Experimental and clinical studies on peritoneal physiology and morphology during chronic peritoneal dialysis
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Citation for published version (APA):

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Introduction
Peritoneal dialysis

Popovich and Monchrief [1] first introduced continuous ambulatory peritoneal dialysis as treatment modality for chronic renal failure. The principle of peritoneal dialysis is the transfer of excess water and waste products from the circulation to the peritoneal cavity, using the peritoneum as a dialysis membrane. The continuous presence of a dialysis solution in the peritoneal cavity allows transfer of solutes. To achieve the removal of superfluous fluid in the body an osmotic agent, most commonly glucose, is added to the dialysis solutions.

Since the peritoneum is a biological membrane, it may be affected by exogenous and endogenous factors, e.g. the continuous exposure to non-physiological dialysis solutions and as a consequence (patho)physiological processes may occur within the body. In this introduction peritoneal anatomy and physiology are discussed in relation to the functional characterization of the peritoneal membrane. The changes that may develop due to long-term peritoneal dialysis are addressed. Furthermore, experimental models for peritoneal dialysis are introduced in which either repeated pharmacological interventions on peritoneal physiology can be conducted, or in which the effect of long-term continuous exposure to dialysis solutions on peritoneal morphology and function can be investigated.

Structures of the peritoneum

The peritoneum is formed by the parietal peritoneum, which covers the inner abdominal walls, and the visceral peritoneum, which contours the majority of the abdominal organs [2]. The serosal space between the visceral and parietal peritoneum is called the peritoneal cavity. Under normal conditions the peritoneal cavity is lined with mesothelial cells and contains a small volume of fluid, most likely to provide lubrication of the internal organs to allow their movement within the peritoneal cavity. During peritoneal dialysis this cavity is filled with approximately 2 L of dialysis solution.

The total anatomical surface area of the peritoneum has been estimated to average 1 m² in adults [3,4]. About 60% of the peritoneal membrane consists of the visceral peritoneum, the parietal peritoneum constitutes 10% and the remaining part is formed by the omentum and mesentery. It is not well elucidated which part of the peritoneum is associated with peritoneal transport, and it may vary in different species. Evisceration in rabbits resulted in marked reduction of the transport rates of creatinine, dextran and para-aminohippuric acid [5,6]. In rats however, evisceration induced an increase in small solute clearances, such as creatinine and urea [7]. In humans, effective peritoneal dialysis has been described after virtually complete resection of the small intestines in a neonate [8]. In these investigations the liver was untouched. The liver surface, however, appears not to make an essential contribution to overall peritoneal transfer of small solutes, such as urea and creatinine. Flessner and Dedrick [9] reported a contribution of the liver surface to the transport of sucrose of 43% to the total transfer for the peritoneal cavity, based on a
theoretical analysis. These findings, however, could not be confirmed in experimental studies using a diffusion chamber fixed to the liver [10,11], sealing the liver surface or performing hepatectomy [12]. Henderson [13] reported that the part of the peritoneum involved in solute transport is markedly less than 1 m² based on observations of urea and inulin clearances obtained during peritoneal and hemodialysis, through membranes of defined surface area. This latter finding implies that the functionally effective peritoneal surface area is smaller than the anatomical one.

The peritoneal membrane is composed of a complex ultrastructure consisting of the peritoneal blood vessels, the interstitial tissue and the mesothelium [14,15]. The parietal peritoneal blood supply occurs via the vasculature of the abdominal wall, whereas the visceral peritoneum is supplied by the mesenteric and coeliac arteries [16]. The peritoneal capillaries and post-capillary venules are located in the interstitium. The peritoneal capillaries consist of a one layer of endothelial cells, lying on a basement membrane. Peritoneal endothelium is mainly of the continuous type, although a limited number of fenestrated capillaries has been found in humans and rabbits [17]. The peritoneal capillary wall is probably the most important barrier in peritoneal exchange of solutes and fluid. The transfer of solutes is generally assumed to be size-selective through a pore system [18]. Peritoneal capillary permeability has been described by a three-pore-model, based on computer simulations [19-21]. The model proposes the presence of many small pores with a radius of 40-55Å, involved in the transport of low molecular weight solutes. The capillary inter-endothelial clefts probably represent the anatomical equivalent of these small pores. A limited number of large pores with radii exceeding 150Å are assumed to mediate the transport of macromolecules like serum proteins. Their morphological representative has not been clearly identified, but venular inter-endothelial gaps may be the equivalent, as these gaps can be induced by certain vaso-active substances [22,23]. The presence of a third, ultra small transcellular pore [21], could clarify the effectiveness of low molecular weight solutes, e.g. glucose, as osmotic agents. Furthermore, the dissociation of water and sodium transport during a hypertonic dwell can only be explained by involvement of ultra small pores in fluid transport [24,25]. Aquaporin-1, a transcellular water channel, has recently been demonstrated in endothelial cells of peritoneal capillaries and venules in rats [26] and the human peritoneum [27,28].

The peritoneal microvessels are embedded in the interstitium. The interstitium is composed of loose connective tissue, containing bundles of collagen, few fibroblasts and few mast cells, within a mucopolysaccharide hydrogel. The restrictive ability of the interstitium is equivocal. No size-selectivity of the interstitium was found in isolated cat mesentery [29], but in vivo microscopy in a rat mesentery suggested the opposite [30]. In peritoneal dialysis patients, the importance of size restriction of the interstitium to macromolecules was unclear [31]. The peritoneal lymphatic vessels are also located within the interstitium. The lymphatics in the subdiaphragmatic area contain gaps or stomata through which fluid and particles can be cleared from the peritoneal cavity. These vessels drain directly via the subdiaphragmatic
lymphatic system into the circulation [32,33]. The lymphatics in the submesothelial tissue are thought to participate to a lesser extent.

The final major constituent of the peritoneum is the mesothelium. A monolayer of mesothelial cells, resting on a basement membrane, contours the visceral and the parietal peritoneum. Mesothelial cells, on the luminal surface covered with microvilli, are active secretory cells which can be concluded from the presence of many microexocytotic vesicles and organelles [34]. They produce among many other substances phosphatidylcholine, a lubricant to prevent adhesions of serosal surfaces [35], and cancer antigen 125. The latter can be used as a marker of mesothelial mass or cell turn-over [36,37]. The mesothelial layer is assumed insignificant as restriction barrier to solutes [38].

Peritoneal permeability

Functional characterization of the peritoneal membrane

The transport across any membrane is determined by its surface area and permeability. In peritoneal dialysis with the peritoneum as dialysis membrane, the effective peritoneal surface area is characterized by the number of perfused peritoneal capillaries [39,40]. The effective peritoneal vascular area is in essence the part of the membrane involved in solute transport. This is considerably smaller than the actual peritoneal vascular surface area [13], as approximately 25% of the peritoneal capillaries are perfused in resting conditions [39]. However, the factors determining the effective peritoneal vascular surface area are not static but dynamic. The mere intraperitoneal instillation of dialysis solution in a cat caused a marked increase in blood flow in its splanchnic organs [41].

The intrinsic permeability is the size-selectivity of the peritoneal membrane. For macromolecules it is mainly dependent on the large pore size. In order to study the two properties of the peritoneal membrane (surface area and permeability), functional characterization of these have been developed. The functional characterization of the intrinsic permeability and the effective peritoneal vascular surface area can be estimated by relating the transport of a solute (mass transfer area coefficient: MTAC, or clearance: C) to its free diffusion coefficient in water ($D_w$) on a double logarithmic scale [42,43]. The slope of this power relationship, MTAC or $C=\text{constant} \cdot D_w^n$, represents the restriction coefficient. A restriction coefficient of 1.0 indicates free diffusion of the solute, a higher restriction coefficient implies the presence of a size selective barrier. The higher the restriction coefficient, the lower the intrinsic permeability. The restriction coefficient to macromolecules was 2.37 [44], based on the serum proteins β2-microglobulin, albumin, IgG and α2-macroglobulin, indicating restricted diffusion of macromolecules from the circulation to the peritoneal cavity. This restriction coefficient expresses the size selectivity and can be used to characterize the peritoneal intrinsic permeability. The transfer of low molecular weight solutes, such as urea, creatinine and urate, is expressed as mass transfer area coefficients (MTAC). The MTAC of a solute is the maximum clearance by diffusion before transport has actually started. A power relationship
also exists when the MTACs are plotted against the molecular weights of these small solutes on a double logarithmic scale [45,46]. The slope of this regression line was 1.24 [44,47]. This is only slightly higher than 1.0 which is obtained when the MTACs were related to their free diffusion coefficients in water. This implies that the transfer of these low molecular weight solutes across the peritoneum mainly occurs by free diffusion, with minimal restriction of the size selectivity of the peritoneal membrane. Therefore, the MTAC of e.g. creatinine can be used as a functional representative of the effective peritoneal vascular surface area. Changes in MTAC of creatinine in individual patients are likely to reflect changes in their peritoneal vascular surface area.

Involvement of nitric oxide
Peritoneal blood flow is unlikely to play a major role in the regulation of the peritoneal vascular surface area or permeability [48-51]. Endogenous substances with vasoactive properties may be involved. Nitric oxide (NO) is the final common pathway for various vasodilating processes. Endothelium derived relaxing factor [52, 53], the equivalent of NO, is rapidly converted to nitrate and nitrite. Nitrite is stable in fresh and spent dialysis effluent, in contrast to nitrite in plasma, where it is directly converted to nitrate [54]. The nitrite concentration in peritoneal effluent is lower than that of nitrate. It appeared that the effluent nitrate concentrations in stable, and uninfected peritoneal dialysis (PD) patients were only dependent on the diffusion of nitrate from the circulation to the peritoneal cavity [55]. Therefore, dialysate concentrations of the NO metabolites nitrite and nitrate in relation to their plasma concentration have been used to investigate the possible involvement of NO in the regulation of peritoneal permeability. Point of interest is the formation of NO. The generation of NO is catalyzed by enzymes, NO synthases [56]. The production of NO through mediation of NO synthases can be inhibited by analogues of L-arginine, the source of NO [57,58], e.g. L-NMMA or L-NAME [59]. Nitrovasodilators, such as nitroprusside [60], induce an increase in the second messenger of NO, cyclic guanosine monophosphate (cGMP), leading to vasodilation [60,61]. The effects of several interventions on the involvement of NO in the peritoneal permeability during experimental peritoneal dialysis will be addressed in chapter 1.3.

Solute transport across the peritoneal membrane
Solute transport across the peritoneal membrane during peritoneal dialysis occurs due to diffusion and solvent drag, or convection. Diffusive transport is determined by the concentration difference between the blood and the dialysis solution in the peritoneal cavity, the concentration gradient. This is the most important transport mechanism for low molecular weight solutes. Convection is determined by the transcapillary ultrafiltration [24], because solvent drag occurs with the transport of water [62]. For low molecular weight solutes the influence of ultrafiltration is limited compared to diffusion during peritoneal dialysis [62], whereas convective transport becomes more important with the increase of the molecular weight of a solute [19,44,63].
Fluid transport during peritoneal dialysis

In peritoneal dialysis, the transport of fluid across the peritoneum is influenced by opposing mechanisms. The osmotic pressure gradient between the blood and the dialysis solution in the peritoneal cavity induces transcapillary ultrafiltration, increasing the intraperitoneal volume. Fluid loss occurs by lymphatic absorption and transcapillary backfiltration. The net ultrafiltration is therefore determined by the transcapillary ultrafiltration and the lymphatic absorption.

The transcapillary ultrafiltration is determined by the hydrostatic pressure gradient, the colloid osmotic pressure gradient, the crystalloid osmotic pressure gradient, the hydraulic permeability and the effective peritoneal vascular surface area. The hydrostatic pressure gradient is determined by the difference between the pressure in the peritoneal capillaries and the intraperitoneal pressure. The latter property probably averages 17 mm Hg [65], which is dependent on the posture because in recumbent position 8 mm Hg has been found [64]. The colloid osmotic pressure gradient of about 26 mm Hg, induced by plasma proteins [65], is directed toward the circulation because the colloid osmotic pressure in the dialysis solutions is negligible due to the low concentration of macromolecules. A dialysis solution containing the glucose polymer icodextrin is an exception because the high concentration macromolecules induces a colloid osmotic pressure gradient in the direction of the peritoneal cavity [66]. The crystalloid osmotic pressure gradient is also determined by the applied osmotic agent in the dialysis solution, most frequently glucose. Despite its small size glucose is an effective osmotic agent. The effectiveness of an osmotic agent depends on the resistance the peritoneal membrane exerts to its transport. This property is expressed as the osmotic reflection coefficient. It can range from 0 in a free passage situation, to 1 in case of total hindrance of a solute by an ideal semi-permeable membrane. Due to its small size, 2.94Å [67], the reflection coefficient of glucose over the large pores is negligible and low over the small pore. Values ranging from 0.02 to 0.05 have been reported [67-69]. However, the reflection coefficient will be 1.0 across the ultrasmall pores, which explains the effectiveness of glucose as osmotic agent. The effect of glucose is most pronounced in the initial phase of the dwell, but this decreases due to glucose absorption during the dwell [25,70]. The peritoneal water transport is mediated by the transcellular water channels i.e. aquaporin-1 [27,71], which are permeable to water but not to solutes. These can be estimated by the sieving of sodium during hypertonic dwells [24,70,72]. Sodium is used to estimate the dissociation between water and small solute transport through transcellular water channels because the sodium concentration in the dialysis solution and uremic plasma are similar. Therefore, when ultrafiltration through aquaporin-1 occurs the dialysate sodium concentration will decrease due dilution in the initial phase of the dwell. The function of aquaporin-1 can be inhibited by mercurial compounds [73]. Carlsson et al. [26] described in vivo inhibition of transendothelial water transport after intra peritoneal administration of mercury chloride during acute peritoneal dialysis in the rat. These authors reported a reduction of the transperitoneal water flow and almost complete blockage of the
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Sieving of sodium. It supports the hypothesis that aquaporin-1 represents the ultrasmall pore system and is involved in glucose induced ultrafiltration during peritoneal dialysis. Amphotericin B has been shown to increase the water, but not the electrolyte permeability of thin lipid membranes formed in vitro from sheep red blood cells dissolved in decane [74]. These observations suggest increased expression or function of aquaporin-1 in the erythrocyte cell membrane. This could explain the observations of Maher et al. [75,76] describing enhanced ultrafiltration after intraperitoneal administration of a high dose of amphotericin B during short dwell exchanges in rabbits. We found similar results in three PD patients treated for fungal infections with amphotericin B intraperitoneally in a therapeutic dose [77]. The greater net ultrafiltration was caused by increased transcapillary ultrafiltration. The influence of amphotericin B and mercury chloride on aquaporin mediated water transport during peritoneal dialysis is further analysed in a chronic peritoneal dialysis model. This is described in part 1, chapters I.1 and I.2.

Direct measurement of the lymphatic flow from the peritoneal cavity is not possible in patients, therefore indirect methods have been developed. Lymphatic absorption can be determined by the disappearance rate of intraperitoneal administered macromolecular tracers, such as radio-iodated serum albumin [70,78] or dextran 70 [79,80]. The disappearance rates of the macromolecular tracers are constant in time [81] and independent of molecular size [82]. Normal values range from 0.4 to 1.2 mL/min [83]. However, increasing the intraperitoneal pressure by external compression [64] or by increasing the administered dialysis volume [84], enhanced the disappearance rate of the volume marker substantially. This indirect measure can be applied as functional characterization of the effective lymphatic absorption rate from the peritoneal cavity. This implies that all pathways of peritoneal lymphatic drainage, both subdiaphragmatic and interstitial.

Effects of long-term peritoneal dialysis
Peritoneal transport
Development of high transport rates of low molecular weight solutes has been reported in patients undergoing long-term peritoneal dialysis, who were investigated during longitudinal follow-up [85-89], in combination with reduced net ultrafiltration. The increase in dialysate-over-plasma (D/P) ratios or MTACs of low molecular weight solutes with prolonged peritoneal dialysis suggests the development of a large peritoneal vascular surface area [43,90,91]. A large effective peritoneal vascular surface induces rapid dissipation of the osmotic gradient [89], and consequently the net ultrafiltration will decrease. Net ultrafiltration failure is the most important transport abnormality in long-term peritoneal dialysis [87,89,90,92]. Net ultrafiltration less than 400 mL after a 4 hour dwell using 3.86% glucose based dialysate solution was reported as clinically important ultrafiltration failure, and proposed as definition of net ultrafiltration failure [93]. Besides a large peritoneal vascular surface area, impaired aquaporin mediated water transport has been reported to induce ultrafiltration failure.
Accurate estimation of aquaporin mediated water transport by the sieving of sodium is of great importance, especially in the situation of a markedly higher plasma sodium concentration than the one in the dialysate. Diffusion of sodium will occur from the circulation to the peritoneal cavity, blunting the sieving and consequently overestimating the impairment of the aquaporin-1 mediated water transport as cause of ultrafiltration failure. The rational of the diffusion correction of sodium is discussed in the appendix of this thesis.

A high lymphatic absorption, determined by high disappearance rate of intraperitoneally administered macromolecules, can also be the cause of ultrafiltration failure [92-96], but no effect of the duration of peritoneal dialysis treatment on this parameter has been reported. The presence of an extremely small effective peritoneal vascular surface area due to numerous adhesions, e.g. in peritoneal sclerosis, also leads to impaired ultrafiltration. Combinations of the causes of ultrafiltration failure have been reported too [93].

An increase in the restriction coefficient to macromolecules has been found with the duration of peritoneal dialysis [47,90]. An increase in the restriction coefficient to low molecular weight solutes however was not present. This implies an increase of the peritoneal size selectivity with the duration of peritoneal dialysis. Whether this was induced by alterations in the interstitial tissue, e.g. by cross linking of the interstitial matrix with advance glycosylation end products (AGEs), or reduction of the pore size of the large pore has not yet been elucidated.

The changes in peritoneal transport that have been reported in long-term peritoneal dialysis patients were also present in many patients with peritoneal sclerosis [97,98]. These observations suggest that the peritoneal membrane alterations found in long-term peritoneal dialysis patients may eventually progress to peritoneal sclerosis.

**Peritoneal morphology**

Investigations of alterations in the peritoneal membrane morphology in patients treated with peritoneal dialysis have mainly focussed on the mesothelial cells and the interstitium in relation to peritonitis [34, 99-107]. However, the development of peritoneal sclerosis, the most serious complication of peritoneal dialysis, appeared not to be directly related to peritonitis incidence [108,98]. The mesothelial cell changes are characterized by a reactive and degenerative expression, and they also show signs of metabolic activity [15,103,109]. Loss of organization of stromal collagen bundles and a patchy appearance of the extracellular matrix can be present [14]. Diabetiform reduplications of the basement membranes of the peritoneal capillaries have been described in peritoneal dialysis patients [34,104,106]. In some cases a tanned discoloration and 'leathery' appearance of the peritoneum is seen [110]. This is caused by advanced glycosylation end products (AGEs) accumulation in the long-lived structure proteins such as collagen and elastin [111], in combination with the presence of an acellular rind of hyalinized collagen, often completely replacing the mesothelium, and fibrotic changes of the peritoneal vessels [101]. Extensive interstitial fibrosis and hyalinization of the media of the vascular wall has been
reported especially in patients with ultrafiltration failure [107]. The interstitial fibrosis most likely consists of type I, III and IV collagen. AGEs in the mesothelium, interstitium and peritoneal capillaries have been described shortly after the start of peritoneal dialysis with glucose containing dialysis solutions [111-114]. Peritoneal sclerosis can develop when these fibrosing processes progress extensively [116]. AGEs formation is addressed in a later paragraph.

Causes of the membrane alterations

Peritonitis

Both continuous exposure to dialysis fluids and recurrent peritonitis have been suggested as mediators in the pathogenetic process of the development of the functional and morphological alterations of the peritoneal membrane during long-term peritoneal dialysis. In the acute phase of infectious peritonitis mesothelial cell denudation of the peritoneal membrane can occur [102,117,118]. In uncomplicated episodes these cells normally regenerate during recovery [108]. However, this process of regeneration may be impaired or perhaps sometimes does not occur at all [111], thereby inducing more permanent alterations of the peritoneal morphology, e.g. after severe Pseudomonas [119] or Staphylococcus aureus infections[116].

Continuous exposure to dialysis solutions

The continuous exposure to unphysiological dialysis solutions is most likely the more important risk factor for the development of peritoneal tissue abnormalities. The bio-incompatibility in most applied dialysis solutions is attributable to the low pH 5.5, the lactate buffer, hypertonicity (334-486 mosm/L) and the glucose concentration (75-220 mmol/L). In vitro studies revealed toxicity to mesothelial cells and neutrophils by glucose and/or the combination of low pH and lactate, as reviewed by Topley et al. [120]. In vivo, low pH is probably less important because pH normalizes shortly after instillation of the dialysis solution [121]. However, the peritoneal tissues are continuously exposed to the extremely high glucose concentrations, as the mesothelial cell layer presents no barrier [122], and more than 65% is absorbed from the peritoneal cavity during a dwell [70]. This is likely to mediate the development of morphological alterations as described in diabetes mellitus, e.g. deposition of AGEs [113], reduplication of vascular basement membranes [34,104,106] and deposition of type IV collagen [107]. The vascular and interstitial changes of the peritoneal membrane that develop with the duration of peritoneal dialysis, and in patients with peritoneal sclerosis are addressed in part III.1. An experimental chronic peritoneal infusion model in the rat was designed to investigate the development of alterations in the peritoneal membrane as has been found in the long-term peritoneal dialysis situation in patients. In this model the development of the structural and functional changes of the peritoneum could be investigated simultaneously. This model is introduced and discussed in part II.2.
**AGES formation**

AGES form after a complex series of reactions [114,115,123,124], starting with the Maillard reaction which results in a Schiff base by the nonenzymatic interaction of the carbonyl group of a sugar with the amine group of a protein. The Schiff base undergoes rearrangement into Amadori products, a reversible reaction, and finally irreversible crosslinks of proteins form AGEs, such as pentosidine [125] and Ne(carboxymethyl)lysine [126]. This process of non enzymatic glycation and oxidation is accelerated by oxidative [127] and carbonyl stress [128]. Carbonyl stress is the process of protein modification by carbonyl compounds, such as malondialdehyde, methylglyoxal and 3-deoxyglucosone, through glycoxidation and lipoxidation [129]. These carbonyl compounds are derived from carbohydrates and lipids. In diabetic patients hyperglycemia is associated with the progression of AGEs formation, the pathogenesis of diabetic complications and aging [130,131]. In normoglycemic uremic patients the increase in AGEs cannot be attributed to hyperglycemia, or just to a decreased removal by glomerular filtration. Interestingly, the AGEs increase is higher in normoglycemic uremias than in diabetic patients. This implicates that uremic plasma contains either precursors or mediators to the Maillard reaction, or both [129]. In peritoneal dialysis an additional factor is the extremely high glucose concentration in the dialysis solution, initiating the Maillard reaction. Furthermore, during heat sterilization of the dialysis solution glucose converts to reactive carbonyl compounds, such as glucoaldehydes also precursors of AGEs. Recently, evidence has been obtained that the pentosidine concentration in peritoneal effluent increased in patients treated with glucose based dialysis solutions, and decreased when treated with glucose free dialysis solutions whereas the plasma concentrations were not altered [132]. This suggests washout of glycated proteins from the peritoneal tissue. The pentosidine concentration of the effluent may reflect the glycation of peritoneal membrane proteins.

**Involvement of the growth factors VEGF and TGF-β in peritoneal alterations**

The combination of extracellular matrix proliferation and the development of a large peritoneal vascular surface area resembles the abnormalities found in diabetic microangiopathy [133]. Vascular endothelial growth factor, VEGF a.k.a. vascular permeability factor [134], is a potent endothelial cell mitogen which promotes formation of new blood capillaries from existing vessels. VEGF also mediates vascular hyperpermeability to serum proteins [135,136]. Its importance in neoangiogenesis has been demonstrated by investigations in tumor models applying antibodies that neutralize VEGF, thereby averting vessel new formation and inhibiting tumor growth and metastasis [137].

VEGF was found to be a mediator in the development of neoangiogenesis in proliferative diabetic retinopathy [133,136,138-140]. Experimental models support this observation as retinal neovascularisation could be inhibited by soluble VEGF-receptor blocking proteins [141]. Although, hypoxia-induced expression of VEGF is a likely mechanism in a number of neovascularising diseases, it seems plausible that high glucose levels [142,143] and advanced
glycation end-products [144,145] contribute to the up-regulations of VEGF expression and progression of the diabetic retinal angiogenesis. The expression of VEGF can be upregulated by several growth factors and cytokines, for example transforming growth factor β (TGF-β) [133,146,147].

TGF-β plays a major role in regulation of repair and regeneration in tissue after injury [148-150]. TGF-β release initiates a sequence of events that promotes the process of healing, such as chemotraction of monocytes, neutrophils and fibroblasts [151], induction of angiogenesis [133] and cell proliferation, and increases deposition of extracellular matrix [152]. In diabetic nephropathy TGF-β has been identified as the growth factor involved in the accumulation of extracellular matrix with deposition of type IV collagen [152-154]. High glucose concentrations and the presence of advanced glycosylation end products enhance TGF-β expression, inducing expansion of the extracellular matrix and the development of fibrosis [153,154]. The proteoglycan decorin binds TGF-β and consequently blocks its biological activities. Exogenous administration of decorin has been reported to suppress TGF-β expression and inhibit matrix deposition in injured glomeruli [154]. TGF-β, on the other hand, inhibits the synthesis of decorin. In the peritoneum decorin also appears to be involved in the regulation of TGF-β activity and collagen fibril formation [155]. In line with these observations, the presence of VEGF and TGF-β in peritoneal effluent of dialysis patients and their relationship with peritoneal permeability characteristics are of great interest in the scope of the morphological alterations that develop in long-term peritoneal dialysis. These subjects are addressed in part III.

Different dialysis solutions
The use of more biocompatible dialysis solutions may prevent the development of structural and functional changes of the peritoneal membrane. Vanholder and Lameire [156] and Feriani [157] reviewed a variety of solutions that differ either in buffer (lactate versus bicarbonate), in sterilization procedure, (heat versus filter sterilization), in composition (sodium and calcium concentrations) or in osmotic agents. Macromolecules, e.g. icodextrin, have been suggested as alternative osmotic agent. Icodextrin has proven to be very effective during long dwell [158-160], especially in patients with a large effective peritoneal vascular surface area [161]. Icodextrin is metabolized by amylase to maltose in the circulation [162]. Blood however, does not contain maltase to convert maltose into glucose. To prevent tissue accumulation of maltose, icodextrin is only used once daily [163]. Low molecular weight solutes have also been suggested, e.g. glycerol. Glycerol can be given four times a day [164], but it has less ultrafiltration capacity than glucose which may limit its application [165]. Development of hyperosmolar syndrome resulting from insufficient metabolism of glycerol has been reported [166], especially in patients with peritoneal absorption rates and no residual renal function. So far, there has not been a single solution identified as effective, safe and affordable as alternative to glucose [156], despite its harmful affects. A regime of combinations with the
most biocompatible solutions seems too simple to solve the problem: a single solution without side effects has not been identified yet, and long-term effects of solution combination treatment are still under investigation.

Experimental models for peritoneal dialysis
Long-term peritoneal dialysis has limitations and side effects. Its future therefore depends on the understanding of the pathogenesis of the side effects in order to, at least, reduce the complications, and on innovations that will make the treatment more effective. A reproducible experimental model could provide this information.

Many experimental models of peritoneal dialysis have been reported in a variety of animals, but the majority has been described in rats and rabbits. Rat models of chronic renal failure have been described in detail, as rats are easy to handle and affordable. However, rats are relatively small, leaving limited amounts of peritoneal effluent for analysis. Most rat models [21,30,38,167,168] have focussed on peritoneal physiology in acute experiments without an indwelling catheter in animals with normal renal function. Only recently, chronic rat models have been introduced. Wieczorowka-Tobis et al. [169] reported a chronic non-uremic model in which twice daily peritoneal dialysis was performed for up to four weeks, e.g. to investigate biocompatibility of dialysis solutions. Peritoneal permeability could be assessed and peritoneal morphology was evaluated. Hekking et al. [170] described a chronic peritoneal infusion experiment for a period of 12 weeks, developed as joined project with our group. Once a day 10 mL of normal saline or 3.86% glucose containing dialysis solution was infused via a subcutaneous reservoir in the neck with attached peritoneal catheter. These experiments were performed to investigate the effect of exposure to dialysis solutions on antibacterial defense mechanisms. Miller et al. [171] and Lameire et al. [172] first reported a uremic model for peritoneal dialysis. The latter study reported preliminary results of dialysis up to 8 weeks after uremia was induced, however with major drop out due to insufficient dialysis, infections and catheter malfunction.

The majority of the rabbit models were aimed on either short-term transport studies in anesthetized rabbits [76,173,175], or focussed on clinical parameters and/or histopathological changes in a long-term experimental set up [176-180]. Recently, Frascasso et al. described the beneficial effects of glycosaminoglycans on functional and morphological dearrangement in a rabbit model [181]. Gotloib et al. described a uremic rabbit model [182] but mortality and morbidity were marked, similar to that of the uremic rat models [171,172]. Concluding from the above a longitudinal model was required to allow repeated investigations in a longitudinal manner. The rabbit was likely to be the most appropriate model to investigate transport characteristics due to the volume of effluent available for investigation. The development of a non-uremic and non-omental rabbit model was undertaken by us, because uremia and omentectomy [182] were likely to interfere with the longevity of the model. This model and its applications are discussed in part I. To study simultaneously
morphology and function in relation to the changes that occur with long-term duration of peritoneal dialysis, a non-uremic and non-omentectomized rat model was developed. The choice of a rat model with normal kidney function was made to be able to maintain continuous peritoneal infusion for a long period of time. In the models described in literature catheter occlusion due to entrapment or wrapping of the omentum was a major complication. Although the omentum is very reactive, we did not perform omentectomy because of possible interference with the longitudinal set up of the model due to protein loss and development of scar tissue and adhesions. This long-term exposure model is introduced in part II.

Aim and outline of the thesis
Adverse alterations in peritoneal physiology and morphology have been reported to develop during long-term peritoneal dialysis treatment with glucose based dialysis solutions. The pathogenesis of these complications is not completely elucidated yet. Therefore, the aim of the studies presented in this thesis was to develop an experimental model in the rabbit, in which the peritoneal physiology could be studied and interventions could be performed in a longitudinal manner to gain a better understanding of the peritoneal physiology. Furthermore, an attempt was made to clarify the development and extent of structural alterations of the peritoneal membrane with the duration of peritoneal dialysis. Growth factors VEGF and TGF-β were also studied in clinical studies as possible mediators involved in these peritoneal alterations. The application of a chronic peritoneal infusion model in the rat allowed simultaneous monitoring of the changes in peritoneal morphology and function with the prolonged exposure to glucose based dialysis fluid.

In part I a chronic peritoneal dialysis model for repeated studies on peritoneal physiology in the rabbit will be described. In chapter I.1 the development of the rabbit model is presented. The methodology to assess the peritoneal permeability characteristics in this model, the standard peritoneal permeability analysis in the rabbit (SPAR), is introduced. A comparison was made between CAPD patients and the rabbit model. In chapter I.2 this model is applied to investigate the effect of pharmacological interventions on peritoneal ultrafiltration through intraperitoneal administration of amphotericin B, a drug which enhances transcellular water transport, and mercury chloride, an inhibitor of aquaporin mediated water transport. In chapter I.3 the rabbit model is used to investigate the effects of the intraperitoneal administration of L-arginine, a substrate for NO synthesis, and L-NMMA, a NO synthase inhibitor, on peritoneal transport and parameters of NO synthesis during 1-hour dwells.

In part II an experimental model in the rat will be described in which the effects of long-term exposure to a glucose based peritoneal dialysis solution on peritoneal morphology and function are investigated (chapter II.1). In chapter II.2 the rational is discussed of fluid supplementation and supplementation
modality in relation to hypovolemia and haemococoncentration, and peritoneal permeability characteristics during 4 hour dwells in an acute rat model using a standardized methodology.

In part III clinical studies effects will be described of long-term peritoneal dialysis on the peritoneal membrane. In chapter III.1 vascular and interstitial changes in the peritoneum of CAPD patients are described in relation to the duration of the peritoneal dialysis treatment and compared to CAPD patients with peritoneal sclerosis. In chapter III.2 vascular endothelial growth factor and transforming growth factor-β1 are analyzed in relation to peritoneal permeability characteristics in a cross sectional design. In chapter III.3 local peritoneal production of VEGF is addressed during glucose based peritoneal dialysis and after the switch to glucose free peritoneal dialysis treatment in relation to parameters of the vascular peritoneal surface area during a longitudinal follow-up.

In the appendix the rational to correct sodium sieving for diffusion from the circulation is discussed in order to assess an accurate estimation of aquaporin mediated water transport.

References


Introduction


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