Understanding and preventing the peritoneal damage caused by conventional dialysis solutions
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Chapter 1

**GENERAL INTRODUCTION**

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1. Renal Replacement Therapy

In 2000, the National Kidney Foundation (NKF) Kidney Disease Outcome Quality Initiative (K/DOQI) Advisory Board approved the following operational definition of chronic kidney disease: 1. Kidney damage for \( \geq 3 \) months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifest by either: pathological abnormalities or markers of kidney damage. 2. GFR (glomerular filtration rate) \(< 60 \text{ mL/min/1.73 m}^2\) for \( \geq 3 \) months, with or without kidney damage. Also 5 stages of chronic kidney disease were defined, ranging from kidney damage with normal or increased GFR (stage 1), to either kidney damage with mild decreased (stage 2), moderate decreased (stage 3) or severe decreased GFR (stage 4) and stage 5 was defined as kidney failure, with a GFR \(< 15 \text{ mL/min/1.73 m}^2\). Also clinical actions to be taken were stated in these so called K/DOQI guidelines [1].

Symptoms of chronic kidney function loss only occur in a relatively late stage, usually when GFR is below 25 mL/min. Amongst other things, water and waste products like creatinine and urea, that are normally excreted with urine, accumulate in the body and renal replacement therapy is indicated.

In January 2005 6292 patients with a functioning renal graft were living in the Netherlands and 5259 patients were treated with chronic dialysis. Of these dialysis patients 26% received peritoneal dialysis, 61% of these were treated with continuous ambulatory peritoneal dialysis (CAPD) (data from RENINE database, Dutch review 28-07-2005). These numbers however only represent the tip of the iceberg: it has been estimated that about 15,000 to 20,000 people have a GFR ranging from 10 to 30 mL/min and therefore renal replacement therapy is very likely to become more and more important in the future [2].

2. Peritoneal Dialysis

In 1976 Popovich and co-workers published an abstract in which they described a method for permanent peritoneal dialysis during which the patient performed normal activities. Except for five daily periods of drainage and instillation of fresh dialysis solution, two litres of dialysate were continuously present in the abdominal cavity [3]. The name of the procedure was later changed in continuous ambulatory peritoneal dialysis (CAPD). Nolph et al. published on the theoretical and practical implications of CAPD [4] and in Canada the technique was improved by introducing plastic bags instead of glass bottles. As the empty bags could stay connected to the peritoneal catheter until the next exchange, the number of disconnections could be reduced hereby also reducing the peritonitis incidence [5]. From 1980 on the numbers of patients treated with peritoneal dialysis treated with CAPD increased rapidly all over the world.
3. **Peritoneal Membrane**

The peritoneum is the largest serous membrane in the body, embryologically derived from mesenchyma. The peritoneal cavity is the space formed by parietal peritoneum, covering the inner abdominal wall and the visceral peritoneum, lining the majority of the internal organs. The peritoneal cavity is lined with a layer of flattened mesothelium and lubricated by a small quantity of serous fluid. This allows movement of the viscera with a minimum of friction [6]. Normally, the peritoneal cavity contains less than 100 mL of fluid, but in peritoneal dialysis, peritoneal dialysis fluid with volumes of 2 L or more are used. The peritoneal surface area for the adult human being is considered to average about 1 m$^2$ [7,8] or even less.

Only part of the anatomical peritoneal surface area is thought to be involved in peritoneal transport, but it is not clear which part is most important in peritoneal transport [9,10]. The peritoneal membrane consists of three main anatomical structures: the mesothelium, the peritoneal blood vessels and the interstitium [11]. The peritoneal cavity is lined with a monolayer of mesothelial cells, which are connected to each other via intracellular junctions [12]. The human omentum has not yet been studied in great depth. However, some studies performed in rodents indicate that there may be little variation between species [13].

The submesothelial basement membrane is a hyaline, one-layered and continuous structure under the mesothelial layer of parietal and visceral peritoneum [14]. The interstitium consists mainly of connective tissue with the fibroblast as the main connective tissue cell [15]. Peritoneal microvessels, arterioles, capillaries and postcapillary venules, represent the third structure of the peritoneal dialysis membrane [16].

4. **Peritoneal Permeability**

Diffusion and convection are the mechanisms involved in the transport of solutes during peritoneal dialysis. Diffusion through a membrane occurs when a concentration gradient is present. According to Fick's first law of diffusion, transport can be described as

$$J_x = (D \cdot A \cdot \Delta C),$$

in which $J_x$ is the rate of solute transfer, $D$ is the free diffusion coefficient, $\Delta x$ is the diffusion distance, $A$ is the surface area and $\Delta C$ the concentration gradient. $D \cdot A / \Delta x$ is called the permeability surface area product or the mass transfer area coefficient (MTAC).

Convective transport or solute drag occurs in conjunction with the transport of water, and thus during ultrafiltration. It is determined by water flux ($J_v$), the mean solute concentration in the membrane ($C$) and the membrane and the solute reflection coefficient $(\sigma)$. This reflection coefficient is the fraction of the maximal osmotic pressure a solute can exert across a semipermeable membrane. It equals 1.0 for an ideal semipermeable membrane and 0 when the membrane offers no resistance to the transport of that solute. With an isoporous membrane $\sigma=1-S$, in which $S$ is the sieving coefficient. $S$ is the ratio between the concentration of a solute in the filtrate divided by its concentration in the plasma when no diffusion occurs. The peritoneum
is not an isoporous membrane which explains why sieving coefficients during peritoneal dialysis range from 0.8 (urea) to 0.7 for glucose. This would reflect a reflection coefficient of 0.2 to 0.3. However much lower values are found in CAPD patients: 0.05 [17], 0.03 [18] and a value of 0.02 was calculated using a distributed model of peritoneal transport [19]. It is likely that these marked differences are caused by the heteroporosity of the peritoneum, especially the presence of transcellular water channels and restricting properties of the interstitium.

The time-course of the diaysate/plasma concentration ratio for low molecular weight solutes shows a hyperbolic curve, due to saturation of the dialysate during the dwell. This implies that peritoneal clearances of low molecular weight solutes are highest during the initial phase of the dwell and gradually decrease during the subsequent hours. The peritoneal equilibration test (PET) as developed by Twardowski et al. [20] is the most simple approach to estimate the transport properties of the peritoneal membrane. It gives the dialysate/plasma concentration (D/P) ratio of urea and creatinine in a standardized way. Also the ratio between the dialysate glucose concentration at the end of the dwell and that before inflow is calculated. In addition the drained volume after 4 hrs and the residual volume can be assessed. The calculation of the mass transfer coefficient i.e., the theoretical maximal clearance by diffusion at time zero, before solute transport has started, is the preferred way to characterize peritoneal permeability.

Solute.s passing from the blood in the peritoneal capillaries to the dialysate in the peritoneal cavity have to pass at least three structures than offer resistance: the mesothelial cell layer, the interstitium and the capillary wall. A three pore model has been suggested to describe peritoneal transport across the peritoneal membrane in which a large number of small pores (radii of 40 A), transport of water and low molecular weight solutes, a low number of large pores (radii>150 A, transport of macromolecules) and ultrasmall or transcellular pores (radii<5A, only permeable for water) have been suggested [21]. This three-pore model provides a quantitative description of sodium and water removal across the peritoneal membrane. In particular, this approach is able to account for the phenomenon of sodium sieving that results in an early fall in the dialysate sodium concentration, due to its dilution by osmotically driven free water transport [22], occurring via aquaporins [23,24]. Several indirect methods to assess free water transport have been suggested. The difference in net ultrafiltration (NUF) between a 3.86% and a 1.36% solution is a rough indication, easy to calculate, but time consuming for patients [25,26]. Another way to estimate free water transport is to measure the dip in dialysate/plasma (D/P) sodium in the initial phase of a 3.86% exchange. Dilution from the circulation occurs by free water transport from the circulation to the dialysate [22,28-29].

The above mentioned tests are tools to assess the function of the peritoneum, but they give no information on peritoneal morphology. Biochemical markers have been described that are thought to be reflective of the peritoneal membrane status, such as for example CA125 [29], IL6 [30-32], hyaluronan [33], metalloproteinases [34,35] tPA [36-38], fibrinogen [39-41], urokinase [42], growth factors as VEGF [43-49], CTGF [50,51] and PAI-1 [52]. Unlike other markers like VEGF, TGF-β is secreted in a soluble form to a very limited extent, and it circulates in plasma mainly as an inactive complex with α2-macroglobulin [53-55]. Also, in tissues it resides as an inactive latent protein-bound form. Although it was observed that TGF-β was
locally released in the peritoneal cavity of most patients during peritoneal dialysis, the lack of a relationship with peritoneal transport parameters and the duration of PD suggests that this was biologically inactive TGF-β1 [55].

5. **Long-term Peritoneal Dialysis**

Ultrafiltration failure, that is the inability to remove sufficient fluid from the body by peritoneal dialysis, is the most important complication of long-term PD [56-62]. It was the main reason for drop out in a study from Japan [63]. This functional deterioration goes hand in hand with histological changes in the peritoneum [64-67]. Reasons for this could be peritonitis, especially serious recurrent peritonitis with micro-organisms like staphylococcus aureus, pseudomonas and yeasts. But even in patients who have never had any peritonitis, this phenomenon occurs. Eventually ultrafiltration failure can result in the necessity to prescribe a restriction of fluid and to use higher dialysate glucose concentrations, short cycle PD, or incidental ultrafiltration with a hemodialyzer. Based on its clinical definition, its prevalence increased from 3% after 1 year on PD to 31% for patients treated with PD for more than 6 years [68]. A cross sectional study in PD patients reported a prevalence of 23% [69], but a significantly higher percentage of 35% was observed in long-term PD patients [63].

In principle four main causes of ultrafiltration failure can be distinguished: 1. The presence of a large vascular surface area (characterized by a high dialysate/plasma ratio and MTAC of creatinine). This leads to a rapid disappearance of the osmotic gradient. 2. A decreased osmotic conductance to glucose (which leads to a decrease in peritoneal free water transport and therefore less sodium sieving). 3. The presence of a high disappearance rate of intraperitoneally administered macromolecules (lymphatic absorption) and 4. A small peritoneal surface area, e.g. due to multiple adhesions, which is rare. The presence of a large vascular area is the most frequent cause of ultrafiltration failure, especially in long-term patients [70-72]. Also, a recent study has shown that patients with early UFF had a higher effective peritoneal surface area, while the occurrence of decreased osmotic conductance to glucose was most evident in the long-term patients. In addition, it appeared that increased lymphatic absorption from the peritoneal cavity was associated with shorter duration of PD [71]. However, a longitudinal analysis has not shown a decrease or an increase in effective lymphatic absorption in time [73]. Exposure to bioincompatible dialysis fluids is generally thought to be of great importance in the development of peritoneal damage on a structural level and subsequent functional deterioration. The dialysis fluids commonly contain 1. Glucose in high amounts, which serves as osmotic agent (1.36% to 3.86% in 1.5 or two liter bags, which will mean an average i.p. exposure to glucose of around 400 grams per day), 2. Lactate as a buffer, which causes a 3. Low pH (dialysis fluid has an average pH of 5 to 5.3), 4. Glucose degradation products, which are formed when the dialysis fluids are heat sterilized, and have proven to be damaging in in vitro and animal studies. Another important feature is that the fluids are 5. Hyperosmolar (490 vs a physiological osmolality of 290).
6. A Peritoneal Exposure Model in the Rat

In patients it is not easy to obtain peritoneal biopsies, as this requires an operation. Most studies using human peritoneal biopsies therefore use the pieces of peritoneum that are taken when inserting the catheter into the peritoneal cavity, during its removal and sometimes during kidney transplantation. Many experimental models of peritoneal dialysis have been described, most of them make use of rats, and most models are short-time, in general not longer than 4 weeks [74,75]. Most rat models [76-80] have focussed on peritoneal physiology in acute experiments without an indwelling catheter in animals with normal renal function. Some chronic rat models have been introduced. Wieczorowka-Tobis et al. [81] described a non-uremic model in which peritoneal dialysis was performed twice daily for 4 weeks, to investigate biocompatibility of dialysis solutions. Peritoneal permeability was assessed and peritoneal morphology was evaluated. Most models are peritoneal exposure models: no fluid exchanges are performed. Peritoneal dialysis fluid is instilled and volumes ranging from 10 mL [82] to 25 mL [83] are used. A long-term peritoneal exposure model in Wistar rats has been developed in our unit [84]. A peritoneal catheter is implanted and tunneled subcutaneously to the neck, where it is connected to a subcutaneous device (Rat-o-Port) allowing repeated puncture through the skin. This allows daily administration of 20 mL of dialysis fluids for a period up to 20 weeks, which is a substantial part of a rat's life span. Drainage is not possible in this model because it leads to blocking of the catheter. At the end of the experiment the function of the peritoneum is assessed by the standard peritoneal permeability analysis adapted for the rat (SPARa), whereafter peritoneal tissues are obtained that are histologically examined. This allows the analysis of functional and morphological relationships. The studies that are part of this thesis have all been performed using this rat model.

7. Aim and Outline of the Thesis

Alternative peritoneal dialysis fluids will be discussed in chapter 2. Also the polyol or sorbitol pathway is introduced in this chapter. Our investigations had three main objectives: the first part of the thesis describes studies on some methodological issues in the chronic peritoneal exposure model in the rat. This part starts with chapter 3 in which the assessment of fibrosis in the peritoneal exposure model is described and two methods, that is the conventional method to assess peritoneal fibrosis with light microscopy and the hydroxyproline measurement, which is a quantitative method and is considered to be the gold standard in assessment of fibrosis, are compared in different peritoneal tissues. In chapter 4, alpha-2-macroglobulin and albumin were investigated as serum markers for peritonitis thus enabling to rule out peritonitis as a confounder in our studies.

In the following part of this thesis studies on the administration of pharmaceuticals during chronic peritoneal exposure in the rat either in drinking water or via administration with the dialysis fluid are discussed. The diabetiform character of particularly the vascular peritoneal
changes and the expansion of extracellular matrix make it very likely that the chronic exposure of peritoneal tissue to the extremely high glucose concentrations in dialysis fluid is of major importance in their pathogenesis. The polyol pathway for intracellular metabolism is involved in the pathogenesis of diabetic microangiopathy. In chapter 5, inhibition of the polyol pathway by an aldose reductase inhibitor, one of the enzymes that catalyzes this pathway, in the rat model is described. Chapter 6 describes effects of angiotensin converting enzyme inhibition which has been reported to protect against pathological formation of fibrosis as has been extensively reported on in renal disease and cardiovascular research areas. In this study lisinopril was supplied in the drinking water of a group of rats during chronic peritoneal exposure to dialysis fluids to investigate a possible protective effect against the pathological development of fibrosis, which is an important development in peritoneal tissues exposed to dialysis fluids. In chapter 7, the opposite question was investigated. In order to try and understand the process of fibrosis development better, we performed a study to try and make a peritoneal fibrotic model, by exposing rats to cyclosporin A during peritoneal exposure to dialysis fluids. This was based on the reports on the pro-fibrotic properties of cyclosporin-A in animal models and also on the clinical observation that 6 out of 18 peritoneal sclerosis patients, diagnosed between 1984 and 1995 at our center had used cyclosporin as immunosuppressant after kidney transplantation.

The last part of the studies in the rat model focuses on different experimental dialysis fluids that were infused in the chronic peritoneal exposure model in the rat, containing pyruvate as a buffer compared to lactate (chapter 7), and in chapter 8 the results obtained with a new solution called PYRAGG are described. This solution contained pyruvate as a buffer, 0.5% aminoacids, 1.4% glycerol and 1% glucose. Chapter 10 consists of a general discussion, A review is given in chapter 11.

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Chapter 1


