Understanding and preventing the peritoneal damage caused by conventional dialysis solutions
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Chapter 10

**General Discussion**
INTRODUCTION

Over the past 30 years, the focus of peritoneal dialysis research has changed from the technical issues related to the establishment of clinical peritoneal dialysis to complex problems of peritoneal membrane biology. Up to the mid 1980s, dialysis fluids and methods of access had to be developed along with the clinical procedures. Treatment was directed primarily at treatment efficacy and infection control. After that period, the first research articles on PD fluid biocompatibility were published, most using peripheral blood leucocytes as model system to assess their viability and function following exposure to dialysis solutions [1]. After that, peritoneal cell cultures were developed, which have been a major step in the development of new peritoneal dialysis solutions. Also, an increasing number of research groups now have sophisticated animals models to more closely simulate the specific constellation of the dialyzed peritoneum [2]. The traditional peritoneal dialysis solutions are bioincompatible because of their low pH, extremely high glucose and lactate concentrations, hyperosmolality and the presence of glucose degradation products formed during heat sterilization of the solutions [3], (chapter 2 in this thesis). The combination of low pH and lactate appeared to be most toxic in vitro studies, because it caused a decrease in the intracellular pH [4]. This effect is probably less important in patients, because the instilled solution is diluted immediately with the intraperitoneal residual dialysate volume, leading to a rapid increase in pH [5]. Glucose in high concentrations is toxic both in in vitro and in animal studies [6,7]. Deposition of advanced glycosylation end-products (AGEs) has been described in peritoneal tissues [8]. Glucose is also likely to be involved in the development of peritoneal neangiogenesis. This is supported by the diabetiform alterations of the microvessels, that are present in patients [9] and that can be induced in animal models [10,11].

THE CHRONIC PERITONEAL EXPOSURE MODEL IN THE RAT

Compared to the first studies in this model we have investigated some additions. These include the use of hydroxyproline as an over-all measure of peritoneal fibrosis, the use of acute phase proteins to exclude peritonitis, which can be a confounding factor, detailed computerized vessel measurements that were applied to measure the wall and luminal thickness of the peritoneal vessels and quantitative realtime PCR analysis performed for VEGF, CTGF and TGF-β.

It appeared that especially the omental hydroxyproline content correlated well with the semiquantitative fibrosis score. Higher values were found for parietal peritoneum, probably due to contamination with muscle fragments form the anterior abdominal wall. We conclude from our studies that the omental hydroxyproline content should always be part of studies aimed at assessment of peritoneal fibrosis.

Most animals that develop peritonitis during the exposure period have overt clinical manifestations. However, in some asymptomatic animals extensive infiltrates are found after sacrifice. We investigated whether serum concentrations of α2-macroglobulin and albumin could identify animals that developed peritonitis without clinical manifestations. It appeared that the sensitivity for albumin was 62% and for α2-macroglobulin 14%, the specificities were
100%. It could therefore be concluded that peritonitis can still be present despite normal serum concentrations of α2-macroglobulin and albumin. However, when α2-macroglobulin is elevated and serum albumin is low, peritonitis is certain and the experiment in such an animal should be terminated.

Using image analysis software the thickness of the vascular wall was measured and surface areas of peritoneal capillaries and small arteries were analyzed. The values for total surface area and luminal area of all vessels together were generated and utilizing these values the wall/total ratio could be calculated to assess wall thickness.

Quantitative real time PCR measurements showed that the relative mRNA content in peritoneal tissue had 4-, 10-, and 58-fold increased for TGFβ1, CTGF and VEGF, respectively, in cyclosporine-treated animals compared to controls. This was a confirmation of the more extensive amount of fibrosis as histologically assessed in these animals compared to controls.

Morphological and Functional Relationships

Such relationships are difficult to study in humans, because of the limited availability of peritoneal tissue. Such studies are possible in our model, but the results has been severely disappointing. Extensive morphological changes were only to a limited extent reflected in parameters of peritoneal transport. This might be due to a number of reasons: (1) The model is not uremic. Therefore low plasma and dialysate concentrations are present for urea and creatinine. Furthermore, these concentrations are often almost equal. This makes the calculation of mass transfer area coefficients extremely susceptible to small errors in the laboratory measurement of these solutes. (2) Although the vascular wall is the most important transport barrier in peritoneal dialysis, relationships between the number of vessels and transport characteristics were not always present. Two explanations are possible. First, also the state of constriction of vasodilatation of the vessels is probably important, because peritoneal transport is not related to splanchnic blood flow, but rather the splanchinc blood volume [12]. Second, we used the α-SMA staining, to calculate the number of vessels in all but one study. This staining might to some extent underestimate the number of capillaries because at least one pericyte has to present in a vessel to obtain positivity.

Pharmaceuticals in PD

Long-term exposure to peritoneal dialysis fluids, commonly used, is a major factor in affecting peritoneal membrane integrity and thereby function. Therefore, PD in its present form may jeopardize the integrity of the organ, on which the whole procedure is based. In the third part of this thesis administration of pharmaceuticals during chronic peritoneal exposure to dialysis fluids have been described to investigate a possible protective effect on the peritoneum by applying the ACE inhibitor lisinopril or by inhibition of the polyol pathway. Also an aggravation of fibrosis by applying cyclosporin A in the rat model has been studied. Inhibition of the polyol pathway with zopolrestat (chapter 6), an aldose reductase inhibitor, showed that this intervention,
when administered intraperitoneally, was effective in preventing the peritoneal damage caused during chronic exposure to conventional glucose/lactate based dialysis fluid. However, when zopolrestat was administered orally during peritoneal exposure to conventional dialysis fluid, it even decreased the amount of vessels with 76% and also resulted in less fibrosis. Our finding that oral administration of zopolrestat was even more effective in preventing peritoneal angiogenesis and fibrosis than intraperitoneal administration, implies that in future studies in peritoneal dialysis patients, oral administration may be applied, which is of course most convenient. At this moment a clinically applicable aldose reductase inhibitor is not yet available.

The ACE inhibitor lisinopril was administered orally during chronic peritoneal exposure to conventional dialysis fluid and resulted in a reduction of the amount of peritoneal vessels of 52% after 16 weeks and 62% after 20 weeks compared to exposure only. Also, the amount of peritoneal fibrosis was significantly less. So far only one short-term study in PD patients has been conducted [13] in which no effect on peritoneal function was found. The short-term character of this study and the limited amount of patients could have accounted for this. A bigger group of patients could be investigated and a longer follow-up would be necessary. Given the protective effect on the kidney, ACE inhibitors are amongst the first choice antihypertensives in dialysis patients. They have a protective effect on residual GFR and might therefore be prescribed in every PD patient. A cross-sectional study comparing PD patients that did use ACE inhibition and the effects on peritoneal function is currently carried out at our center.

In chapter 7 it was shown that the known profibrotic and angiogenic effects of cyclosporin-A augment the morphological peritoneal abnormalities induced by dialysate in the rat model. To evaluate the implications for use of cyclosporin-A for immunosuppression after a renal transplantation on long-term peritoneal dialysis patients further study is required. At present, nephrologists should be aware that the conventional peritoneal dialysis solutions and cyclosporin-A induce similar growth factors that may enhance the development of peritoneal angiogenesis and fibrosis, possibly leading to sclerosis. This might have consequences for the choice of new immunosuppressants in patients after renal transplantation.

In conclusion, the administration of pharmaceutical agents to prevent peritoneal damage seems a promising step. Inhibition of the polyol pathway during peritoneal dialysis in patients will be investigated when a clinically applicable aldose reductase inhibitor becomes available. Also, a cross-sectional study on ACE inhibition has been started.

**Experimental Solutions**

The development of dialysis fluids of a more biocompatible nature to prevent this peritoneal damage is very important. The newly developed lactate/bicarbonate buffered fluid proved to be superior to conventional peritoneal dialysis fluids in *in vitro* tests, and later also in clinical trials [14-16].

However, these fluids are not the complete answer to the problem as they have to be combined with high concentrations of osmotic agents, usually glucose. The combination of introducing dialysis fluids with additives is an interesting option, but it is not very likely that all the damaging characteristics will completely be blocked.
In this thesis, in the fourth part, dialysis solutions using pyruvate as a buffer instead of lactate, were described. It was shown that pyruvate reduced peritoneal neoangiogenesis with 50% and had a less dramatic effect on the prevention of peritoneal fibrosis (chapter 8). However, the use of filter sterilized conventional dialysis fluid (to avoid the formation of advanced glycosylation end products) had a similar effect on angiogenesis and fibrosis in the chronic peritoneal exposure model in the rat (chapter 9). PYRAGG, an experimental dialysis fluid, that was developed especially for our study, contained a combination of three osmotic agents (aminoacids 0.5%, glycerol 1.4% and glucose 1%), was filter sterilized, and buffered with pyruvate. From a theoretical point of view this is the most biocompatible PD solution. This fluid not only reduced the amount of vessels per field with 59% compared to infusion with conventional dialysis fluid, but also tissues from rats infused with PYRAGG showed significantly less fibrosis.

Pyruvate has never been used on a large scale, despite its theoretical advantages. However, intravenously administered pyruvate in high dosages has been used for years in the so-called pyruvate loading test, aimed to detect mitochondrial defects in oxidative metabolism in children, for instance in those suspected to have Leigh syndrome [17]. In this test a high dose of 910 mmol is administered in 10 minutes. This was usually well tolerated although tachycardia and trembling developed in some patients. Lately, pyruvate has been reported to have cardioprotective effects [18,19]. Pyruvate has been found to improve cardiac function in NYHA class II-III heart failure patients [20]. Also, it was shown that pyruvate, but not lactate, had a cardioprotective effect during cardiopulmonary bypass surgery in patients [21]. The above studies indicate that dialysis solutions buffered with pyruvate are not only beneficial for the peritoneum, but that the absorbed pyruvate may also have beneficial systemic effects. Whether this leads to a reduction in cardiovascular death in PD patients should be subject for further investigations.

References


