The endothelial surface layer: a new target of research in kidney failure and peritoneal dialysis

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Chapter 1

Introduction
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1. The endothelial glycocalyx

1.1 Structure and functions

The endothelial glycocalyx is a negatively charged mesh of glycoproteins, proteoglycans and glycosaminoglycans (GAGs) coating the luminal side of the endothelium (Fig. 1A). Together with associated plasma molecules it forms the endothelial surface layer, that can restrict the flow of plasma, and can exclude red blood cells and various macromolecules. Visualized for the first time in 1966 by Luft et al after ruthenium red staining, this layer is an important regulator of endothelial function in the systemic circulation and exerts various vasculoprotective effects. These include mechanosensing and shear-induced release of nitric oxide, regulation of coagulation and redox state, prevention of leucocyte adhesion, regulation of endothelial permeability, angiogenesis and protection of the vasculature against accelerated atherosclerosis. This complex and dynamic structure harbours various enzymes (e.g., extracellular super oxide dismutase), and binds several growth factors and therefore modulates their signaling capacity. In addition, the endothelial glycocalyx reversibly binds Na⁺ via the negatively charged GAGs and acts as a buffer for sodium at the endothelial level.

Heparan sulfate (HS) is the main glycosaminoglycan within the endothelial glycocalyx accounting for up to 60% of the total GAGs. Chondroitin sulfate (CS), dermatan sulfate and hyaluronan (HA) account for the rest. It has been shown that HS is distributed in the luminal part of the EG, more distal from the plasma membrane, whereas CS and HA are attached to the proteoglycans at sites closer to the membrane.

Figure 1A. Staining of the mesenteric microvasculature with Lycopersicon esculentum lectin (red: the lectin binds to N-acetylgalcosamine- (GlcNAc) 2-4 within the endothelial glycocalyx; green: autofluorescence of the erythrocytes present in the vascular lumen; blue: DAPI for the nuclei) in rats with normal kidney function. A continuous lining is visible at the luminal side of the endothelium.
Hyaluronan is the only non-sulfated GAG and is not attached to a proteoglycan but to its receptors CD44 and RHAMM (receptor for hyaluronan mediated motility). Amongst the proteoglycans, syndecans and glypicans are the most common molecules on the endothelial surface (Fig. 1B). The glycocalyx dimension increases with the vascular diameter and is dependent upon the balance between biosynthesis and shedding of its components into the blood. A hydrodynamically relevant glycocalyx dimension of approximately 0.5 µm has been measured by Vink et al in hamster cremaster muscle capillaries using a fluorescent dye-exclusion technique, whereas in rat mesenteric small arteries its thickness reached 3 µm.

1.2 Consequences of glycocalyx damage
The endothelial glycocalyx is an important regulator of vascular homeostasis. Degradation of glycocalyx structures was found to occur after provocation with inflammatory and atherogenic stimuli such as ischemia-reperfusion, infusion of oxidized low-density lipoproteins, administration of tumor-necrosis factor-α and hyperglycemia. Consequences of glycocalyx damage include increased leucocyte and platelet adhesion, activation of coagulation, increased vascular permeability, impaired signal transduction followed by reduced release of nitric oxide in response to shear stress, and accelerated atherosclerosis. Pathologic loss of glycocalyx may therefore be associated with increased endothelial vulnerability and an impaired vascular wall protection throughout the circulatory system. Disruption of the endothelial glycocalyx may be the first step in the development of atherosclerosis.

1.3 Methods for the assessment of the endothelial glycocalyx in vivo
Given the role of the endothelial glycocalyx in vascular homeostasis, the development
of non-invasive methods that allow the assessment of this structure \textit{in vivo}, has become of major importance. Although successfully used in experimental settings,\textsuperscript{16} intravital microscopy is not suitable for studies of the endothelial glycocalyx in humans. Nieuwdorp \textit{et al.} reported for the first time measurements of the systemic glycocalyx volume in humans by comparing the distribution volume of dextran 40, a glycocalyx permeable tracer, with that of fluorescently labelled erythrocytes, which cannot penetrate into the glycocalyx. Using this method, the authors measured a systemic glycocalyx volume of 1.7 liters in healthy individuals.\textsuperscript{17} However, this method is invasive and cannot be easily implemented in clinical studies. Recently, a new imaging method, Sidestream Darkfield microscopy, has been developed based on the observation that in normal conditions the red blood cells (RBC) are largely excluded from the endothelial cell by the endothelial surface layer.\textsuperscript{18} Only the luminal part of the ESL transiently allows the access of erythrocytes. With this non-invasive method, movies of the sublingual microvasculature are recorded and measurement sites are placed automatically every 10 µm along each visible microvessel. An extensive description of the method is given in chapter 2 of this thesis. The RBC column width is measured at each site in consecutive frames. The position of the outer edge of the RBC perfused lumen at each measurement site (DPerf) is derived from the RBCW distribution, and the perfused boundary region is defined as the distance of the median RBCW to the outer edge of the perfused diameter. Importantly, this parameter reflects the erythrocyte permeable region of the endothelial surface layer and therefore, it is not a measure of its anatomic thickness.

In addition, determinations of plasma levels of glycocalyx constituents have been performed in various clinical conditions. The most widely used are hyaluronan and syndecan-1, but also heparan sulfate. Although most of these molecules, for instance hyaluronan, which is the main constituent of the extracellular matrix, are not specific for the endothelium, high plasma levels have been associated with alterations of the endothelium.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{endothelial_surface_layer_diagram.png}
\caption{Schematic illustration of endothelial surface layer imaging method. RBC width = median red blood cell column width.* DPerf = perfused diameter (RBC perfused lumen). ** PBR = perfused boundary region (RBC permeable part of cell free layer including cell permeable glycocalyx). *** Cell free layer.}
\end{figure}
However, the interpretation remains challenging, also because effects of kidney function on plasma concentrations have not been clearly established.

2. Chronic kidney failure

2.1 Endothelial surface layer in chronic kidney failure and after successful renal transplantation

Dialysis patients have increased morbidity and mortality, which is only partly caused by their increased risk for cardiovascular disease. Endothelial dysfunction, increased endothelial permeability, oxidative stress, inflammation, overhydration, and accelerated vascular disease are common features in patients with chronic renal failure. Therefore, considering its vasculoprotective effects, the endothelial glycocalyx may be relevant for the development of vascular complications in chronic kidney disease, and it is reasonable to hypothesize that changes in the endothelial glycocalyx may occur in this condition. Successful renal transplantation improves the survival of patients with end-stage renal disease but is accompanied by only a modest decrease in cardiovascular mortality. Successful transplantation solves the uremia related toxicity but cannot erase the pre-existent cardiovascular burden in these patients. In addition, the side effects of various immunosuppressive regimens add to this pre-existent burden. It is not known if successful kidney transplantation restores the endothelial glycocalyx and thereby improves endothelial function, contributing to an improved risk profile of transplanted patients.

2.2 The endothelial glycocalyx in peritoneal dialysis

Peritoneal dialysis (PD) makes use of peritoneal tissues as a dialysis membrane to remove waste products and an excess of fluid from the body. The capillary wall of peritoneal blood vessels represents the initial resistance to solute transport from the plasma through the interstitial tissues and the mesothelial layer to the dialysate in the peritoneal cavity. Low molecular weight solutes are removed from the blood through the endothelial small pore system, probably interendothelial clefts with radii of 40-55 Å. Circulating macromolecules pass through a limited number of so-called large pores, the anatomic substrate of which has not been established with certainty, but may be interendothelial gaps that can be present in the venular endothelium, especially in situations of vasodilation, and have radii >250 Å. The transport of macromolecules is size-dependent, but independent of electric charge. Transcapillary ultrafiltration of fluid occurs through the interendothelial pores and through the endothelial water channel aquaporin-1 (ultramasmall pore, radius<5Å) that is present in peritoneal endothelial cells, and allows the transport of water only. Whereas solute removal occurs mainly by diffusion through the pores, fluid removal is by filtration, either by hydrostatic gradients (small pores) or by crystalloid osmosis (both small and ultrasmall pores). The latter is generated by the extremely high glucose concentrations present in the conventional dialysis solutions.
Endothelial permeability is in part dependent on the endothelial glycocalyx. Therefore, this structure is the primary barrier in transendothelial solute and water transport in the systemic circulation. The role of this delicate layer of polysaccharides in transcapillary transport has been extensively demonstrated in several studies which showed that various insults to the glycocalyx result in increased vascular permeability to solutes of different size or to water, followed by development of tissue edema. Therefore, it has been argued that the state of the peritoneal glycocalyx might also be of major importance to PD. This has led to new hypotheses concerning the factors that influence the transport characteristics of the peritoneal membrane, however without any proof. The peritoneal tissues in patients treated with PD are chronically exposed to extremely high glucose concentrations present in PD fluids, which cause neoangiogenesis, and contribute to the development of peritoneal fibrosis. Similar to the situation for the over-all glycocalyx in diabetes mellitus, the peritoneal endothelial glycocalyx may be altered in patients treated with chronic peritoneal dialysis. Therefore, it can be hypothesized that the changes in the systemic endothelial glycocalyx in patients with diabetes mellitus due to hyperglycemia might also occur in the peritoneal microcirculation of PD patients due to the high dialysate glucose concentrations, although the exposure routes are different: in diabetes the endothelial exposure is luminal, while in PD it is from the basolateral side. Also, the glucose concentrations are of a different magnitude: from 20 mmol/L in blood in hyperglycemic diabetics to 200 mmol/L in the dialysate of a PD patient. In addition, neoangiogenesis occurs during PD and new vessel formation is associated with disrupted endothelial glycocalyx at the luminal side of a blood vessel. All this makes it likely that changes in the peritoneal endothelial glycocalyx occur during PD and they are distinct from the changes present in the systemic microcirculation. Endothelial glycocalyx damage in PD may be of importance not only with regard to peritoneal transport. Peritoneal alterations, such as angiogenesis and interstitial damage,
are promoted by the release of growth factors, cytokines and various proteins into the peritoneal tissues, caused by damage to the endothelial glycocalyx. Therefore, defining the role of the glycocalyx in this condition will guide further strategies on its preservation.

3. Peritoneal dialysis - effects on the peritoneum

Long-term treatment with peritoneal dialysis can induce morphological and functional alterations of the peritoneal tissues. Structural changes include: loss and degeneration of the mesothelium, thickening of the submesothelial compact zone, fibrosis, and a variety of vascular abnormalities, like diabetiform reduplication of the basement membrane of peritoneal capillaries, subendothelial hyalinization with luminal narrowing or obliteration, deposition of type IV collagen within the arterial wall, neoangiogenesis and lymphangiogenesis. The extremely high concentrations of glucose in the dialysis fluids together with heat sterilization-induced glucose degradation products (GDPs), both resulting in peritoneal deposition of advanced-glycation end-products (AGEs), and the high lactate concentration are probably the most important factors responsible for these alterations. The aforementioned changes may lead to an increase in peritoneal solute transport and, in some patients, to ultrafiltration failure, which sometimes leads to cessation of this method of renal replacement therapy. Therefore, in order to reduce the PD-related peritoneal alterations and prolong the treatment with PD, new dialysis solutions with lower GDPs content, neutral pH or using different osmotic agents than glucose, have been developed. The effects of different solutions have been investigated mainly in experimental studies, and most of those studies reported favorable effects of the new solutions on the peritoneum, showing less fibrosis and less angiogenesis compared to the conventional solutions. Importantly, many of these studies either used animals with normal kidney function, or the follow-up period was very short. Surprisingly, the significant favorable effects on peritoneal morphology have not been confirmed by functional data in either patients or animals with renal failure and a long exposure duration. No significant favorable effect on peritoneal transport parameters was found in these studies and therefore, the question arose whether these models can actually mimic the human situation and many questions remained unanswered. The long-term peritoneal exposure in a rat model with chronic kidney failure, as developed by our group, allows to include both the effects of renal failure and those of long-term peritoneal exposure. Therefore we used this model for our further investigations.

Ultrafiltration failure may develop in some patients treated with PD, and is characterized by insufficient removal of excess of fluid from the body. Whereas the presence of a large vascular surface area, associated with high transport rates of small solutes and a rapid decrease in the osmotic gradient, is the major cause of ultrafiltration failure, another factor that can contribute to the development of this complication is a high
lymphatic reabsorption of intraperitoneally administered dialysis solutions, leading to a decrease in the intraperitoneal volume. Lymphatic drainage from the peritoneal cavity occurs mainly via the subdiaphragmatic lymphatics, but also by lymphatics that accompany the peritoneal interstitial vasculature. The lymphatic absorption rate can be assessed in patients during a standard peritoneal permeability analysis, by measurement of the disappearance rate of Dextran 70, which is used as volume marker, from the peritoneal cavity. Lymphangiogenesis has been described to develop in rats during peritoneal exposure and is related to peritoneal fibrosis. However in PD patients no effect of long term exposure to dialysis solutions on the lymphatic absorption rate from the peritoneal cavity has been found. Therefore, the relationship between the degree of lymphangiogenesis and the lymphatic reabsorption, as measured by a peritoneal function test, is unknown.

4. Objectives and outline of the thesis

The objectives of this thesis are to identify the alterations of the endothelial surface layer in the systemic microvasculature associated with end-stage renal disease, and to investigate the state of the endothelial glycocalyx in both the systemic and the peritoneal microcirculation during peritoneal dialysis, and explore its relationships with peritoneal transport. In addition, we do an extensive analysis of the peritoneal alterations induced by long-term exposure to glucose-based dialysis solutions.

The first part of the thesis addresses the state of the endothelial surface layer in patients with chronic kidney failure. In chapter 2 we assess the state of the endothelial surface layer in the systemic microcirculation in patients with end-stage renal disease, treated with hemodialysis or peritoneal dialysis, and compare the results with the data in healthy individuals. In all study participants, imaging of the sublingual microvasculature is performed, and plasma concentrations of circulating glycocalyx constituents are measured. The state of the endothelial surface layer in renal transplant recipients with stable renal function is investigated in chapter 3. Chapter 4 addresses the utility of hyaluronan plasma concentrations as marker for the endothelial glycocalyx in patients with chronic kidney disease. The second part of the thesis focuses on the importance of endothelial glycocalyx during treatment with peritoneal dialysis. In chapter 5 we test whether relationships are present between the state of the systemic microvascular glycocalyx as assessed by sidestream darkfield imaging of the sublingual microvasculature, and parameters of peritoneal transport in patients treated with peritoneal dialysis. In chapter 6 we assess the endothelial surface layer in the peritoneal microvasculature in rats with chronic kidney disease, with or without exposure to dialysis solutions and compare it to rats with normal renal function. For this, SDF imaging of the peritoneal microcirculation and immunohistochemistry of peritoneal specimens are performed.
In addition, relationships with peritoneal transport parameters are tested. The third part of the thesis focuses on the peritoneal membrane alterations induced by long-term exposure to dialysis solutions. In chapter 7 we investigate the effects of chronic kidney disease and long-term exposure to a conventional and biocompatible dialysis solutions on the peritoneum in an experimental rat model. The results of the measurement of the effective lymphatic absorption rate during a standard peritoneal permeability analysis in rats, and the relationship with lymphangiogenesis are presented in chapter 8. In chapter 9 all the above mentioned chapters are discussed and a summary of the main findings is formulated.
References


Introduction and Outline of the Thesis


