New insights in the molecular mechanisms regulating peritoneal permeability

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Introduction

Peritoneal dialysis (PD) is used in approximately 15% of dialysis patients worldwide. With the reduction of acute peritonitis, the major problem associated with PD is now the high incidence of ultrafiltration (UF) failure, which can affect up to 50% of PD patients treated for more than 6 years [1]. Cross-sectional and longitudinal studies have shown that peritoneal permeability for small solutes increases with time on PD, which induces a faster absorption of glucose, an early dissipation of the osmotic gradient and, eventually, UF failure [1,2]. The relevance of these modifications is illustrated by the fact that high peritoneal membrane (PM) permeability is a significant risk factor, predicting both technical failure and death in PD patients [3]. In this commentary, we will discuss how recent structural, functional and molecular data provide new insights in our understanding of the modifications of the PM in long-term PD.

Structural modifications of the peritoneum

The endothelium lining peritoneal capillaries is considered to be the functional barrier to water and small solutes transport during PD, whereas the contribution of the mesothelium and interstitial tissue remains to be defined [4]. The amount of perfused peritoneal capillaries determines the so-called ‘effective peritoneal surface area (EPSA)’, i.e. the functional area of exchanges between blood and dialysate. These exchanges rely on the osmotic gradient between dialysate and plasma, allowing water influx, diffusion of small solutes such as urea and creatinine, and counter-diffusion of glucose [4]. The water channel aquaporin-1 (AQP1), which is located in the endothelium lining peritoneal capillaries, probably corresponds to the water-selective, ultrasmall pore involved in the free water permeability explaining the sodium sieving during the first 30 min of an hypertonic dwell [5,6].

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Histopathologic studies have shown that long-term PD is associated with modifications of the PM, including perivascular and submesothelial fibrosis, alterations of the mesothelium, and replication of capillary basement membrane [7,8]. In particular, vascular proliferation and increased endothelial area within the PM have been documented in long-term PD patients, and these changes correlate with PD duration [8,9]. Thus, vascular proliferation and, possibly, vasodilation of peritoneal vessels, might represent the structural basis for increased EPSA in long-term PD [9].

**Nitric oxide and the peritoneum**

Nitric oxide (NO) plays a role in the regulation of vascular tone and permeability [10], and interacts with angiogenic growth factors [11]. It might thus regulate EPSA, and UF, during PD. NO is synthesized from L-arginine by three nitric oxide synthase (NOS) isoforms—the neuronal NOS (nNOS), the endothelial NOS (eNOS), and the inducible NOS (iNOS). Both nNOS and eNOS are constitutively expressed in cells and their activity depends on intracellular Ca\(^{2+}\) levels. By contrast, iNOS transcription is activated by lipopolysaccharides and/or cytokines, and its activity is Ca\(^{2+}\)-independent [12]. NOS activity results in the stoichiometric production of NO and \(\cdot\text{citrulline}\) from \(\cdot\text{arginine}\), a reaction that can be assessed by the specific and highly sensitive \(\cdot\text{citrulline}\) assay.

The \(\cdot\text{citrulline}\) assay has been used to assess NOS activity in the PM. In control conditions, the activity is mainly due to the predominant eNOS isoform [13]. In long-term PD, peritoneal NOS activity increases fivefold above levels observed in control and uraemic subjects prior to the onset of PD [9]. Furthermore, the increased NOS activity correlates positively with PD duration and is solely due to the upregulation of eNOS—itself reflecting the increase in endothelial area [9]. A major increase in NOS activity, due to both eNOS and iNOS, has been observed in a rat model of acute peritonitis [14]. Interestingly, addition of the NOS inhibitor N\(^5\)-nitro-\(\cdot\text{arginine}\) methyl ester (\(\cdot\text{NAME}\)) to the dialysate restores UF in this model [14]. Thus the release of NO, secondary to NO upregulation, might be a major regulator of UF in PD patients. In addition to its vascular effects, NO might also affect the PM by generating peroxynitrite or by modifying critical protein residues, as suggested by increased nitrotyrosine and nitrosocysteine reactivity [9,15].

**Vascular endothelial growth factor and the peritoneum**

Vascular endothelial growth factor (VEGF) is a potent regulator of angiogenesis and vascular permeability [11]. The *VEGF* gene codes for several VEGF isoforms, which arrange into disulphide-linked homodimers. The binding of VEGF dimers to tyrosine-kinase receptors (VEGFR-1 and VEGFR-2) located in endothelial cells initiates a signal transduction cascade responsible for endothelial proliferation and migration, activation of plasminogen and collagenase, and vasodilation, resulting in physiological angiogenesis [11]. VEGF also plays a central role in tumour angiogenesis, and ischaemic retinal diseases [16]. In addition, VEGF binds to the extracellular matrix, and thus induces the release of basic fibroblast growth factor (bFGF), another potent angiogenic factor [17]. Stimuli for VEGF expression include hypoxia, hypoglycaemia, cytokines, growth factors, hormones, and inactivation of the oncogenes VHL and p53 [11,16].

VEGF is expressed in the human PM, and is located in the endothelium lining peritoneal capillaries. Its expression is clearly upregulated in long-term PD patients [9]. VEGF has also been detected in the dialysate, where its abundance correlates with the permeability for small solutes and the loss of UF [18]. The synthesis of VEGF by cultured mesothelial and endothelial cells isolated from the peritoneum has been demonstrated [19]. Thus, by analogy with other angiogenic diseases, upregulation of VEGF may trigger vascular proliferation in the PM in long-term PD. The putative stimuli involved in the regulation of VEGF in the PM include other growth factors, local ischaemia, or inflammatory cytokines [11,16].

Multiple interactions between NO, eNOS and VEGF occur within endothelial cells. Both NO and eNOS are required for VEGF-driven angiogenesis and vascular permeability [20]. On the other hand, VEGF is known to upregulate eNOS and NO production [21]. In turn, NO modulates and even suppresses the hypoxic induction of VEGF, which creates a negative feed-back between NO and VEGF induction [11]. Interestingly, such cross-talk exists in the PM, since upregulation of eNOS in a rat model of acute peritonitis is associated with down-regulation of VEGF [15].

**Reactive carbonyl compounds and advanced glycation end products in the peritoneum**

Chronic exposure of the PM to high glucose concentrations is associated with enhanced glycation of PM proteins. It is possible that advanced glycosylation end products (AGEs) participate in the pathogenesis of PM alteration during PD [22]. Advanced glycation of proteins is induced by reactive carbonyl compounds (RCOs), including glyoxal, methylglyoxal (MGO), and 3-deoxyglucosone [22]. These RCOs, which lead to formation of AGE epitopes such as pentosidine or carboxymethyllysine, originate from both the peritoneal dialysate and the uraemic circulation, irrespective of diabetes [23]. During PD, the peritoneum is thus exposed to high concentrations of RCOs derived from both the uraemic circulation and glucose-based PD fluids, with the resulting accumulation of AGEs within the PM [9,19].

Thus RCOs promote AGE modifications of proteins, which initiate a range of cellular responses including stimulation of monocytes, secretion of inflammatory...
cytokines, proliferation of vascular smooth muscle cells, stimulation of growth factors, and secretion of matrix proteins [24]. Independently of their AGE-mediated effects, RCOs may also, at least in vitro, increase VEGF expression in cultured mesothelial and endothelial cells [19]. Furthermore, a link between RCOs/AGEs and VEGF is supported by demonstration of increased VEGF expression in the PM of rats chronically injected with MGO [19], as well as by colocalization of pentosidine and VEGF in peritoneal capillaries from long-term PD patients [9].

**Does chronic uraemia per se contribute to peritoneal changes?**

By analogy with the increased permeability of serosal membranes such as the pleurae or the pericardium, it has been suggested that uraemia per se might increase PM permeability [25]. That hypothesis has recently been supported by the association of several molecular mechanisms—upregulation of NOS, high levels of circulating RCOs and AGEs, increased growth factors—with higher peritoneal permeability in a chronic uraemic rat model [26]. The permeability changes and increased NOS activities correlate with the degree of renal failure, and are not prevented by correction of uraemia-associated anaemia with erythropoietin [26]. These results suggest an independent contribution of uraemia in peritoneal changes during PD and indicate that the peritoneum is indeed a serosal membrane that might be modified by uraemia.

**Conclusion: molecular mechanisms for PM dysfunction in long-term PD**

Altogether these data fit a hypothetical framework outlined elsewhere [27] and illustrated in Figure 1.

Chronic uraemia is associated with high levels of circulating RCOs, which initiate AGE protein modifications in the PM. During PD, RCOs contained in glucose-based dialysate will amplify the AGE formation in the PM. RCOs and AGEs initiate a number of cellular responses, including stimulation of VEGF expression. In turn, VEGF interacts with endothelial cells and, together with eNOS and NO, stimulates angiogenesis and increases vascular permeability. These combined modifications increase EPSA, and eventually impair UF. Fibrosis will also be involved in PM dysfunction. Of note, uraemia itself might stimulate VEGF and bFGF, the latter being also released by VEGF interaction with the extracellular matrix.

Novel therapeutic strategies to protect the PM against the consequences of long-term PD arise from these hypothetical mechanisms [27]. These strategies include (i) reduction of RCOs and AGEs levels in the dialysate, by using fluids different from heat-sterilized glucose or by adding inhibitors of AGE formation to the latter [23]; (ii) inhibition of the l-arginine:NO pathway, for instance with l-arginine analogues [28]; (iii) modulation of angiogenesis with agents that inhibit endothelial cell growth, adhesion or migration, or interfere with factors such as VEGF and bFGF and their receptors [29]. The potential benefit of these strategies will have to be carefully assessed, for inhibition of an ubiquitous mediator such as NO may have a double-edged sword effect [28]. In addition, trials with anti-angiogenic compounds in non-cancerous diseases are thus far limited and there is little information on safety, long-term side-effects and impact of such therapy on normal physiological processes [29].

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References


