Angiogenesis in Peritoneal Dialysis

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Key Words

Peritoneal dialysis · Angiogenesis · Ultrafiltration capacity

Abstract

Long-term exposure to peritoneal dialysis fluid induces morphological alterations, including angiogenesis, leading to a loss of ultrafiltration (UF) capacity. We discuss the effect of different factors in peritoneal dialysis (PD) on angiogenesis. In addition, we describe the process of angiogenesis and the possible role of different cell types in the peritoneum upon PD contributing to new blood vessel formation. Furthermore, we review several interventions used in our rat PD exposure model to decrease angiogenesis in PD. Moreover, we show new data on the use of sunitinib to inhibit angiogenesis in this rat model. Although various interventions seem to be promising, well-randomised clinical trials showing absolute prevention of angiogenesis and UF failure are, yet, still missing. To make real progress in PD treatment, the aim should be to prevent angiogenesis as well as peritoneal fibrosis and PD-induced inflammation.

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Introduction

Peritoneal dialysis (PD) has been a kidney replacement therapy for end-stage renal disease for approximately 30 years [1]. In PD, the ability of the peritoneal membrane to

function as a semi-permeable membrane is exploited to allow diffusive exclusion of uraemic toxins and exchange of solutes between the circulation and the PD fluid (PDF) [2, 3]. Currently, in Europe approximately 10% of the dialysis patients are using PD [4]. Advantages of PD include a more continuous removal of waste products, which results in an improved well-being of the PD patients, when compared to haemodialysis where the waste products accumulate in between dialysis. In addition, PD patients show increased mobility compared to haemodialysis patients, and PD is less expensive. Major disadvantages are the risk of PD-induced peritonitis and peritoneal tissue remodelling and angiogenesis, which can lead to technique failure.

Daily instillation of PDF causes morphological and functional alterations of the peritoneal membrane. Changes observed after PD are detachment of the mesothelial layer, increased submesothelial extracellular matrix (ECM) deposition, fibrosis, and angiogenesis (fig. 1). The changes arising due to PD ultimately lead to ultrafiltration (UF) failure (UFF) [5]. In this review, the focus will be on new blood vessel formation upon PD.

The peritoneal membrane consists of three layers, namely a mesothelial monolayer which covers the submesothelial compact zone – comprising connective tissue, ECM, a few fibroblasts, immune cells as macrophages and mast cells – and a third deeper loose adipose layer in which peritoneal capillaries and peritoneal lymphatic vessels are found [6–8].

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1420–4096/11/0344–0245\$38.00/0

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Fig. 1. Histological changes after long-term PD treatment. Toluidine blue staining of the omentum showing induction of milky spots and angiogenesis in a PD-treated rat (**b**) and a control rat (**a**). Staining of the mesentery showing blood vessel formation (CD31, red) and macrophage influx (ED2, green) in a PD-treated rat (**d**) and a control rat (**c**). Adapted from Schilte et al. [59]. For colours see online version.

The mesenteric and celiac arteries provide the visceral peritoneum with blood. Peritoneal microvessels exchange oxygen, hormones, water, nutrients, antibodies and inflammatory mediators in the peritoneal membrane and cavity [2].

Angiogenesis results in an increased effective surface area exchange whereas lymphangiogenesis leads to raised lymphatic absorption rates. The expanded vascular network results in a decrease in the glucose-driven osmotic pressure of the PDF leading to UF loss. In addition, vascular wall changes such as thickening and increased permeability cause an increase in small solute transport and thus in reduction time for exchanging waste products [9, 10].

Peritoneal Dialysis Fluids

PDF is instilled in the peritoneal cavity via a permanent catheter and rat studies have shown a minor increase in vessel density when a rat-o-port was implemented for 5 weeks [11]. Briefly, in our rat PD exposure model the rats undergo surgery whereby a small incision is made in the skin of the abdomen and in the linea alba. A silicon catheter (± 12 cm) attached to a mini vascular access port is tunnelled subcutaneously from the abdominal incision

to the neck of the rat. The other end of the catheter is introduced into the peritoneal cavity. In the first week, the animals receive 2 ml of saline with 1 U/ml heparin. Thereafter, during a 5- to 10-week period, the rats are injected once daily with 10 ml PDF [12].

Under normal conditions, the human peritoneum contains approximately 100 ml of fluid which serves as a lubricating moisture. During continuous ambulatory peritoneal dialysis, around 2 litres of PDF, thus 20 times as much, is instilled with 5 exchanges per day.

Using our rat exposure model, Zareie et al. [13] showed an increase in the number of blood vessels in the omentum, the mesentery and parietal peritoneum upon PD treatment with lactate buffer. Although the blood vessel formation was less in the lactate buffer control in comparison to the glucose containing PDF, this indicates that only the instillation of a large amount of fluid results in angiogenesis but also marks the importance of the composition of the PDF (fig. 2). The effect of different components in PD, including catheter, uraemia, peritonitis, buffer, glucose and glucose degradation products (GDPs), on angiogenesis is summarised in table 1.

Therefore, research has been performed to study the relation between UFF and the composition of the PDF. Conventional PDFs have a low pH value, a high glucose concentration in a lactate-buffered solution, and contain

Fig. 2. Blood vessel formation in a rat exposure model upon treatment with different PDFs. The number of blood vessels per cm² in the omentum (**a**) and in the mesentery (**b**) after toluidine blue staining on spread preparations. **c** The number of blood vessels within the submesothelial ECM after CD31 staining on frozen sections, adapted from Zareie et al. [13]. Experimental groups: heat (dianeal), filter (filter-sterilised lactate-buffered PDF with the same electrolyte composition and pH as the heat-sterilised

solution, and also containing 3.86% glucose), buffer (lactate buffer with the same electrolyte composition and pH as the other two solutions but without glucose), untreated. Data are presented as median and interquartile ranges. ^aHeat versus buffer and untreated: $p < 0.04$; ^bfilter versus buffer and untreated: $p < 0.002$; ^cheat, filter and buffer versus untreated: $p < 0.02$; ^dheat versus untreated: $p < 0.02$.

Table 1. The effect of different components in PD on angiogenesis

	Extrinsic factors			Fluid characteristics		
	catheter	uraemia	peritonitis	(lactate) buffer	buffer+ glucose	buffer+glucose +GDPs
Angiogenesis	+	+	+	+	++	+++

Peritoneal changes are indicated as: + = moderate; ++ = strong; +++ = very strong, compared to control rats.

GDPs which are formed during heat sterilization of the fluids. In vitro studies indicate impairment of the host defence mechanism due to the bioincompatibility of PDFs [14]. More physiological PDF bicarbonate and bicarbonate/lactate-buffered PDF with a physiological pH value and less GDPs have been developed. Evidence has been found that supports the benefit of switching from conventional to bicarbonate/lactate-buffered PDFs [15, 16]. Hekking et al. [15] compared a bicarbonate/lactate-buffered PDF (Physioneal) with a lactate-buffered PDF (Dianeal) in a rat PDF instillation model. Reduced neo-vascularisation and fibrosis was observed after 9–10 weeks of bicarbonate/lactate-buffered PDF compared to the lactate-buffered PDF-treated group.

Moreover, a comparable rat study showed a higher vascular density and more progressive fibrosis, associated with an upregulation of transforming growth factor β

(TGF- β) and vascular endothelial growth factor (VEGF), in the group treated with conventional dianeal versus the bicarbonate/lactate-buffered physioneal-treated group. In addition, the dianeal group showed higher accumulation levels of advanced glycation end products (AGEs) [16]. Biopsy specimens from continuous ambulatory peritoneal dialysis patients show AGE accumulation in the interstitium and in the microvessels, which was correlated with progression of interstitial fibrosis and vascular sclerosis and showed an inverse relationship with UFF [17]. AGEs can upregulate TGF- β and VEGF expression [18, 19]. Besides, AGEs have been found to be a chemoattractant for monocytes. In addition, monocytes cultured on a matrix composed of collagen IV mixed with AGE-albumin have been found to produce platelet-derived growth factor, a mitogen for vascular smooth muscle cells [20].

Patient studies also show a correlation between time on glucose PD, increased VEGF production and UFF. When the patients changed to glucose-free PDF, the levels of VEGF dropped, which indicates a role of high glucose concentration in the induction of VEGF production [21].

Vascular Endothelial-Specific Growth Factors

VEGFs are key players in angiogenesis and, as mentioned before, also in peritoneal angiogenesis. Vessel formation is a multistep process. It begins with vasculogenesis, a stage in which endothelial cells (ECs) differentiate and proliferate in an area without any vascular tissue followed by the formation of a primitive tubular network. Angiogenic remodelling takes place where the vessels enlarge and mature. ECs interact with the supporting cells and the surrounding matrix. Sprouting of vessels can also occur. In this process, mature vessels destabilise first after which new vessels are formed.

Besides VEGFs, which are important in the initiation of the formation of immature vessels, also other vascular endothelial-specific growth factors including 4 members of the angiopoietin (Ang) family and at least 1 of the ephrin (Eph) family are necessary for appropriate angiogenesis. Ang1, a Tie2 ligand, is needed for vessel stabilisation, integration with supporting cells, and maturation. Ang2 is involved in destabilisation of the vessels [22, 23]. Reduction in Tie2, an Ang receptor, and increased levels of Ang2 in a uraemic rat PD model have been shown to correlate with peritoneal angiogenesis [23]. It is speculated that EphB2 and EphB4 are important in the venous versus arterial identity and EphB2 is important in the maintenance of interaction between endothelial and smooth muscle cells. To our knowledge, there is no direct link between Ephs and angiogenesis in PD; however, in some tumours, the endothelium of newly formed vessels highly expresses EphB2 [22].

Peritoneal Cells Involved in PD-Induced Angiogenesis

Angiogenesis is not simply regulated by one mechanistic switch. A lot of factors are involved in the formation of new blood vessels. These factors are produced by many different cells. Briefly, some important cells and their potential role in angiogenesis will be described.

The predominant cell type in the human and rat peritoneal cavity are the macrophages [24, 25]. Macrophages, together with polymorphonuclear leucocytes, are the first-line defence against invading micro-organisms during PD. Activated macrophages release prostaglandin E₂, interleukin (IL)-1 β , IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor α (TNF- α). Prostaglandin E₂ is involved in angiogenesis by enhancing endothelial cell migration and contributing to cell survival [26]. MCP-1 not only attracts other monocytes from the blood but has also been shown to be directly involved in angiogenesis. In vitro experiments show an enhanced MCP-1-induced protein expression by human umbilical vein ECs upon MCP-1 stimulation. MCP-1-induced protein in turn enhances endothelial cell apoptosis, proliferation, migration, and the induction of other angiogenesis-related genes resulting in capillary-like tube formation [27]. In vivo, it has been demonstrated that overexpression of IL-1 β leads to a sustained angiogenesis and submesothelial thickening and fibrosis [28]. In addition, IL-1 β increases vessel-like structures, through enhancing VEGF production and downregulation of Ang1, and augments proliferation when added to ECs [29, 30]. Also, IL-6 and IL-8 can be involved in angiogenesis since IL-6 stimulates endothelial progenitor cell proliferation, migration and Matrigel tube formation and IL-8 enhances EC survival, proliferation and capillary tube formation [31, 32]. Macrophage-produced TNF- α causes induction of capillary-like blood vessel formation both in vitro and in vivo [33].

In healthy peritoneum, mast cells are present and the number of mast cells increases upon infection [34, 35]. Activated mast cells can induce new vessel formation by producing proangiogenic factors like TGF- β , basic fibroblast growth factor, VEGF, TNF- α and IL-8 (see above) [36, 37]. In addition, mast cells are capable of producing tryptase, stimulating vascular tube formation, and chymase [38, 39]. Although the relative number of peritoneal mast cells in the PDF of rat PD exposure models declines, mast cells are strongly associated with PD-induced angiogenesis. This is probably due to mast cell accumulation in the omentum [40]. The mast cells are found near blood vessels and the number of mast cells in the omentum strongly correlates with the number of blood vessels. PDF exposure in mast cell-depleted wild-type rats and mast cell-deficient *Ws/Ws* rats shows a significant reduction in omental angiogenesis also indicating a compelling role for mast cells in PD-induced angiogenesis [41].

ECs are very important cells in PD because of their prominent role to create a barrier for solute transport during PD. Upon PD, ECs of the vessels in milky spots of

PD-treated animals change in morphology. The ECs enlarge and become activated [15]. Activation of ECs, for example by surgery in the abdomen, induces IL-6 and MCP-1 production, which leads to the attraction of immune cells but also supports angiogenesis [42].

One of the adverse effects in PD is the loss of mesothelial cells (MCs); nevertheless, MCs have an important role in new vessel formation. The remaining MCs become activated [43]. Basic fibroblast growth factor, heparin-binding epidermal growth factor and VEGF mRNA is constitutively expressed by resting MCs *in vitro*. These growth factors are known to be involved in angiogenesis and vascular permeability [44, 45]. In culture, MCs upregulate VEGF expression upon stimulation with TNF- α , IL-1 β or GPDs [46, 47]. GPDs also increase VEGF production by ECs [47]. In addition, human MCs can produce the chemokines IL-8, huGRO- α , γ IP-10, RANTES and MCP-1, which either stimulate angiogenesis directly or might enhance vascularisation via attracting macrophages to the peritoneal cavity [48].

So, a number of pro-angiogenic factors have been shown in association with high peritoneal solute transport rates [49]. However, not only direct pro-angiogenic factors produced by the cells described above cause an increase in vessel formation, also other mediators in PD can influence the vascularisation. Important is also the epithelial-to-mesenchymal transition (EMT), under the influence of TGF- β . During EMT, MCs lose their epithelial phenotype and acquire myofibroblast-like characteristics. MCs that had undergone EMT augmented the ability to invade and migrate into the submesothelial layer. Besides the change in morphological features, the non-epithelioid MCs produce higher amounts of VEGF than the non-EMT MC counterparts. In PD patients, a correlation has been found between the overexpression of VEGF by non-epithelioid MCs and high transport rates [50]. Moreover, damaged MCs also lead to peritoneal fibrosis due to the secretion of ECM macromolecules consisting of collagen, fibronectin, laminin, proteoglycans and amongst others the production of TGF- β_1 . TGF- β_1 has a kind of positive feedback loop and enhances the production of ECM leading to further new vessel formation [51].

Prevention of PD-Induced Angiogenesis

Over the last few years, research has focused on the prevention of UFF and peritoneal membrane changes during PD treatment. The use of angiogenesis inhibitors may lead

to reduced vessel formation resulting in sustained UF and improved well-being of the patients due to a more continuous removal of waste products. Several studies have been performed to reduce angiogenesis in PD.

Cyclooxygenase (COX) enzymes are involved in prostaglandin synthesis. COX-2 is known to be an angiogenesis stimulator by upregulating the mRNA transcription and the production of VEGF. Furthermore, it enhances the production of thromboxane A₂, prostaglandin E₂ and prostacyclin. Studies have shown a possible role for COX-2 inhibitors in the reduction in fibrosis and angiogenesis both *in vitro* and *in vivo*, although data are contradictory in some experiments [52, 53]. Fabbrini et al. [54] have shown the influence of the COX-2 inhibitor, celecoxib, in PD in a rat exposure model. Celecoxib treatment prevented PD-induced angiogenesis in both the omentum and parietal peritoneum. New lymphatic vessel formation was totally prohibited. Although the prostaglandin E₂ levels were reduced, VEGF levels were unaffected by celecoxib treatment. Most importantly, UF was restored upon celecoxib treatment. Celecoxib may therefore be of interest in the prevention of UF in PD patients; however, cardiovascular safety must be taken into account.

Another interesting possibility to prevent angiogenesis is the use of tyrosine kinase inhibitors. Sunitinib is a tyrosine kinase inhibitor and involved in the inhibition of VEGF and platelet-derived growth factor receptor signalling. A case study performed by Tapiawala et al. [55] showed a positive effect of a PD patient treated with sunitinib against metastatic renal cell carcinoma. The patient's peritoneal permeability remained stable even over 10 years. Our animal studies show prevention of new vessel formation in the omentum as well as the mesentery by sunitinib after 5 weeks of PD treatment (fig. 3). However, sunitinib treatment further increased the PD-induced VEGF levels. This has also been published by others and could be explained by a lack of degradation due to blocking of the VEGF receptor signalling [55, 56]. Interestingly, a loss of mast cells in the mesentery was observed in the sunitinib-treated animals.

Together with previous studies, in which mast-cell-deficient rats also showed significantly reduced angiogenesis in the omentum and in which strong correlations between omental angiogenesis and mast cells have been found, this suggests an important role for mast cells in new blood vessel formation upon PD [41].

There are not only studies on the effect of direct angiogenesis inhibitors in PD but also on the prevention of the onset of angiogenesis. For example, Sandoval et al.

Fig. 3. Prevention of angiogenesis by sunitinib treatment. Percentage of vessel surface area in the omentum (**a**; * $p < 0.01$) and mesentery (**b**; * $p < 0.05$) measured by CD31. Box plots indicate the median and 5–95% level. Representative examples of CD31 stain-

ing of the omentum (**c**). Toluidine blue staining of the mesentery confirms CD31 measurements (**d**). Magnifications $\times 10$. C = Control; C + S = control + sunitinib; PD + S = PD + sunitinib. For colours see online version.

[57] investigated the administration of rosiglitazone, a peroxisome proliferator-activated receptor- γ agonist, in a mice exposure model. Peroxisome proliferator-activated receptor γ agonists are known to control glycaemia by improved insulin sensitivity and have anti-inflammatory, and therefore probably also antifibrotic, properties. Administration of the drug led to reduced AGE formation and inflammation, a preserved mesothelial cell layer, and improved UF compared to the PD control mice. However, although promising for inhibiting the alterations due to PD, rosiglitazone treatment of patients with type II diabetes has been linked to several severe side effects as shown in a meta-analysis study by Singh et al. [58].

Since EMT also leads to angiogenesis, there has also been a focus on the prevention of EMT upon PD. Loureiro et al. [24] studied the effects of bone morphogenic protein-7, which antagonises TGF- β_1 and can promote mesenchymal-to-epithelial transition. Inhibition of bone morphogenic protein-7 in a rat PD exposure model inhibited angiogenesis as well as the number of cytokeratin-positive cells, indicating a positive correlation between EMT and angiogenesis.

Whether these positive interventions used in animal studies can be translated into clinical medicine remains to be elucidated. Currently, the COX-2 inhibitor celecoxib is under study in a clinical trial.

Conclusion

PD has improved over the years, for example by the use of new PDF; however, further research on the prevention of the alterations of the peritoneum upon PD is necessary. Although some interventions are promising, well-randomised clinical trials showing complete reduction in angiogenesis and UFF are lacking. To improve PD treatment, research should not only focus on the prevention of angiogenesis but on a combination of angiogenesis,

peritoneal fibrosis, as well as PD-induced inflammation. This will ultimately result in future PD treatment regimens with new inhibitory drugs without undesirable side effects.

Acknowledgement

This work was supported by the Dutch Kidney Foundation, grant No. C05-2142 and C09-2331.

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