Experimental and clinical studies on peritoneal physiology and morphology during chronic peritoneal dialysis
Zweers, M.M.

Citation for published version (APA):
The growth factors VEGF and TGF-β1 in peritoneal dialysis

MM Zweers¹, DR de Waart², W Smit¹, DG Struijk¹,³, RT Krediet¹

¹Department of Nephrology, ²Department of Clinical Chemistry, Academic Medical Center Amsterdam, and ³Dianet Foundation, Utrecht-Amsterdam, The Netherlands

J Lab Clin Med 1999;134:124-132
Abstract
The morphological alterations in the kidney and the retina that can be present in patients with diabetic microangiopathy are mediated by growth factors. Vascular endothelial growth factor (VEGF) is a mediator of neoangiogenesis in diabetic retinopathy. Transforming growth factor β (TGF-β) is involved in the extracellular matrix proliferation in diabetic nephropathy. The aim of the present study was to investigate the presence of VEGF and TGF-β1 in peritoneal effluents of continuous ambulatory peritoneal dialysis (CAPD) patients treated with glucose containing dialysis solutions, in relation to parameters of peritoneal transport.

Standard peritoneal permeability analyses using 3.86% glucose dialysate, were performed in 16 stable PD patients (median duration of PD 39 months, range 1-104). The power relation that is present between dialysate/serum (D/S) ratios of serum proteins that are only transported across the peritoneal membrane and their molecular weights (mol wt) was used to predict the D/S ratio's when diffusion would be the only explanation of the measured dialysate concentration. It was appreciated that all TGF-β1 in the circulation was bound to α2-macroglobulin. D/S ratio's of VEGF (p<0.0005) and TGF-β1 (p<0.015) were significantly higher than expected when VEGF and TGF-β1 would only have been transported from the circulation by diffusion. No relationship was present between the effluent concentration attributed to local production of VEGF (LVEGF) and that of TGF-β1 (LTGF-β1). LVEGF correlated with the mass transfer area coefficient (MTAC) creatinine (r=0.69, p<0.007), MTAC urate (r=0.60, p<0.02) and the glucose absorption (r=0.75, p<0.004), all reflections of the peritoneal vascular surface area. A negative correlation was observed between the transcapillary ultrafiltration (926 mL/4 hour, 394-1262 mL/4 hour) and LVEGF (r=-0.52, p<0.045). This negative tendency was also observed between the net ultrafiltration (622 mL/4 hour, -43-938 mL/4 hr) and LVEGF (r=-0.48), but did not reach significance. LVEGF and duration of treatment did not correlate, possibly because the relative small number of patients. LTGF-β1 showed no relation with transport parameters or duration of treatment.

In conclusion we found evidence for local production of both VEGF and TGF-β1 in the peritoneal membrane of patients using chronic peritoneal dialysis with glucose based dialysate solutions. The analogy with VEGF in diabetic retinopathy suggests a pathogenetic role of high dialysate glucose concentrations in the development of these alterations in the peritoneal membrane.

Introduction
Various diabetiform alterations have been described in the peritoneum of chronic peritoneal dialysis patients treated with glucose containing dialysis fluids. These include basement membrane reduplications of peritoneal capillaries [1,2], expansion of extracellular matrix [3] with extensive deposition of collagen IV [4] especially in the vascular wall, and the deposition of advanced glycosylation end products [5,6]. The high solute transport rates that have been described in long-term peritoneal dialysis patients [7,8], suggest the development of a large vascular peritoneal surface area in relation to the duration of peritoneal dialysis treatment. Recently we could confirm this neoangiogenesis in a histomorphometric study [9].
The development of diabetic microangiopathy is mediated by growth factors. TGFβ is likely to be the main growth factor for the extracellular matrix proliferation with deposition of collagen IV in the kidney in the presence of diabetes mellitus [10]. TGFβ may therefore also be involved in the alterations in the peritoneal membrane as it is upregulated in mesothelial cells after exposure to glucose [11]. Furthermore, TGF-β1 induces proliferation of extracellular matrix in vitro [12]. Diabetic retinopathy is another important manifestation of diabetic microangiopathy. Vascular endothelial growth factor (VEGF) has been demonstrated to play a key role in the neoangiogenesis of proliferative diabetic retinopathy [13-16]. The expression of VEGF can be upregulated by ischemia, high glucose concentrations, and other growth factors and cytokines, e.g. TGFβ [17]. In analogy, involvement of both TGFβ and VEGF can be hypothesized in the pathogenesis of glucose induced diabetiform alterations of peritoneal tissue.

The aim of this cross sectional study was to investigate the presence of TGF-β1 and VEGF in peritoneal dialysis effluents of CAPD patients treated with glucose containing dialysis fluids in relation to peritoneal transport parameters.

Patients and methods

*Patients*
Sixteen non-diabetic CAPD patients (6 males and 10 females) were examined with a standard peritoneal permeability analysis. The median duration of peritoneal dialysis treatment was 39 months, range 1-104. All patients used commercially available, glucose based dialysis solutions (Dianeal, Baxter BV, Utrecht, The Netherlands). None of the patients had peritonitis at the time of the study or in the four preceding weeks. Informed consent was obtained from all patients after an explanation of the aim and the methods of the study.

*Study design*

The standard peritoneal permeability analyses were performed during 4 hour dwell periods with 3.86% glucose containing dialysate (Dianeal) as described previously [18]. In brief, the peritoneal cavity was rinsed with 1.36% glucose dialysate prior to the instillation of the test solution. Dextran 70 (Hyskon, Medisan Pharmaceuticals AB, Uppsala, Sweden) was added to each test bag as a volume marker, for the calculation of fluid kinetics [19]. Dialysate samples were taken from the test dialysate before inflow and 10, 20, 30, 60, 120, 180 and 240 minutes after instillation of the test solution. These samples were collected after a temporal drainage of 100 to 200 mL to avoid a dead-space effect. Subsequent to the drainage at 240 minutes the peritoneal cavity was rinsed again, this time with 1.36% Dianeal. Samples taken from this bag were used to calculate the residual volume. Blood samples were drawn at the start and at the end of the test. Dextran 1 (20 mL, Promiten, NBPI, Emmer-Compascuum, The Netherlands) was given intravenously after the first blood sample was drawn, to prevent a possible anaphylaxis to dextran 70 [20].

*Assays*
Urea, creatinine and urate were measured by means of enzymatic methods on an automated analyzer (Hitachi H747, Boehringer Mannheim, Mannheim, Germany).
VEGF and TGF-β1 in peritoneal dialysis

Glucose concentrations were determined by the glucose oxidase-peroxidase method using an auto analyzer (SMA-II, Technicon, Terrytown, USA). β2-Microglobulin was determined with an IMx system using a microparticle enzyme immunoassay (Abbot Laboratories, North Chicago, USA). Albumin, transferrin, IgG and α2-macroglobulin were measured with nephelometry (BN 100, Behring, Marburg, Germany).

VEGF and TGF-β1 were measured in serum and dialysate with commercially available enzyme-linked immunosorbent assays (human VEGF and human TGF-β1 Quantikine™, R&D Systems, Minneapolis, USA). The lower detection limit of the VEGF assay was 15.0 ng/L and of the TGF-β1 assay 30.0 ng/L. Prior to the determination of VEGF and TGF-β1, dialysate samples were concentrated 9.6 times (median, range 1.82-28.52) by means of positive pressure ultrafiltration using a 250 mL cell and a YM-10 membrane with a molecular cutoff point of 10,000 Da (Amicon Corp., Danvers, USA). The concentration factor was defined as the albumin concentration in the concentrate divided by the albumin concentration in the original dialysate sample. Total dextran was determined by high performance liquid chromatography [21].

Calculations
To express the transport of low molecular weight solutes, the mass transfer area coefficients of urea, creatinine and urate were calculated according to the model of Waniejewski [22]. The solute concentrations in serum were corrected for plasma water [23]. The mass transfer area coefficient of a solute is the maximum theoretical clearance of the solute at time point zero, before the transport has actually started. Glucose absorption was calculated as the difference between the amount of glucose instilled and recovered, relative to the amount instilled. Peritoneal handling of the macromolecules β2-microglobulin, VEGF, albumin, transferrin, IgG, α2-macroglobulin and TGF-β1 was expressed as dialysate/serum (D/S) ratios. In tissues TGFβ is mainly present in a biological inactive form, bound to a latency associated protein. α2-Macroglobulin is the TGFβ binding protein in serum [24,25]. A peritoneal transport line was computed for each patient based on the least squares regression analysis of the D/S ratio of β2-microglobulin (MW = 11,800 Da), albumin (MW = 69,000 Da), transferrin (MW = 85,000 Da), IgG (MW = 150,000 Da), α2-macroglobulin (MW = 820,000) and their molecular weights when plotted on a double logarithmic scale [26]. These proteins are transported from the circulation to the peritoneal cavity. The slope of the regression line represents the size-selectivity of the peritoneal membrane. By interpolation of the molecular weight of VEGF (MW = 34,000 Da) and extrapolation of the molecular weight of α2-macroglobulin bound TGF-β1 (MW = 845,000 Da) in the regression equation, the expected D/S ratios were calculated, assuming that their dialysate concentrations would only be determined by transport from the circulation. The concentration of these growth factors attributed to local production was defined as the difference between the measured and expected dialysate concentration.

The peritoneal fluid parameters were calculated as previously described by Krediet et al [19]. Briefly, fluid transport across the peritoneal membrane during peritoneal dialysis is influenced by opposing mechanisms. The transcapillary ultrafiltration increases the intraperitoneal volume. Fluid loss from the peritoneal cavity is assumed to occur by backfiltration and lymphatic absorption in the lymphatic system. The resultant of these mechanisms is the net ultrafiltration. The
transcapillary ultrafiltration was calculated as the dilution of the volume marker dextran 70. The transcapillary ultrafiltration rate was assessed by dividing the transcapillary ultrafiltration by the dwell time. Convective disappearance of the volume marker was used as an indirect method to quantify the contribution of the peritoneal lymphatics in the absorption of fluid from the peritoneal cavity. These calculations include all pathways of uptake into the lymphatics, both interstitial and subdiaphragmatic. The change in intraperitoneal volume during the dwell can be calculated from the dilution of the volume marker after correction for incomplete recovery. The net ultrafiltration rate was expressed as the change in intraperitoneal volume divided by the dwell time.

The results are presented as median values and ranges as most of the data were distributed asymmetrically. The differences between the measured and expected dialysate concentrations of VEGF and TGF-β1 were tested by a modified t-test to determine whether the deviation from the regression line was significant. This test takes the variability of the regression lines into account [27]. To investigate the absolute differences between the measured and expected dialysate concentrations of these growth factors Wilcoxon matched pairs rank sum test was applied. Spearman rank correlation analysis was used for calculation of correlations.

Results
*Solute transport*
Patient characteristics are presented in Table I. VEGF and TGF-β1 could be detected in concentrated effluents of all patients. However, the effluent TGF-β1 concentration in 2 patients was less than could be expected on the basis of transport from the circulation. The mass transfer area coefficients of the low molecular weight solutes urea, creatinine and urate, and the glucose absorption are summarized in Table II. The D/S ratios of the macromolecules β2-microglobulin, transferrin, albumin, IgG and α2-macroglobulin are also presented in Table II. Regression lines were calculated for each individual patient based upon the power relation between the D/S ratio of the serum proteins and their molecular weights (see methods). The r-values of the individual regression lines exceeded 0.93 (p<0.02) in all patients. Based on these regression lines, D/S ratios of VEGF and TGF-β1 could be predicted when their presence in the effluent would be determined by transport from the circulation only. Figure 1 shows the regression line between the D/S ratios of serum proteins and their molecular weights and also the measured D/S ratios of VEGF and TGF-β1. The measured D/S of VEGF was significantly (p<0.001) higher than could be expected based upon the transport line, taking the variability of this line into account. The D/S of TGF-β1, however, did not differ significantly from the regression line (p>0.2). Investigating the expected and measured D/S ratio of TGF-β1 as paired data for each patient, showed that the measured D/S ratio's were significantly greater (p<0.01) than the expected. The expected and measured D/S ratio's of VEGF and TGF-β1 computed for each patient are given in Figure 2. The dialysate and serum concentrations of VEGF and TGF-β1, and their values in serum of healthy individuals are presented in box and whisker plots in Fig 3. The median value in the effluent attributed to local production was 15.8 ng/L (6.5-28.4 ng/L) for VEGF and 31.1 ng/L (0-293.8 ng/L) for TGF-β1. Effluent VEGF attributed to local production was not correlated with that of TGF-β1 (r=−0.01, p>0.9), nor was
Table I. Patients demographics.

<table>
<thead>
<tr>
<th>pts</th>
<th>sex</th>
<th>months on PD</th>
<th>prim. kidney disease</th>
<th>glucose exposure (\text{mmol/24 hour})</th>
<th>urine production (\text{mL/24 hour})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>56</td>
<td>CIN</td>
<td>804</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>40</td>
<td>HN</td>
<td>756</td>
<td>1000</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>56</td>
<td>GCN</td>
<td>702</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>24</td>
<td>A</td>
<td>2361^b</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>37</td>
<td>CGN</td>
<td>1108</td>
<td>550</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>52</td>
<td>W</td>
<td>878</td>
<td>500</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>104</td>
<td>CIN</td>
<td>1156</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>12</td>
<td>SLE</td>
<td>1156</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>6</td>
<td>CIN</td>
<td>752</td>
<td>750</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>8</td>
<td>PCKD</td>
<td>804</td>
<td>820</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>103</td>
<td>PCKD</td>
<td>1156</td>
<td>1000</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>27</td>
<td>SLE</td>
<td>1260</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>1</td>
<td>PCKD</td>
<td>804</td>
<td>2200</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>1</td>
<td>HN</td>
<td>804</td>
<td>1150</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>57</td>
<td>CIN</td>
<td>804</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>84</td>
<td>CGN</td>
<td>1284</td>
<td>0</td>
</tr>
</tbody>
</table>

HN: hypertensive nephropathy, A: amyloidosis, CGN: chronic glomerulonephritis, W: Wegener's glomerulomatosis, CIN: chronic interstitial nephritis, PCKD: polycystic kidney disease; SLE: systemic lupus erythematosus. ^a at the time of the investigation. ^b patient treated with CCPD.

Table II. Parameters of peritoneal solute transport of 16 CAPD patients

<table>
<thead>
<tr>
<th>low molecular weight solutes</th>
<th>median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTAC urea (mL/min)</td>
<td>18.0 (12.8-21.3)</td>
</tr>
<tr>
<td>MTAC creatinine (mL/min)</td>
<td>9.9 (6.6-15.2)</td>
</tr>
<tr>
<td>MTAC urate (mL/min)</td>
<td>7.6 (4.8-12.4)</td>
</tr>
<tr>
<td>glucose absorption (%)</td>
<td>64 (49-77)</td>
</tr>
</tbody>
</table>

D/S ratios serum proteins

| β2-microglobulin            | 0.090 (0.077-0.523) |
| albumin                     | 0.009 (0.003-0.138) |
| transferrin                 | 0.006 (0.003-0.015) |
| IgG                         | 0.005 (0.003-0.028) |
| α2-macroglobulin            | 0.001 (<0.001-0.004) |

The total effluent concentration of VEGF related to that of TGF-β1 (r=0.24, p>0.3). The duration of peritoneal dialysis treatment showed no relation with the total effluent concentrations of VEGF or TGF-β1 or those attributed to local production. There was also no relationship present between the duration of peritoneal dialysis and the parameters of solute transport with exception of the MTAC of urate (r=0.66, p=0.01). However, the MTAC of creatinine, a reflection of the vascular peritoneal surface area, showed a marked relationship with the VEGF dialysate concentration attributed to local production, r=0.69 and p<0.007 (Figure 4). A similar correlation was present between the MTAC of urate and the effluent concentration of VEGF attributed to local production, r=0.59 (p<0.02), and
Figure 1. The regression line based on the power relationship between the D/S ratio of β2-microglobulin, albumin, transferrin, IgG and α2-macroglobulin (●) and their molecular weights. The measured D/S ratios of VEGF and TGF-β1 (○) are given in relation to their molecular weights. Means and SEM are presented.

Table III. Correlation coefficients and levels of significance of effluent VEGF and TGF-1 in relation to parameters of small solute transport

<table>
<thead>
<tr>
<th></th>
<th>MTACurea</th>
<th>MTACreat</th>
<th>MTACurate</th>
<th>glucose absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>total dialysate VEGF</td>
<td>r=0.12</td>
<td>r=0.62</td>
<td>r=0.59</td>
<td>r=0.58</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.6</td>
<td>p&lt;0.016</td>
<td>p&lt;0.02</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td>effluent VEGF attributed to local production</td>
<td>r=0.05</td>
<td>r=0.69</td>
<td>r=0.60</td>
<td>r=0.75</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.8</td>
<td>p&lt;0.007</td>
<td>p&lt;0.02</td>
<td>p&lt;0.004</td>
</tr>
<tr>
<td>total dialysate TGF-β1</td>
<td>r=-0.08</td>
<td>r=0.01</td>
<td>r=-0.07</td>
<td>r=-0.24</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.7</td>
<td>p&gt;0.9</td>
<td>p&gt;0.78</td>
<td>p&gt;0.3</td>
</tr>
<tr>
<td>effluent TGF-β1 attributed to local production</td>
<td>r=-0.14</td>
<td>r=-0.08</td>
<td>r=-0.17</td>
<td>r=-0.36</td>
</tr>
<tr>
<td></td>
<td>p&gt;0.5</td>
<td>p&gt;0.7</td>
<td>p&gt;0.5</td>
<td>p&gt;0.1</td>
</tr>
</tbody>
</table>

between the glucose absorption and the locally produced VEGF, r=0.75 (p<0.004) (Figure 4). The daily glucose exposure at the time of the investigation (702-1284 mmol/24 hr) showed no relationship with the effluent concentration of VEGF attributed to local production of VEGF nor with that of TGF-β1. The correlation coefficients and the levels of significance determined for total effluent concentrations of VEGF and TGF-β1, and those attributed to local production of VEGF and TGF-β1 in relation to transport parameters of small solutes are summarized in Table III.

Peritoneal fluid transport
The transcapillary ultrafiltration rate was 3.86 mL/min (1.64-5.26 mL/min), the effective lymphatic absorption rate 1.18 mL/min (0.76-3.09 mL/min) and the net ultrafiltration rate 2.66 mL/min (-0.18-3.91 mL/min). No relationship was present between these parameters and the duration of peritoneal dialysis. A negative correlation was observed between the transcapillary ultrafiltration and the dialysate concentration of VEGF attributed to local production (r=-0.52, p<0.045), but with a wide scatter (Figure 5).
VEGF and TGF-β1 in peritoneal dialysis

Figure 2. The individual data in 16 patients of the expected and the measured D/S ratios of VEGF (left panel) and TGF-β1 (right panel).

This negative tendency was also seen between the net ultrafiltration and the local production of VEGF (r = -0.48, p < 0.06), but did not reach significance (Figure 5). When the total dialysate concentration of VEGF was related to the transcapillary ultrafiltration and the net ultrafiltration, these trends were less obvious, r = -0.30 and r = -0.14. No relationships were present between the total effluent concentration of TGF-β1 or that attributed to local production and the fluid transport parameters.

Discussion
In this study the presence of the growth factors VEGF and TGF-β1 was investigated in peritoneal effluent in relation to peritoneal transport parameters. It was demonstrated that the total VEGF concentration in the effluent and that of TGF-β1 were significantly higher than could be expected, based on transport from the circulation. This implies that besides the transport from the circulation, also local production of these growth factors in peritoneal tissues, followed by release in the dialysate, is likely to contribute to their effluent concentrations. The magnitude of locally produced VEGF and TGF-β1 could be estimated by comparing their D/S ratios to those of macromolecules known to be transported across the peritoneal membrane without local production. However, it is possible that the magnitude of VEGF attributed to local production is an underestimation of the actual amount locally produced VEGF in the peritoneum. VEGF is known for its high affinity for two endothelial receptors [15]. Furthermore, VEGF has been reported to bind to tumor neovasculature resulting in a concentration effect on the locally produced VEGF [36]. It is not unlikely that the neovasculature of the peritoneal membrane is able to exert a similar concentrating effect and thus remove VEGF from the peritoneal effluent.

Diabetic microangiopathy is characterized by the deposition of extracellular matrix [10] and by neoangiogenesis [15]. Extracellular matrix expansion is also present in the peritoneum after long-term peritoneal dialysis [3]. Functional studies on peritoneal transport of various solutes have made it likely that high peritoneal transport rates of low molecular weight solutes indicate the presence of a large
effective (or functional) vascular peritoneal surface area, i.e. a large number of perfused peritoneal microvessels [28,29]. An increase in the vascular surface area is often temporary, for instance during peritonitis [30] or after intraperitoneal administration of nitroprusside [31]. However, the enhancement in the transport rates of low molecular weight solutes described during follow-up of long-term CAPD patients [7,8] points to the development of structural alterations in the number of peritoneal microvessels. Recently, we were able to show that neoangiogenesis was indeed present in long-term CAPD patients [9]. High concentrations of VEGF in ocular fluid of patients with diabetic retinopathy are associated with proliferative retinopathy [13,14]. These concentrations decreased after successful laser coagulation [13]. This suggests that VEGF plays a key role in neoangiogenesis in proliferative diabetic retinopathy. The strong correlations that we observed between the MTACs of creatinine and urate, and the glucose absorption on one hand, and total dialysate VEGF or locally produced VEGF on the other hand, make it very likely that VEGF is also involved in peritoneal neoangiogenesis. This results in an increased vascular surface area, leading to high diffusional solute transport rates. The above mentioned correlations were absent for the MTAC of urea and the D/S ratios of serum proteins. The former can be explained by the near equilibrium between plasma and dialysate urea concentrations after 4 hours, making the MTAC of urea a less sensitive measure for differences in peritoneal surface area than the MTACs of creatinine and urate. The absence of a correlation between dialysate VEGF and D/S ratios of serum proteins is probably caused by the size-selectivity of the peritoneal membrane: the transport of macromolecules is not only dependent on the number of pores in the vascular wall but also on their size [31]. However, VEGF has been shown to permeabilize a number of vascular beds, including those of the peritoneal wall, mesentery and diaphragm [32], probably by increasing the large pore radius [15]. A correlation between effluent VEGF would therefore not have been unlikely.
VEGF and TGF-β1 in peritoneal dialysis

**Figure 4.** The effluent concentration of VEGF attributed to local production, was positively correlated with the mass transfer area coefficient of creatinine, \( r=0.69, p<0.007 \) (left panel) and the glucose absorption, \( r=0.75, p<0.004 \) (right panel).

The fact that we did not find it may have been caused by the presence of interstitial alterations, increasing the size-selectivity of the peritoneal membrane, and counteracting the increased vascular permeability. This is of course speculative and needs confirmation by experimental studies.

A negative correlation with a wide scatter was seen between the transcapillary ultrafiltration and the effluent VEGF concentration attributed to local production. The transcapillary ultrafiltration is dependent on the product of the peritoneal ultrafiltration coefficient and the transperitoneal pressure gradient. The latter is composed of the hydrostatic, the colloid osmotic and the crystalloid osmotic pressure gradient [33]. Of these, only the decrease of the crystalloid osmotic pressure gradient during a dwell is dependent on the vascular surface area. Therefore, inter individual differences in the other pressure gradients could explain the scatter. A similar negative, but not significant tendency was observed between the effluent VEGF concentration and the net ultrafiltration per 4 hours. The net ultrafiltration is determined by the effective lymphatic absorption and the transcapillary ultrafiltration. Inter individual differences in the effective lymphatic absorption rate can explain why a statistically significant correlation between VEGF and the net ultrafiltration was not found.

For all correlations studied between peritoneal transport and VEGF, Spearman's correlation coefficient was greater for effluent VEGF corrected for diffusion from the circulation, than for total effluent VEGF. This also supports our hypothesis that locally produced and released VEGF is involved in peritoneal neoangiogenesis. VEGF is expressed by various cells, both at mRNA and protein level and it is to a large extent secreted in soluble form [15,16]. This may explain that, although at present it is unknown which cells in the peritoneum produce VEGF, its effluent concentration may reflect the peritoneal vascular surface area.

The total effluent concentration of TGF-β1 or that attributed to local production did not show any relation with peritoneal transport parameters nor with the effluent concentrations of VEGF. However, this does not imply that TGF-β1 is not involved in the pathogenesis of various diabetiform alterations of the peritoneal membrane in patients treated with glucose based dialysis fluids. TGFβ has been reported as main mediator involved in the expansion of the extracellular matrix with
deposition of collagen IV in diabetic nephropathy [10]. Similar diabetiform alterations as extensive extracellular matrix proliferation [3] with the deposition of collagen IV [4] have been described in CAPD patients. Unlike VEGF, TGFβ is secreted in a soluble form to a very limited extent, and circulates in plasma mainly as an inactive complex with α2-macroglobulin [24,25,34]. Also, in tissues it resides as an inactive latency-protein bound form. Activation, e.g. by acidification or enzymatic cleavage is necessary for its biological effects. Although we observed that TGF-β1 was locally released in the peritoneal cavity of most patients during peritoneal dialysis, the lack of a relationship with peritoneal transport parameters suggests that this was biologically inactive TGF-β1. This is supported by the observation that not only plasma had to be acidified to be able to measure TGF-β1 (according to the instructions of the manufacturer), but also the concentrated dialysate (unpublished). Whatever the mechanism, our results make it unlikely that dialysate TGF-β1 concentrations can be used as a reflection of extracellular matrix expansion in peritoneal tissues. Lin et al. reported a study on the expression of TGF-β1 during peritonitis in peritoneal dialysis patients, with a possible relation to peritoneal fibrosis [35]. TGF-β1 and its mRNA were detectable during peritonitis in effluents and in the cells in it. However, the TGF-β1 concentration was below detection limit in dialysate after recovery. A possible explanation for the difference between the two studies could be that these authors did not concentrate the effluents before ELISA analysis.

The lack of relationship between the duration of peritoneal dialysis and the concentrations of growth factors, or the majority of the peritoneal transport parameters is most likely due to the limited number of patients and the cross-sectional design of the study. Based on the increases of solute transport rates with the duration of CAPD reported in two prospective longitudinal studies [7,8] it can be
expected that this may also be the case for VEGF. However, because of great interindividual variations, a large group of patients followed longitudinal will probably be required to support this hypothesis.

It can be concluded that evidence was found for local peritoneal production of both VEGF and TGF-β1 in the peritoneum of patients treated with peritoneal dialysis with glucose based dialysis solutions. Our results showed that the TGF-β1 concentration in the effluent was not related to transport parameters, probably because of secretion in an inactive form. In contrast, we obtained reasonable evidence that VEGF could be a mediator in the peritoneal neangiogenesis in chronic peritoneal dialysis patients. Dialysate VEGF may therefore reflect the vascularization of the peritoneal membrane.

References
Chapter III.2


31. Douma CE, de Waart DR, Struijk DG, Krediet RT. The nitric oxide donor nitroprusside
intraperitoneally affects peritoneal permeability in CAPD. Kidney Int. 1997; 51:1885-1892.


