Glucotoxicity of the peritoneal membrane: the case for VEGF

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Introduction

Although nephrologists are well aware of the devastating consequences of chronic hyperglycaemia in patients with diabetes, the continuous exposure of the peritoneum to the high glucose concentrations present in peritoneal dialysate has been approached with aloof concern. This is rather surprising, as dialysate glucose concentrations are 15 to 40 times the physiological levels and peritoneal dialysis (PD) patients may be exposed to the equivalent of 100 kg of glucose per year.

Long-term PD is associated with the progressive development of functional and structural alterations of the peritoneal membrane. Longitudinal reports suggest that ultrafiltration tends to decrease with time on dialysis [1–2]. In the majority of cases, loss of ultrafiltration capacity can be attributed to a rise in small solute transport, resulting in a rapid dissipation of the transperitoneal osmotic gradient. The presence of high small solute transport rates points to an expansion of the effective peritoneal vascular surface area [3]. Its morphological substrate may be the neoangiogenesis that has been observed in the peritoneum of long-term PD patients [4–6]. Other structural changes include reduplication of the basal lamina of the mesothelium and the blood vessels, interstitial fibrosis and hyalinization of the blood vessel media, with preferential deposition of collagen type IV [4–9]. The diabetiform nature of these morphological changes has bolstered the hypothesis that the continuous exposure to the high glucose concentrations in dialysate is an important pathogenetic factor.

The recognition that glucotoxicity contributes to the development of the functional and structural
alterations of the peritoneum is essential, as the initial clinical approach to a patient with failing ultrafiltration capacity is to increase the number of hypertonic exchanges or to switch to cyclic continuous PD. These interventions further increase the cumulative glucose exposure and the patient may thus be precipitated into a vicious circle with progressively worsening peritoneal membrane damage. The present communication briefly reviews the extant evidence that glucose, either directly or indirectly through the generation of glucose degradation products (GDP) or the formation of advanced glycation end-products (AGE) is responsible for these changes. In particular, the role of vascular endothelial growth factor (VEGF) as a downstream mediator is highlighted.

**Glucose**

VEGF is a heparin-binding cytokine that plays a prominent role in physiological and pathological angiogenesis and potently induces microvascular hyperpermeability [10]. Several lines of evidence implicate VEGF as a mediator of glucose-induced tissue damage. VEGF expression is upregulated in diverse cell types cultured in a high glucose environment, as well as in various tissues of experimental animals and humans with diabetes. Microvascular dysfunction induced by topical application of elevated glucose levels in a granulation skin chamber was attenuated by administration of neutralizing anti-VEGF antibodies [11]. Finally, VEGF blockade prevented early renal dysfunction in experimental diabetes [12].

Considering its biological properties and its upregulation by high ambient glucose concentrations, VEGF is an attractive candidate to provide a mechanistic link between chronic glucose exposure on the one hand, and peritoneal neoangiogenesis with resultant loss of ultrafiltration on the other. Human peritoneal mesothelial cells harvested from spent dialysate and cultured in vitro have the capacity to produce substantial amounts of VEGF, but no correlations were found between supernatant VEGF levels and time on PD, solute transport characteristics, ultrafiltration rate or accumulated dose of glucose [13]. Other cell types present in the peritoneal cavity that are capable of producing VEGF, include peritoneal macrophages [14] or capillary endothelial cells [6]. The concentration of VEGF in peritoneal effluent was higher than what could be attributed to transport from the circulation, implying local production [15]. The amount of locally synthesized VEGF correlated with the mass transfer area coefficient of creatinine and urate, glucose absorption and transcapillary ultrafiltration, and increased with time on PD [15]. In addition, VEGF effluent concentrations decreased after switching to glucose-free dialysate, with a commensurate effect on the parameters of effective peritoneal surface area [16]. Although these studies present ample circumstantial evidence that VEGF mediates glucose-induced damage of the peritoneal membrane, they do not provide proof of causality. We have studied experimental diabetes as a model for chronic exposure of the peritoneum to high ambient glucose levels [17]. The peritoneal microcirculation in early streptozotocin-induced diabetes in the rat is characterized by pronounced neoangiogenesis, associated on the functional level with an elevated small solute transport rate, similar to the alterations found in long-term PD patients. The hyperglycaemia-induced microvascular alterations were largely prevented by treatment with a monoclonal anti-VEGF antibody, whereas treatment with an isotype-matched control antibody was without effect (Figure 1). These results are thus the first to support an aetiological role for glucose in the development of peritoneal neoangiogenesis and identify VEGF as an important downstream mediator [17].

**Glucose degradation products**

Glucose in PD fluid is known to degrade during heat-sterilization and, to a lesser extent, during prolonged storage giving rise to a variety of GDP. The toxic effects of these components on viability and function of peritoneal leukocytes, fibroblasts and mesothelial cells are now fully recognized [18]. There is, however, a dearth of information on the potential vascular consequences of chronic exposure to GDP. Cultured rat mesothelial and human endothelial cells expressed VEGF in response to methylglyoxal, but not to glyoxal or 3-deoxyglucosone [19]. Intraperitoneal exposure to

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![Fig. 1](Image). The vascular density of the visceral peritoneum was significantly higher in diabetic rats than in controls animals. The vascular proliferation was prevented by chronic treatment with anti-VEGF antibodies (Ab), but not by control Ab. VEGF blockade did not decrease vascular density in control rats. *$P < 0.01$ vs controls. **$P < 0.01$ vs diabetes and diabetes + control Ab.
methylglyoxal increased VEGF expression in peritoneal tissue of experimental animals [19]. Although these results need confirmation, they suggest that GDP may augment local production of VEGF and thus contribute to peritoneal neoangiogenesis.

Advanced glycation end-products

Glucose and a variety of other reactive carbonyl compounds have the potential to bind non-enzymatically to free amino groups on proteins or to lipids, resulting in the formation of AGE [20]. AGE formation is accelerated when the ambient glucose levels are elevated or when the prevailing oxidant stress is high, as for instance in uraemia [20]. Furthermore, GDPs are known to facilitate the formation of AGE [21,22]. As residual renal function deteriorates, low molecular weight AGE are retained in the circulation and may be actively transported into the peritoneum [23]. The peritoneal cavity of PD patients thus harbours optimal conditions for a dramatically accelerated AGE formation and accumulation. AGE have, indeed, been detected immunocytochemically in the mesothelium, submesothelial stroma and vascular wall of PD patients [6,9,24–27].

The pathogenicity of AGE relates to their ability to accumulate in tissues with the formation of cross-links, and to generate oxygen-derived free radicals. Another important biological action of AGE is the induction of VEGF expression in diverse cell types [28]. AGE may thus have the potential to promote peritoneal neoangiogenesis.

Attempts have been made to correlate the extent of AGE accumulation with functional parameters [9,25,26]. Peritoneal staining for AGE increased along with time on PD and was associated with a higher permeability to various solutes [25,26]. In another study, the degree of interstitial fibrosis and vascular sclerosis correlated with interstitial and vascular AGE accumulation, respectively [9]. An inverse correlation was found between these peritoneal histological changes and ultrafiltration volume [9]. The results incriminate AGE accumulation in the pathophysiology of ultrafiltration failure, although the precise mechanisms underlying this association remain unclear. It is generally acknowledged that the interstitium does not contribute importantly to the barrier function of the peritoneal membrane [29]. It is unknown whether interstitial fibrosis can be associated with a decreased hydraulic permeability or with a more rapid transport of small solutes. On the other hand, a correlation has been reported between the degree of interstitial fibrosis and vascular density in the membrane of long-term PD patients [4]. It may be more logical to postulate that peritoneal AGE accumulation is associated with both interstitial fibrosis and neoangiogenesis, while the latter phenomenon is primarily responsible for the increased small solute transport and loss of ultrafiltration capacity. Unfortunately, no morphometrical analysis of vascular density was performed in the studies on peritoneal AGE accumulation [9,25,26].

Strategies to reduce peritoneal exposure to glucose and GDP

Non-glucose-based dialysates, including icodextrin, glycerol and amino acids, as well as double-chamber dialysates with low GDP content have become available for clinical use. Whereas extensive in vitro testing has suggested that these new dialysates may be more biocompatible than conventional dialysate [18,30], their superiority on in vivo peritoneal membrane function remains to be fully demonstrated.

Icodextrin and amino acid solutions contain lower concentrations of GDP than conventional dialysate [31]. Less in vitro glycation and AGE formation occurs in icodextrin than in conventional heat-sterilized glucose-based dialysate [27,32,33]. In accordance, AGE accumulation was lower in the peritoneal membrane of experimental animals dialyzed with icodextrin than in those treated with glucose-based solutions [34]. A switch to icodextrin- and glycerol-based dialysis in small group of patients with severe ultrafiltration failure decreased pentosidine dialysate levels and modestly improved ultrafiltration rate [35]. In contrast, no difference in peritoneal transport characteristics and peritoneal membrane markers were found in CCPD patients randomized to either icodextrin or standard dialysate [36].

Double-chamber dialysates with low GDP content induce lower in vitro AGE formation than conventional dialysate [37]. In keeping with these findings, the peritoneum of rats exposed to these dialysates is characterized by lower staining for AGE and a better preserved ultrafiltration capacity [38], as well as by less submesothelial thickening [39].

Conclusion

Several lines of evidence support the involvement of dialysate glucose and GDP concentrations with resultant peritoneal AGE accumulation in the pathophysiology of peritoneal membrane alterations. High glucose concentrations, as well as GDP and AGE, have the potential to upregulate VEGF expression. Even though the cell type that generates VEGF in response to dialysate exposure is unknown, strong evidence implicates local VEGF production in the pathogenesis of peritoneal neoangiogenesis and loss of ultrafiltration capacity. This knowledge has given impetus to the extensive testing of new dialysate solutions, either non-glucose based or with low GDP content. Although promising preliminary results have been obtained, further work is necessary to demonstrate that these new dialysates better preserve peritoneal membrane integrity in vivo.
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