates the advanced glycation end product (AGE) and advanced lipoxidation end product (ALE) modification of the peritoneal membrane. AGEs as well as their RCO precursors initiate a number of cellular responses, including cytokine and growth factors [vascular endothelial growth factor (VEGF), basic fibroblast growth factor-2 (FGF2) and transforming growth factor-β (TGF-β)] production, vascular smooth muscle cell proliferation, and specific nitric oxide synthase (NOS) up-regulation. Enhanced VEGF and FGF2 expression, together with an augmented nitric oxide (NO) release, stimulate angiogenesis and vasodilation and increase the permeability of peritoneal capillaries. These combined modifications increase the effective peritoneal surface area (EPSA). The latter augments the permeability for small solutes and glucose, stimulates glucose reabsorption, and results in faster than normal dissipation of the osmotic gradient across the peritoneal membrane with an eventual loss of ultrafiltration. The up-regulation of FGF2 and TGF-β stimulates interstitial fibrosis of the peritoneum.

INNOVATIVE APPROACHES TO MORE EFFECTIVE AND BIOMEDICALLY MORE SUITABLE PERITONEAL DIALYSIS TECHNOLOGIES

Carbonyl stress, increased NO secondary to NOS up-regulation, and up-regulation of VEGF and FGF2 cause some of the morphologic and molecular alterations of the peritoneal membrane. Their manipulation might provide some therapeutic benefits (Fig. 2).

Reduction of the RCO content in peritoneal dialysis fluid or in peritoneal cavity

As previously stated, peritoneal protein modifications are determined not only by RCOs originating from PD fluid, but also by RCOs originating from the uremic circulation. Some strategies have been designed to reduce the RCOs present in PD fluid, and may prove rewarding despite the fact that they do not fully eliminate the peritoneal carbonyl stress.

Glucose-free PD fluids. Major efforts have been undertaken over the last few years to sustain ultrafiltration and to improve nutrition by replacing glucose with icodextrin or amino acids [88–90]. An advantage of these newer solutions accrues from their lower RCO content. GO, MGO, 3-DG as well as total RCO levels are markedly reduced in fresh icodextrin and amino acid PD fluid when compared with heat-sterilized glucose PD fluid [91]. As a consequence, pentosidine and CML generation during incubation is lower in icodextrin and amino acid PD fluid than in conventional glucose PD fluid [91, 92].

The benefits of a lower RCO content of PD fluid are mitigated by the changes in peritoneal fluid RCO content occurring during dwell time [91]. Individual RCOs, such as GO, MGO and 3-DG, fall progressively in icodextrin effluents or remain undetectable in amino acid effluent. By contrast, with dwell time, total RCOs accumulated in uremic serum diffuse within the peritoneal cavity with an attendant increase of the AGE formation potential of PD fluid. While the clinical benefits of these fluids for the preservation of the peritoneal membrane remain to be documented in long-term studies, these findings do

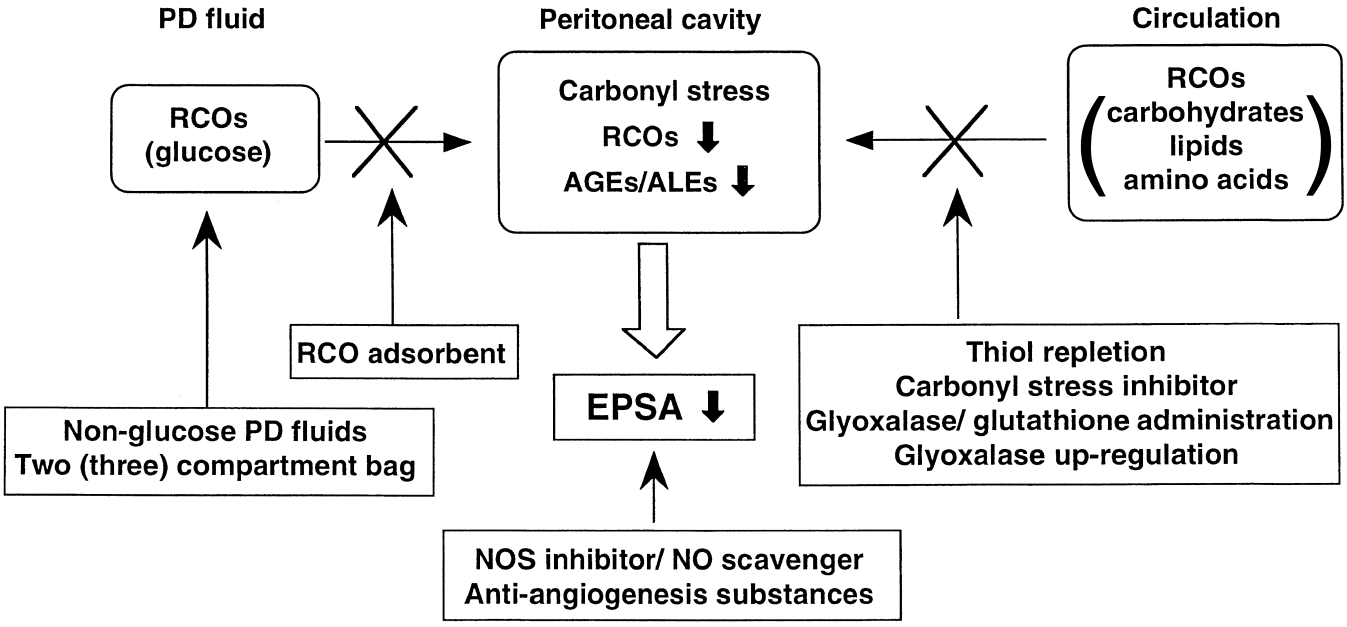

Fig. 2. Innovative approaches to more effective and biomedically more suitable peritoneal dialysis technologies. Peritoneal membrane modifications are determined by RCOs originating from both PD fluid and uremic circulation. PD fluid RCOs are lowered in non-glucose fluids or in multi-compartment bag systems. Peritoneal cavity fluid RCOs metabolism is enhanced by thiol repletion and the addition of glyoxalase I and glutathione. They are trapped by inhibitors of carbonyl amine chemistry or by RCO adsorbents. The increased release of NO, either by constitutive endothelial nitric oxide synthase (eNOS) or by inducible nitric oxide synthase (iNOS) induced by peritoneal inflammation, increases the effective peritoneal surface area (EPSA). These changes are reversible by NOS inhibitors, NO scavengers, and anti-angiogenic substances. The preservation of the peritoneal membrane might require a combination of several therapeutic approaches.

not detract from the clinical merits of these glucose free PD fluids [88]. Icodextrin fluid sustains ultrafiltration profile that is beneficial for long dwells by employing colloidal, rather than crystalline, osmotic pressure, whereas amino acid fluid improves nitrogen balance in patients with malnutrition and avoids the glucose overload, an advantage in diabetic and obese patients.

Multi-compartment bag. An interesting approach to lower the RCO content of glucose-containing PD fluid has been recently provided by a multi-compartment bag system [93, 94]. In this system, glucose is kept at a low pH, separate from the electrolyte buffer (bicarbonate-based) solution at a neutral pH. When both bags are mixed, the final solution has a physiologic concentration of bicarbonate, a reduced concentration of lactate, and a physiologic pH. Its RCO content and AGE generation potential are very low despite conventional heat-sterilization and subsequent storage [95, 96].

The clinical effects of this new fluid have been recently assessed up to two years [97, 98]. Both studies demonstrated elevated effluent levels of cancer antigen (CA) 125 and reduced levels of hyaluronic acid in their overnight effluent, suggesting reduced proinflammatory potential and improved peritoneal membrane integrity. After two years, membrane transport characteristics and ultrafiltration capacity were similar in the double bag and in the control group [97]. Although encouraging, these results should be assessed with caution as a much longer study period may be required to reveal any changes in these parameters.

Inhibitor of carbonyl amine chemistry. An alternative therapeutic strategy might rely on compounds known to inhibit AGE formation. Compounds, such as aminoguanidine, OPB-9195, and probably biguanides [99], contain a hydrazine nitrogen atom that is able to react with carbonyl groups and eventually form hydrazone [63]. Trapping of RCOs by these compounds thus should inhibit the RCO modifications of proteins. Indeed, both aminoguanidine and OPB-9195 inhibit in vitro the formation of AGEs (CML and pentosidine) as well as that of ALEs (MDA-lysine and HNE-protein adduct). AGE generation (pentosidine) from incubated uremic plasma also is inhibited, demonstrating that both compounds trap precursor RCOs of pentosidine accumulating in uremic serum [60].

We have demonstrated that addition of OPB-9195 or aminoguanidine to commercial glucose PD fluids reduced the generation of pentosidine and CML [63], in agreement with a previous study by Lamb et al [100], who reported that aminoguanidine inhibited the fluorescence intensity on albumin incubated in heat-sterilized glucose PD fluid. We further documented a dramatic fall in GO, MGO and 3-DG levels within 24 hours of incubation of PD fluids with OPB-9195 or aminoguanidine [63].

These carbonyl-scavenging agents have further benefits. They block carbonyl stress-mediated intracellular signaling [80, 83]. Oral administration of OPB-9195 to rats, after balloon injury of their carotid arteries, effectively reduces neointima proliferation in arterial walls [62].

Clinical trials of aminoguanidine as well as of OPB-9195 in diabetic patients have been hampered, however, by their neurotoxicity largely due to the trapping of pyridoxal [101]. Less toxic and more specific carbonyl stress inhibitors need to be developed.

Glyoxalase detoxification. Reactive carbonyl compounds partly are detoxified by the glyoxalase pathway. We recently observed a patient in whom a deficiency of glyoxalase I was associated with unusually elevated levels of AGEs (pentosidine and CML) and their precursors [102]. The causes of this deficiency remain unknown. Nevertheless, the association of very low levels of glyoxalase I with strikingly elevated levels of AGEs and RCO precursors offers new therapeutic insights in that maneuvers augmenting glyoxalase I activity might lower RCO and AGE levels.

Preliminary observations in vitro support this approach. Detoxification of RCOs by glyoxalase I is markedly impaired by a decreased thiol concentration (such as glutathione) [103]. Thiol compound such as glutathione, cysteine or *N*-acetylcysteine added to mixtures of GO, MGO, 3-DG decreases their levels. Added to heat-sterilized glucose PD fluid, they lower AGE generation after incubation. Glutathione is undoubtedly less toxic in vivo than aminoguanidine but, unfortunately, less efficient at least in vitro. Addition of glyoxalase I overcomes this limit. Utilized jointly, both compounds dramatically accelerate and intensify the in vitro lowering of GO and MGO both in RCO mixtures and in glucose-based PD fluid. A similar effect is observed when glyoxalase I is replaced by lysates of glyoxylase I transfected cells or by tissue extracts of human glyoxalase I transgenic mice [103]. These results open the exciting prospect of lowering the peritoneal carbonyl stress in humans by raising glyoxalase I activity in peritoneal cells by genetic engineering (described below) or by the concomitant administration of recombinant glyoxalase I with glutathione.

RCO adsorbent. Compounds with RCO binding properties might be immobilized on beads or in a cartridge in order to adsorb RCOs in conventional heat-sterilized glucose PD. Polystyrene sulfonyl hydrazide beads or diaminoguanidine agarose beads added to heat-sterilized glucose PD fluid decrease the levels of GO and MGO as well as the generation of pentosidine and CML after incubation (Miyata T and Ueda Y, unpublished observation).

Inhibitor of the L-arginine-NO pathway

Modulation of the activity of NO may have considerable therapeutic value. The L-arginine analogs, which interfere competitively with the binding of L-arginine to NOS, are well-characterized NOS inhibitors [104]. Despite their lack of specificity, N^G-monomethyl-L-arginine (L-NMMA) and its prodrug N^G-nitro-L-arginine methyl ester (L-NAME) have proved to be the most useful NOS inhibitors [104]. Newer compounds that are more selective toward iNOS [105] and alternative strategies to modulate the biological activity of NO have been considered, but remain to be characterized [106, 107].

Only a few studies have investigated the potential of NOS inhibitors on peritoneal membrane characteristics. Addition of L-NMMA to PD fluid does not alter peritoneal permeability in a chronic PD model in rabbits [108]; however, in different rat models of peritonitis, the addition of L-NAME to PD fluid increases net ultrafiltration with a dose-dependent effect on the permeability for small solutes and proteins [38, 109]. The usefulness of NOS inhibition in long-term PD remains to be defined.

Manipulation of an ubiquitous mediator such as NO raises a number of potential problems and may actually have a double-edged sword effect. Current efforts aim towards improving the selectivity of NOS inhibitors, taking into account differences in expression levels, cofactor utilization and location [104, 105].

Inhibitor of angiogenesis

Based on recent insights [110], a spectrum of strategies for the modulation of angiogenesis has emerged, with the most successful approach to date being the use of agents that inhibit endothelial cell growth [111]. Interference with factors such as VEGF and FGF2 and their receptors may provide another approach. A third approach is to interfere with endothelial cell adhesion and migration [111].

Although more than 30 anti-angiogenesis compounds have been tested in human clinical anti-cancer trials, trials for noncancerous diseases are scarce and limited in scope [111]. No studies have been undertaken in long-term PD, probably because appropriate animal models are lacking [112]. It must be stressed that trials using anti-angiogenic substances require specific approaches to overcome investigator bias [113], and that little information on safety and long-term side-effects of this type of therapy is available.

Gene therapy

Some of functional and structural changes associated with long-term PD might be targeted by gene therapy [114]. Ex vivo gene therapy involves harvesting peritoneum samples to isolate mesothelial cells that will be genetically modified before re-implantation into the peritoneal cavity [115]. The feasibility of this procedure has been demonstrated in animal models by the production of anti-inflammatory and anti-oxidant proteins from genetically modified cells following experimental denuda-

tion of the membrane or chronic exposure to dialysis solutions [115]. While reimplantation of autologous cells in PD patients has been accomplished, conditions for reseeding the peritoneal membrane on an acute or chronic basis still need to be defined. In vivo gene transfer is based on direct gene delivery and in situ genetic modification. The adenovirus system appears to be the most efficient for delivering genes to a significant percentage of mesothelial cells [115]. However, its use in vivo may be limited by local changes resulting from inflammatory and immune responses.

Future prospects of gene therapy in the peritoneum include using either non-viral systems or viruses with low potential for immunogenicity, increasing gene transfer efficiency, and regulating transgene expression through use of mesothelial cell-specific promoters [116].

CONCLUSIONS

Peritoneal dialysis has, among many other factors, the merit of having modified our concept of the peritoneal membrane. Known as a mere envelope of abdominal organs a few decades ago, the peritoneal membrane is now a topic of active research that has already underscored its complex, very active nature.

Evidence is either obtained directly from peritoneal cells and tissue or extrapolated from other cell types to their peritoneal counterparts, and includes information on the composition of peritoneal cavity fluids and their dependence on the uremic environment.

This review focuses on reactive carbonyls and their association with a number of molecular changes observed in peritoneal tissues and with several alterations of peritoneal membrane function. This hypothetical approach will require further testing. Taking into account the complexity of the various systems involved in the maintenance of the peritoneal membrane, it is likely that many of today's assumptions or simplifications will have to be revised and that the present hypothesis will require substantial amendments.

Whatever the fate of the proposed hypothesis, the insights gained on the peritoneal membrane offer a new paradigm to assess the effect of uremic toxins on serosal membranes.

Finally, the progress accomplished in the dissection of the molecular events leading to peritoneal membrane failure open new avenues towards the development of a safe, more biocompatible peritoneal dialysis.

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APPENDIX

Abbreviations used in this paper are: AGEs, advanced glycation end products; ALEs, advanced lipoxidation end products; AQP-1, aquaporin-1; CML, N^{ε}-carboxymethyllysine; 3-DG, 3-deoxyglucosone; EPSA, effective peritoneal surface area; FGF2, basic fibroblast growth factor; GO, glyoxal; HNE, 4-hydroxynonenal; MDA, malondialdehyde; MGO, methylglyoxal; NO, nitric oxide; NOS, nitric oxide synthase; PD, peritoneal dialysis; RCO, reactive carbonyl compound; VEGF, vascular endothelial growth factor.

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