Regulation of NO synthase isoforms in the peritoneum: implications for ultrafiltration failure in peritoneal dialysis

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Abstract

Background. Ultrafiltration (UF) failure often complicates peritoneal dialysis (PD). At least two molecules might be involved in UF failure: aquaporin-1 (AQP1), a water channel thought to be the ultrasmall pore of the peritoneal membrane (PM), and nitric oxide (NO), which might regulate effective peritoneal surface area and microvascular permeability.

Methods. The contributions of AQP1 and NO in UF failure were evaluated by combining different experimental approaches. Specific antibodies were used to investigate the expression (immunoblotting) and localization (immunostaining) of AQP1 and NO synthase (NOS) isoforms in the peritoneum, in correlation with: (i) morphometric analyses; (ii) the l-citrulline assay, which specifically measures NOS enzymatic activities; and (iii) permeability parameters across the PM.

Results. AQP1 is located in the endothelium lining peritoneal capillaries, and its expression is remarkably stable in samples ranging from normal to highly inflamed peritoneum and even when transcellular water permeability is absent (loss of sodium sieving). A significant NOS activity, mediated by specific NOS isoforms, can be assayed in the peritoneum. The NOS activity significantly increases in conditions such as peritonitis and long-term PD, and this increase is mirrored by up-regulation of NOS isoforms, as well as angiogenesis and increased endothelial area.

Conclusions. These data suggest that the NO-mediated increase in effective peritoneal surface area, followed by a dissipation of the osmotic gradient, is a major mechanism accounting for the loss of UF in PD. Other biological consequences of increased NO levels in the peritoneum might include initiation of angiogenesis or modification of functionally important proteins such as AQP1.

Keywords: aquaporin-1; nitric oxide; nitric oxide synthase; peritoneal dialysis; peritoneum; ultrafiltration

Introduction

Ultrafiltration (UF) failure often complicates acute or chronic peritoneal dialysis (PD). Functionally, UF failure might be explained by three mechanisms: (i) an increase in the effective peritoneal surface area (EPSA), with an ensuing increase in glucose absorption and dissipation of the osmotic gradient; (ii) a decrease in the ultrasmall pores located in the capillary endothelium, with a decrease in free-water permeability; and (iii) an association of both these mechanisms [1].

At the molecular level, at least two candidate molecules might be involved in UF failure. The first is aquaporin-1 (AQP1), a water channel that is located in the endothelium lining capillaries, venules and small veins in the peritoneum [2,3]. Several lines of evidence support the hypothesis that AQP1 is the ultrasmall pore [3,4], a structure responsible for up to 50% of the UF during hypertonic dwell. The second molecule thought to play a significant role in regulating UF is nitric oxide (NO). In addition to its involvement in neurotransmission and defence against infection, NO controls systemic vasodilatation and affects microvascular permeability [5]. Furthermore, addition of the NO donor nitroprusside to the dialysate increases EPSA in stable PD patients [6], whereas NO synthase (NOS) inhibitors such as N^G^-nitro-l-arginine methyl ester (l-NAME) increase UF [7].

In this brief review, we will focus on the experimental work that has been initiated to evaluate the potential contributions of AQP1 and NO in UF failure.

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Expression and location of AQP1 in the peritoneum

In addition to its abundant expression in the kidney, AQP1 is located in the apical and basolateral membranes of endothelial cells lining non-fenestrated capillaries in numerous tissues including the peritoneum [2]. This location is consistent with the predicted topology of the ultrasmall pore, and recent studies performed in knockout mice provided evidence that osmotically driven water transport across the peritoneum is significantly decreased by AQP1 deletion, whereas it remains unchanged in the case of AQP4 deletion [4]. Staining for AQP1 has also been observed in the mesothelium, where its functional role remains obscure [2,3].

Immunostaining and immunoblotting studies demonstrated the remarkable stability of AQP1 expression and localization in samples ranging from normal to highly inflamed peritoneum [3]. This observation was strengthened by the demonstration of an apparently normal expression of AQP1 in the peritoneal capillaries of a long-term PD patient with UF failure and abolition of the sodium sieving (i.e. a disappearance of the transcellular water permeability) [8]. It is of note that the apparent lack of modulation of AQP1 expression contrasts with the up-regulation of the endothelial NOS in peritoneal samples obtained from patients with loss of UF [3,8]. This observation of a differential regulation of two endothelial proteins led to a systematic investigation of NOS isoforms in the peritoneum.

Determination of NOS activity and expression of NOS isoforms in the peritoneum

NO is synthesized from L-arginine by a family of three NOS isoforms: the neuronal NOS (nNOS, NOS1), the inducible NOS (iNOS, NOS2) and the endothelial NOS (eNOS, NOS3), which are expressed in a large variety of cells and tissues [5]. Both nNOS and eNOS are constitutively expressed in cells, in which their activity is controlled by intracellular Ca$^{2+}$ levels, whereas iNOS remains quiescent until its transcription is activated [5].

The NOS activity, which results in the stoichiometric production of NO and L-citrulline from the substrate L-arginine, is the basis of the L-citrulline assay—a simple, specific and highly sensitive method that recently has been adapted [9] to demonstrate significant NOS activities in human and rat peritoneum (Figure 1). In parallel, the availability of monoclonal antibodies allowed the characterization of the expression of the three NOS isoforms within the peritoneum (Figure 1).

Variations of NOS expression in clinical conditions associated with a loss of UF

The loss of UF that accompanies acute peritonitis is a common problem in PD, and the finding of increased NO metabolites in the dialysate suggested that modifications of NOS expression and/or NO liberation might play a role in the permeability changes [10]. Recent studies in a rat model of acute peritonitis [11] demonstrated that functional changes including increased permeability for small solutes and decreased UF are paralleled by a 10-fold increase in NOS activity (mediated by both iNOS and eNOS), whereas AQP1 expression remains unchanged. Morphological studies in rats with peritonitis showed that up-regulation of iNOS is related to macrophage infiltration, whereas up-regulated eNOS is paralleled by a significant increase in vascular density, endothelial surface area and reactivity for nitrotyrosine [11].

Similar experimental methods were used to test the hypothesis that NO and NOS might play a role in the increased EPSA observed in long-term PD patients [12]. In a large series of peritoneal biopsies from control subjects and uraemic patients treated or not with PD, the peritoneal NOS activity was found to be increased 5-fold in long-term (>18 months) PD patients compared with controls and uraemic subjects prior to the onset of PD. In uraemic patients, NOS activity is correlated positively with the duration of PD and, in the absence of peritonitis, is mediated solely by an up-regulation of eNOS. The latter finding reflects a significant increase in vascular density.

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**Fig. 1.** Demonstration of NOS activity and expression of NOS isoforms in the peritoneum. Top left: time course analysis of the formation of 1$^{-}$[H]citrulline in the peritoneum, following addition of a reaction mixture containing cofactors and 1$^{-}$[H]arginine substrate for NOS. The formation of L-citrulline reflects NOS activity and the equimolar production of NO. The 30 min incubation time is located in the linear part of the curve. A sample from rat with peritonitis was used to perform this analysis, in order to show the kinetics of total and Ca$^{2+}$-independent NOS (each determination was done in duplicate). Top right: immunoblot analysis of NOS isoforms. Control lysates (nNOS, rat pituitary; eNOS, bovine aortic endothelial cells; iNOS, mouse macrophages; 2 μg each) and lysates from human kidney and infected rat peritoneum (40 μg of protein per lane) were submitted to SDS–PAGE, transferred to nitrocellulose and probed with a monoclonal antibody against NOS1, eNOS or iNOS, respectively. Each antibody recognizes the appropriate isoform expressed in the control, as shown by the major band at 155 kDa, 140 kDa and 130 kDa for NOS1, eNOS and iNOS, respectively. The infected peritoneum contains the three NOS isoforms. Note that the monoclonal anti-iNOS cross-reacts with an upper band corresponding to eNOS in kidney and peritoneum samples. Bottom: immunostaining for NOS isoforms in rat peritoneum. A positive signal for eNOS is detected in the endothelium lining peritoneal blood vessels, whereas the mesothelium is not stained (A). The intensity of the signal, as well as the density of stained structures for eNOS, are markedly increased in the peritoneum of a rat with peritonitis (B). A faint signal for nNOS can be detected in nerves located within the peritoneum (C), but also in the media of large peritoneal arteries (D). Staining on serial sections demonstrates the absence of cross-reactivity between nNOS (D) and eNOS (E), the latter being restricted to endothelial cells lining the arterial wall. Staining for iNOS is only found in the peritoneum of rats with peritonitis, typically located in infiltrating macrophages in both the parietal (F) and visceral (G) peritoneum. Magnification (A–G) × 350. (Modified from references [9] and [11]).
and endothelial area in the peritoneum of long-term PD patients [12].

Potential roles of NO in the peritoneum

The data summarized above suggest that increased NO, secondary to NOS up-regulation, might be a major regulator of UF during PD. Indeed, NO is known to: (i) mediate vasodilation; (ii) promote angiogenesis; and (iii) interact with cytokines to increase microvascular permeability [5]. The result of these combined modifications might be increased EPSP, stimulation of glucose absorption and a faster than normal dissipation of the osmotic gradient—resulting in a loss of UF. The increase in nitrotyrosine immunoreactivity that consistently is associated with increased NOS activity in the peritoneum [11,12] illustrates another potentially important effect of NO, i.e. the biochemical modification of residues (formation of nitrotyrosine or nitrosocysteine) within the peritoneum. In that respect, the intense reactivity for nitrotyrosine observed in the capillary endothelium might suggest that NO could modify plasma membrane proteins such as AQPI and interfere with their function in endothelial cells [8,11].

Conclusions and perspectives

A significant NOS activity, mediated by specific NOS isoforms, can be assayed in human and rat peritoneum. The NOS activity within the peritoneum is significantly increased in clinical conditions such as peritonitis and long-term PD, in association with an increased EPSP and a loss of UF. The increased NOS activity is mirrored by the up-regulation of specific NOS isoforms, as well as angiogenesis and increased endothelial area. In contrast, the expression of the water channel AQPI remains apparently stable in these conditions. These data provide a structural and molecular basis to propose that an NO-mediated increase in EPSP, followed by a faster than normal glucose absorption and dissipation of the osmotic gradient, is a major mechanism accounting for the loss of UF. Future studies should focus on the biological consequences of increased NO levels in the peritoneum, such as initiation of angiogenesis or modification of critical residues within functionally important proteins, as well as the implication of local factors (advanced glycation end-products and growth factors) in that process.

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