Experimental and clinical studies on peritoneal physiology and morphology during chronic peritoneal dialysis
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General discussion
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In the chapters of this thesis various different approaches have been described to investigate the peritoneum as a dialysis membrane. These investigations have been done in peritoneal dialysis patients, in a long-term peritoneal dialysis model in the rabbit and in a chronic peritoneal exposure model in the rat. The general issues that are discussed include (1) changes in the peritoneum, (2) vascular endothelial growth factor, (3) the rabbit model and (4) the effect of dehydration on peritoneal permeability. Finally, possible future investigations have been indicated.

Changes in the peritoneum
Alterations in peritoneal physiology and morphology have been reported to develop during long-term peritoneal dialysis treatment. Increased transport rates of low molecular weight solutes have been associated [1-5] with the development of a large effective peritoneal vascular surface [6-8] in combination with ultrafiltration failure [6]. We found evidence of neoangiogenesis and increased deposition of fibrous tissue in the extracellular matrix with the duration of peritoneal dialysis using conventional dialysis solutions [chapter III.1, 9]. Peritoneal dialysis patients with peritoneal sclerosis compared to controls, matched for age and duration of peritoneal dialysis, had significantly lower ultrafiltration and higher transport rates [10], suggesting a larger peritoneal vascular surface area in the presence of peritoneal sclerosis. This was confirmed in chapter III.1: a significantly greater number of vessels per field of peritoneal tissue was found in peritoneal sclerosis patients compared to their matched controls. Peritonitis incidence was not different between the peritoneal sclerosis patients and the matched controls, but the accumulative glucose exposure was greater in the peritoneal sclerosis group in the Hendriks study [10]. This implies that the continuous exposure to the extremely high glucose concentrations present in the glucose based dialysis solutions is a major pathogenetic factor in the development of alterations in peritoneal morphology and function. This was further studied in our rat model, chapter II.1, in which long-term peritoneal exposure to 3.86% glucose based dialysis solution was compared to Ringers lactate, a solution of a similar composition as the dialysis solution, however without the extremely high glucose concentration. Neoangiogenesis, deposition of fibrous tissue in the extracellular matrix and reduplication of basement membranes of peritoneal capillaries were found in combination with increased transport rates and decreased ultrafiltration in the glucose group, whereas the Ringers lactate infused rats were not different in peritoneal morphology or function from the untreated control rats. The fact that the peritoneal morphological alterations and the changes in transport characteristics developed in our rat model in absence of uremia, suggests that this may not play a major role in the initiation or progression of the peritoneal neoangiogenesis and fibrotic processes during peritoneal dialysis. However, recently Miyata et al. [11] discussed the possibility of precursors or mediators of the Maillard reaction in uremic plasma [12]. The Maillard reaction initiates the advanced glycosylation end product (AGE) formation leading to irreversible
cross-linking of matrix proteins [13], causing the brownish discoloration of the peritoneal membrane found in long-term peritoneal dialysis and in patients with peritoneal sclerosis. The influence of AGEs on peritoneal permeability has not been elucidated yet. It has been hypothesized that the small, but significant increase in the restriction coefficient for macromolecules [6,14] with the duration of peritoneal dialysis was caused by a more rigid structure of the large pores [14]. It could be hypothesized that the restrictive changes of the large pore diameter could be influenced by AGE formation, possibly in combination, with the increase of AGEs in the interstitium, leading to more restrictive transport of macromolecules with the duration of peritoneal dialysis. This remains to be elucidated by further investigations.

**Vascular endothelial growth factor**
The changes in peritoneal morphology show great similarities with the abnormalities found in diabetic microangiopathy [15]. Therefore, it was hypothesized that growth factors mediated by hyperglycemia and involved in the development of diabetic retinopathy and nephropathy, might be involved in the development of the peritoneal alterations too. Evidence was found for local production of both VEGF and TGFβ in peritoneal tissue [16]. TGFβ showed no relationships with the peritoneal transport parameters. This could be explained by the fact that numerous cells produce TGFβ after a variety of stimuli inducing an array of reactions [8,15,17]. VEGF concentration attributed to local production showed positive correlations with the MTAC of creatinine and glucose absorption, and a negative trend was observed with the ultrafiltration. These observations suggested a role for VEGF in the development of a large peritoneal vascular surface area. Investigating VEGF in effluent of 10 peritoneal dialysis patients treated with glucose based dialysis solutions during longitudinal follow-up, showed an increase in the local production with the duration of peritoneal dialysis treatment (chapter III.3). The increase in local VEGF production correlated with the difference in the MTAC of creatinine. This finding suggests a causal relationship between the changes in the transport parameters and VEGF production in the peritoneal cavity. Four of the patients in this study switched to glucose free dialysis treatment due to ultrafiltration failure defined as <400 mL/4 hour during a 3.86% glucose based dwell [19]. In these 4 patients the local peritoneal production of VEGF decreased after the switch to non-glucose dialysis treatment accompanied by improvement of some transport parameters [chapter III.2]. No relationship was however, found between the magnitude of the increase in the local production of VEGF and the duration of peritoneal dialysis or the initial value at the start of the investigation. Furthermore, some patients showed a steep rise in the increase in local VEGF productions while treated with glucose based dialysis solution, where in others the increase was only marginal. These observations suggest mediation of individual susceptibility, e.g. genetic factors such as polymorphisms in different growth factors. For example, PAI-1 polymorphisms have been described as risk factor in the development of progressive diabetic retinopathy [20]. In analogy, VEGF polymorphisms may be involved in the rate and magnitude of the
development of diabetiform neoangiogenesis found in long-term peritoneal dialysis.

**Rabbit model**
The longitudinal rabbit model, investigated in part I, appeared applicable for various pharmacological investigations on peritoneal solute transport and ultrafiltration. The data obtained from the standard peritoneal permeability analysis in the rabbit were in the same order of magnitude as those obtained in patients [17], after correction for body surface area. The inter and intra individual coefficients of variation of the peritoneal permeability characteristics determined in a clinical study [22], were of a similar magnitude in rabbits. However, the mediation of NO in peritoneal permeability is most likely different from that in humans, because the peritoneal vascular surface area was found less sensitive to NO in rabbits. Therefore, when models are applied to investigate effects of pharmacological interventions or to elucidate physiological and pathological mechanisms with the aim to extrapolate to the clinical situation, species inter variability involved in these aspects, have to be taken into account.

**The effect of dehydration on peritoneal permeability**
The functional or effective peritoneal vascular surface area is mainly determined by the number of perfused capillaries, or the number of pores available, in combination with interstitial resistances. So far, no evidence was available that the hydration status of the interstitium would influence the transfer of low molecular weight solutes or macromolecules. It has been hypothesized that the interstitium would dehydrate during a hypertonic dwell and, although the total distance from the peritoneal capillary to the peritoneal cavity may be shortened, the restriction to solute movement would increase due to contortion of the interstitial matrix [23,24]. In chapter II.1 no evidence was found to confirm this hypothesis. In fact dehydration and haemoconcentration did not influence peritoneal permeability parameters. The lower MTAC urea suggested increased restriction but glucose absorption was not affected, nor were the clearances of the macromolecules different among the three groups investigated. The higher plasma concentrations of solutes and the higher osmolarity in the group without fluid supplementation in comparison with the other groups suggested that these parameters, obtained at the end of the dwell of the SPARa, were likely to be lower in the beginning of the experiment. The plasma value at the end of the dwell therefore most likely overestimates the average plasma concentration. Keeping the principle of the MTAC calculation in mind: \((P-D_{10})/(P-D)\), overestimation of the plasma concentration \(P\) will artificially result in a lower ratio.

It is conceivable that the proposed studies in peritoneal dialysis patients, and in the two models in rodents will not only deepen our insight in pathophysiological mechanisms, but will also lead to marked improvements in the application of peritoneal dialysis as a long-term renal replacement therapy.
Future investigations

Focus for further studies may involve two directions.

1. The mechanisms mediating the development of the alterations in peritoneal morphology and function during long-term peritoneal dialysis should be further elucidated. The mediation of growth factors should be targeted, such as VEGF polymorphisms, involvement of TGFβ, and connective tissue growth factor CTGF in extracellular matrix proliferation on mRNA level. Furthermore, effects of AGE formation in the presence or absence of uremia, and the expression of aquaporin-1 and nitric oxide synthase are of interest. These aspects can be investigated in a clinical set up in effluent, plasma and in biopsies.

Prevention of the progress to the development of the abnormalities should be aimed for as well. For instance the application of additives to the dialysis solutions which may inhibit the advance to adverse alterations, for example inhibition of the Maillard reaction and consequently AGE formation could be reduced by the intraperitoneal administration of aminoguanidine; the effect of growth factors could be constrained by intraperitoneal administration of inhibitors (anti-VEGF receptor proteins, octreotide: growth factor inhibitor) and the application of more biocompatible dialysis solutions. Assessment and understanding of the effects caused by long-term treatment with dialysis solutions with different osmotic agents (such as icodextrin, amino acids and glycerol) and/or with other buffers than lactate (such as bicarbonate or pyruvate buffered solutions) should be extended. The influence of combinations of the treatment with these solutions on physiology and morphology, should also be studied in a long-term follow-up. Furthermore, as soon as ultrafiltration problems and morphological abnormalities develop, the effect of peritoneal rest should be examined with respect to recuperation of peritoneal function and morphology. Evidently, a part of the above cannot be studied in peritoneal dialysis patients. The chronic peritoneal infusion model in rat provides simultaneous information on peritoneal morphology and function, and is therefore instrumental for these investigations.

2. The rabbit model could be used to obtain better understanding of the mechanisms that influence peritoneal ultrafiltration, such as hydrostatic pressure gradient (portal versus caval hypertension), colloid osmotic pressure gradients (low versus high, using plasmapheresis) and lymphatic absorption (intraperitoneal administration of phosphatidyl choline).

References


General discussion


