Understanding and preventing the peritoneal damage caused by conventional dialysis solutions
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Chapter 7

Cyclosporin A induces Peritoneal Fibrosis and Angiogenesis during Chronic Peritoneal Exposure to a Glucose-based, Lactate-buffered Dialysis Solution in the Rat

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ABSTRACT

INTRODUCTION

Cyclosporin-A has been described to stimulate the development of fibrosis. The objective of the present study was to investigate whether administration of cyclosporin-A contributes to the development of the peritoneal morphological and functional alterations induced in the peritoneum by long-term exposure to dialysis solutions, in the chronic peritoneal exposure model in the rat.

METHODS

Ten rats received peritoneal infusion of dialysis solution and oral administration of cyclosporin-A for 8 weeks. Eight rats, that received only peritoneal infusion of dialysate for 8 weeks, served as control animals. After the experimental period function of the peritoneum was assessed thereafter the rats were sacrificed and peritoneal tissue was obtained. The number of vessels per field of peritoneal omental tissue section was counted after αSMA-staining and the presence of peritoneal fibrosis assessed after PSR-staining and hydroxyproline content determination. VEGF was measured in plasma and dialysate with ELISA. Quantitative realtime PCR analysis was performed for VEGF, CTGF and TGF-β.

RESULTS

Histology revealed more fibrosis in intersegmental and perivascular areas of cyclosporin exposed animals compared to animals exposed to dialysate only. Also the hydroxyproline content of parietal peritoneum was higher in the cyclosporin treated group than in the infusion only animals. Although peritoneal transport characteristics were not altered, vessels had increased in number and their walls tended to be thicker in the cyclosporin-treated animals compared to controls. The relative mRNA content in peritoneal tissue had 4-, 10-, and 58-fold increased for TGFβ1, CTGF and VEGF, respectively, in cyclosporin treated animals compared to controls.

CONCLUSION

The fibrotic and angiogenic effect of cyclosporin augment the morphological peritoneal abnormalities induced by exposure to high glucose dialysis solutions. The increased expression of both TGFβ1, CTGF and VEGF suggests that the combination may enhance the development of fibrosis and angiogenesis.
INTRODUCTION

Encapsulating peritoneal sclerosis is the most serious complication of peritoneal dialysis. Its prevalence ranges from 0 to 4.4% in single center studies and from 0.7 to 2.8% in registries [1]. However, in patients treated for more than 8 years, its prevalence exceeded 19% [2]. The clinical signs and symptoms of peritoneal sclerosis may become apparent after discontinuation of peritoneal dialysis, for instance because of a kidney transplant [3-5], as was observed also in our department in 6 out of 18 patients with peritoneal sclerosis diagnosed between 1986 and 1995 [unpublished]. The interval between kidney transplantation and the occurrence of bowel obstruction was usually 6 to 12 months, but sometimes exceeded 2 years [4,5]. This observation suggests that other factors than peritoneal dialysis per se may contribute to the development of peritoneal sclerosis. Cyclosporin-A is almost invariably used for immunosuppression after kidney transplantation. Cyclosporin-A can induce expression of the pro-fibrotic transforming growth factor-β1 (TGF-β1) and causes interstitial fibrosis in a rat model for chronic cyclosporin nephropathy [6,7]. We therefore hypothesized that cyclosporin-A might contribute to the development of encapsulating peritoneal sclerosis in patients treated with peritoneal dialysis previously.

The pro-fibrotic effects of TGF-β1 are partly mediated by connective tissue growth factor (CTGF) [8]. In addition to fibrosis we demonstrated previously that angiogenesis occurs during long-term peritoneal dialysis [9] and it seems very likely that vascular endothelial growth factor (VEGF) is of importance in this process [10].

The objective of the present study was to investigate whether administration of cyclosporin-A contributes to the development of the peritoneal morphological and functional alterations induced in the peritoneum by long-term exposure to dialysis solutions, in a chronic peritoneal exposure model in the rat.

MATERIALS AND METHODS

ANIMALS

Twenty-two male Wistar rats (HSD, Harlan, Zeist, The Netherlands), aged 7-8 weeks, weighing 300–320 grams, were used. Group 1 received peritoneal infusion of dialysis solution and oral administration of cyclosporin-A for 8 weeks (n=12), group 2 (control group) only received peritoneal infusion of dialysate for 8 weeks (n=10). The rats were housed solitarily under controlled conditions (temperature 19°C, relative humidity 50±5%, 12/12 hour light/dark cycle) and fed standard chow (Hope Farms, Woerden, The Netherlands) and fluid ad libitum. All rats acclimatized for one week before insertion of a peritoneal catheter under anesthesia with a mixture of ketamine:xyazine:atropine (4:2:1). The length of the catheter was adjusted for each rat and a titanium/silicone device (Rat-o-Port, MTINC, 71S, Access Technologies, Norfolk Medical, Skokie, IL, USA) was attached to the catheter and implanted subcutaneously...
in the neck of the animals. Peritoneal healing was allowed for one week after the catheter insertion by daily infusion of 1 mL of heparinized saline (5 IU/1mL NaCl 0.9%), after which the experimental period started. All rats received a daily infusion of 60 mL/kg body weight (BW) 3.86% glucose containing dialysis solution (Dianead, Baxter, S.A., Castlebar, Ireland) via the Rat-o-Port. Cyclosporin was orally administered in chocolate milk (Neoral suspension, 15 mg/kg BW); the concentration of cyclosporin in chocolate milk was adjusted per rat according to their individual weights and the volume they drunk. In a pilot study through cyclosporin serum levels ranged between 66–101 ng/mL. In each group 2 animals were sacrificed before the end the experimental period because of obstructed catheters: Histology showed signs of peritonitis (extensive infiltrates in all peritoneal biopsies) and these animals were excluded from the study. After the treatment period, a standard peritoneal permeability analysis was performed and rats were sacrificed immediately thereafter. The study was performed in accordance with the regulations required by the local ethics committee for animal experimentation.

**STANDARD PERMEABILITY ANALYSIS IN THE RAT (SPARA)**

The SPARa is based on the human standard peritoneal analysis (SPA) described by Pannekeet et al. [11], and adapted for rats as described by Zweers et al. [12]. During this whole procedure intramuscular administration of anesthetics consisting of a mixture of ketamine, xylazine and atropine was applied. Blood was obtained by tail vein puncture before the experiment and by cardiac puncture at the end of the experiment. Body temperature was kept constant throughout the SPARa by placing the animal on a heating pad (37°C).

**ASSAYS**

Total dextran 70 was measured in all dialysate samples by high performance liquid chromatography [13]. Both in plasma and in dialysate, urea (Hitachi H747, Boehringer Mannheim, Mannheim, Germany) and creatinine (Hitachi H911, Boehringer Mannheim, Mannheim, Germany) were measured with enzymatic assays. The glucose concentration was assessed by the glucose oxidase-peroxidase assay (SMA II, Technicon, Terrytown, NJ, USA). Enzymatic methods for the determination of creatinine are influenced to some extent by high glucose concentrations as are present in glucose based dialysis solutions. A correction factor (CF) was determined for the measurement of creatinine which is used in our laboratory: $\text{CF} = -3.10^{-4} \cdot (\text{gluc})^2 + 0.11 \cdot (\text{gluc}) + 105$, in which (gluc) is the glucose concentration in the effluent, expressed in mmol/L. Sodium was measured by an ion selective electrode (Hitachi H747, Boehringer Mannheim, Mannheim, Germany).

VEGF was measured in dialysate and blood samples taken at the end of the experiment, after the SPARa (240 minutes) by ELISA (MMVOO, mouse VEGF Quantikine kit, R&D systems). The ELISA cross reacts with rat VEGF and recognizes both 164 and 120 amino acid residue forms of VEGF. Prior to the ELISA, the dialysate samples were concentrated 10-fold with a centriprep centrifugal filter device of 15 mL with a YM-10 membrane (Amicon Corp., Danvers,
USA). Also albumin (Nordic, Sigma) IgG (Nordic, Sigma) and fibrinogen (ICN, Sigma) were measured by ELISA (goat anti-rat albumin, goat-anti-rat IgG, goat-anti-rat fibrinogen, Nordic Immunology). A peritoneal transport equation was computed for each rat based on the least squares regression analysis of the D/P ratio and the free diffusion coefficients in water ($D_{20,w}$) of these proteins when plotted on a double logarithmic scale. The following values were used for $D_{20}$: albumin =6.1, fibrinogen=2.19, IgG =4.0 $cm^2 s^{-1} 10^{-7}$. By interpolation of the $D_{20,w}$ of VEGF ($D_{20,w}=5.98$) in the regression equation, the expected D/P ratios were calculated, assuming that the dialysate concentrations would only be determined by transport from the circulation. The quantity of VEGF determined by the difference between the measured and the expected dialysate concentration was attributed to local production.

**Calculations**

Peritoneal fluid and solute kinetics were calculated as described previously [14]. The transcapillary ultrafiltration was calculated from the dilution of the volume marker, i.e. dextran 70. 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 is defined as the change in intraperitoneal volume divided by the dwell time. The mass transfer area coefficients (MTACs) of urea and creatinine were calculated according to the model of Waniewski et al. [15], in which the solute concentration is expressed per volume of plasma water. Glucose absorption was estimated as the difference between the instilled and the recovered amount of glucose, relative to the instilled quantity of glucose. Free water transport was estimated by the sieving of sodium, expressed as the dialysate-to-plasma (D/P) ratio of sodium. A diffusion correction was made because a concentration difference between initial dialysate and plasma concentration causes Na$^+$ diffusion from the circulation to the dialysate, which leads to an underestimation of the actual sodium sieving. This was done using the MTAC of creatinine as described previously [16].

**Histopathology**

Omental tissue was obtained from each rat and processed for light microscopy. The tissues were fixed in freshly prepared 4% paraformaldehyde. Paraffin-embedded tissue was serially sectioned at 5μm thickness, sections were stained with hematoxylin/eosin and picro-sirius red F3B (PSR), the latter providing a brick red staining of all fibrillary collagen. For visualization of vessels, adjacent sections were immunohistochemically stained for α smooth muscle actin-1 (SMA-1, dilution 1:800, DAKO, Denmark), which is expressed in vascular smooth muscle cells and pericytes. For detection a streptavidin-biotin-peroxidase method was used. The sections were deparaffinized in xylene and ethanol, followed by incubation with hydrogen peroxide 0.3% in methanol to block endogenous peroxidase activity. The onset of the staining sequence was a block with 10% normal goat serum followed by incubation with primary antibodies against αSMA (monoclonal, DAKO, Denmark). Subsequently, biotinylated rabbit anti-mouse F(ab')$_2$ against
the monoclonal antibody and biotinylated swine anti-rabbit against the polyclonal antibodies (both DAKO, Denmark) in PBS-NHS containing 5% normal rat serum and horse radish peroxidase-conjugated streptABC complex (DAKO, Denmark) were applied. The peroxidase activity was detected with 1 mg/mL 3,3-diaminobenzidine tetrahydrochloride (Sigma, St Louis MO, USA) and 0.015% H$_2$O$_2$ in 50 mM Tris-HCl buffer, pH 7.6, yielding the brown coloration. All slides were counterstained with Mayer's hematoxylin, dehydrated through a series of ethanol and xylene and mounted with Pertex mounting medium.

The number of vessels per field of peritoneal tissue section was counted in αSMA-stained omental tissue, using a light microscope (Leitz Dialux 20, Leica, Rijswijk, The Netherlands) with a 25x flat field objective (x10 ocular). Five non-overlapping fields from the upper left to the lower right were investigated throughout the specimen, in which all vessels were counted and measured. The thickness of the vascular wall was measured using the sections stained for αSMA. Images generated by light microscopy (Olympus BX 60, U-CMAD-2, Japan) with a x10 Olympus objective were captured using a digital camera (Coolsnap cf, Roper Scientific Inc., Duluth, GA, USA). Surface areas of peritoneal capillaries and small arteries were analyzed using image analysis software (Image Pro Plus 4.5, Media Cybernetics Inc., Silver Spring, MD, USA).

From each specimen, 5 randomly selected images were captured (0.172 mm$^2$ each). The inner and outer border of all vessels was outlined, and the values for total surface area and luminal area of all vessels together were generated using the above mentioned software. Utilizing these values the wall/total ratio (WTR) could be calculated to assess wall thickness. Capillaries (diameter <8 μm), middle sized vessels (8–20 μm) and arterioles/small arteries (>20 μm) were analyzed separately, as described earlier by our group [9].

The amount of fibrosis was measured by semi-quantitative scoring of the extent of PSR staining of omental sections. Briefly, submesothelial (SM), perivascular (PV) and intersegmental (IS) areas of omental tissue were judged separately, using as grades 0=normal presence of fibrous tissue, 1=mild excess, 2=moderate, and 3=severe excess. The semi-quantitative scoring was assessed by two blinded observers and inter-observer variability was less than 10%.

Also, the amount of fibrosis was determined quantitatively by measuring the hydroxyproline content in parietal peritoneum by HPLC [17].

**Quantitative PCR Experiments**

Omental tissue was excised immediately after sacrifice and stored in RNAlater (Ambion, Austin, USA) at −80°C. The tissue was homogenized using a Polytron device and total RNA was isolated using Trizol (Life technologies). cDNA was synthesized using Superscript II Reverse Transcriptase (Invitrogen, Breda, The Netherlands) and subsequently purified with a Qiaquick PCR kit (Qiagen, Venlo, The Netherlands). Quantitative realtime PCR was performed with primers for CTGF

(sense: 5'-CACAgAgTggAgCgCCTgTTC-3',
anti-sense: 5'-gATgCACTTTTTgCCCTTCTTAATg-3'),
TGF-β1 (sense: 5'-ACCAACTACTgCTTCAgCTCCAC-3',
...
and anti-sense: 5'-ggCAAggACCTTgCTgTACTg-3' 
and VEGF-A (sense: 5'-CATgCCAAgTggTCCCCA-3', 
and anti-sense: 5'-CTATCTTTTgTACTgCATTCA-3') with the Light Cycler using the 
Fast Start DNA Master SYBR Green I kit (Roche Diagnostics, Almere, The Netherlands). Message 
RNA of the house keeping gene encoding for TATA-box binding Protein (TBP) was analyzed 
by realtime PCR to correct for variable input (sense: 5'-CAggAgCCAAgAgTgAAgAAC-3', 
anti-sense: 5'-GgAAATAATTCTggCTCATACT-3').

**Statistical Analysis**

Medians and interquartile ranges are given unless stated differently. The Mann-Whitney U test 
was done to investigate differences between the two groups.

**Results**

Peritoneal transport of low molecular weight solutes (Table 1) and of macromolecules (Table 2) 
was not different in animals exposed to dialysate and cyclosporin A in comparison to animals 
exposed to dialysate only, with the exception of fibrinogen that showed a lower D/P ratio in the 
cyclosporin treated animals. Histology revealed more fibrosis in intersegmental and perivascular 
areas of cyclosporin exposed animals compared to animals exposed to dialysate only (Table 3). Corresondingly, the hydroxyproline content in the cyclosporin treated group was higher than 
in the infusion with dialysate only animals: 1243 (944–1511) ng/mg tissue compared to 541 
(467–749) ng/mg (p=0.05).

The number of vessels in the cyclosporin-exposed animals was significantly higher than 
in infusion with dialysate only animals (Table 3). When the vessels were examined in detail it 
appeared that both the total vascular surface area and the luminal area tended to be larger in 
the cyclosporin-treated animals compared to the animals infused with dialysate only, but did 
not reach statistical significance. The wall/total vascular surface area ratio, especially for larger 
vessels, was higher in the cyclosporin treated animals compared to those of the infusion only 
group (0.67 vs 0.61, p=0.036), suggesting thickening of the vascular wall in the presence of 
cyclosporin (Table 4).

Plasma levels of the 164 and 120 isoforms of VEGF-A protein were higher in the cyclosporin-
treated animals than in the rats that received dialysate only. The protein concentrations of these 
VEGF-A isoforms in the dialysate effluent were similar, and, correspondingly, the calculated 
local peritoneal production of VEGF-A 164 and 120 did not differ between both experimental 
groups (Table 5). However, realtime PCR indicated a higher peritoneal expression of VEGF-A 
at the mRNA level in the cyclosporin A-treated rats compared to rats that had received dialysate 
only. Also, the expression of TGF-β1 and CTGF mRNAs was markedly higher in the peritoneal 
tissue from animals treated with cyclosporin compared to those from the infusion only group 
(Table 6).
Table 1: Transport parameters measured during the SPARa. Medians and interquartile ranges are given.

<table>
<thead>
<tr>
<th>Solute transport</th>
<th>Dialysate/CsA (n=10)</th>
<th>Dialysate only (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTAC creatinine (µL/min)</td>
<td>164 (132-195)</td>
<td>149 (132-176)</td>
</tr>
<tr>
<td>Gluc. Abs. (%)</td>
<td>54 (47-61)</td>
<td>59 (57-61)</td>
</tr>
<tr>
<td>D/P sodium (%)</td>
<td>90 (88-90)</td>
<td>89 (89-91)</td>
</tr>
<tr>
<td>Fluid transport (µL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCUFR</td>
<td>54 (41-64)</td>
<td>61 (53-70)</td>
</tr>
<tr>
<td>NUFR</td>
<td>28 (18-44)</td>
<td>37 (34-43)</td>
</tr>
</tbody>
</table>

MTAC creat: mass transfer area coefficient for creatinine, Gluc. Abs.: glucose absorption, D/P sodium: dialysate over plasma ratio of sodium, TCUFR: transcapillary ultrafiltration rate, NUFR: net ultrafiltration rate

Table 2: Dialysate/plasma ratio's for proteins (medians and interquartile ranges)

<table>
<thead>
<tr>
<th></th>
<th>Dialysate/CsA</th>
<th>Dialysate only</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/P Albumin</td>
<td>0.014 (0.011-0.26)</td>
<td>0.012 (0.009-0.015)</td>
</tr>
<tr>
<td>D/P IgG</td>
<td>0.008 (0.006-0.016)</td>
<td>0.009 (0.006-0.011)</td>
</tr>
<tr>
<td>D/P Fibrinogen</td>
<td>0.002 (0.001-0.003)</td>
<td>0.005 (0.003-0.030)*</td>
</tr>
</tbody>
</table>

*p<0.05

Table 3: Quantification of numbers of vessels and amount of fibrosis in omentum of rats. Medians and interquartile ranges are given. Scoring of fibrosis was performed in submesothelial (SM), perivascular (PV) and intersegmental (IS) areas.

<table>
<thead>
<tr>
<th></th>
<th>Dialysate/CsA</th>
<th>Dialysate only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels/field</td>
<td>27 (17-32)</td>
<td>14 (7-23)**</td>
</tr>
<tr>
<td>Fibrosis (0-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>2 (2-3)</td>
<td>1.5 (1.5-2)</td>
</tr>
<tr>
<td>IS</td>
<td>2 (1.5-2.5)</td>
<td>1 (1-1.5)*</td>
</tr>
<tr>
<td>PV</td>
<td>2 (1.5-2.5)</td>
<td>1.5 (1-2)*</td>
</tr>
</tbody>
</table>

*p<0.05, **p=0.05

Table 4: Surface areas of peritoneal vessels.

<table>
<thead>
<tr>
<th></th>
<th>Diameter&lt;8 µm</th>
<th>Diameter 8-20 µm</th>
<th>Diameter&gt;20 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dialysate/CsA</td>
<td>Dialysate only</td>
<td>Dialysate/CsA</td>
</tr>
<tr>
<td>TSA (µm²)</td>
<td>63 (45-78)</td>
<td>56 (46-67)</td>
<td>109 (118-270)</td>
</tr>
<tr>
<td>LA (µm²)</td>
<td>16 (11-25)</td>
<td>14 (11-20)</td>
<td>46 (29-80)</td>
</tr>
<tr>
<td>WTR</td>
<td>0.74 (0.67-0.80)</td>
<td>0.71 (0.63-0.78)</td>
<td>0.71 (0.64-0.79)</td>
</tr>
</tbody>
</table>

*p<0.05 vs. Group 1
TSA: total surface area, LA: luminal area, WTR: wall/total ratio
Table 5: VEGF levels in dialysate, serum and locally produced VEGF (medians and interquartile ranges).

<table>
<thead>
<tr>
<th></th>
<th>Dialysate/CsA</th>
<th>Dialysate only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (ng/L)</td>
<td>153 (113-186)</td>
<td>82 (51-119)*</td>
</tr>
<tr>
<td>Dialysate (ng/L)</td>
<td>21 (3-45)</td>
<td>28 (5-34)</td>
</tr>
<tr>
<td>Locally produced (ng/L)</td>
<td>13 (5-34)</td>
<td>24 (9-33)</td>
</tr>
</tbody>
</table>

*p=0.05

Table 6: Results of quantitative PCR expressed as ratio (each growth factor is divided by the amount of Tata Box binding Protein).

<table>
<thead>
<tr>
<th></th>
<th>Dialysate/CsA (n=7)</th>
<th>Dialysate only (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTGF</td>
<td>2.09</td>
<td>0.20**</td>
</tr>
<tr>
<td>TGFβ-1</td>
<td>3.41</td>
<td>0.83*</td>
</tr>
<tr>
<td>VEGF</td>
<td>26.8</td>
<td>0.46**</td>
</tr>
</tbody>
</table>

*p=0.01, **p<0.01

CTGF: connective tissue growth factor, TGFβ-1: transforming growth factor beta-1, VEGF: vascular endothelial growth factor. The number of animals in the two groups is smaller, because it was not possible to extract enough DNA in each animal.

DISCUSSION

Oral administration of cyclosporin during chronic peritoneal exposure to a standard 3.86% glucose containing lactate-buffered dialysis solution leads to more extensive peritoneal fibrosis and a larger amount of vessels than peritoneal exposure to dialysate only. In addition the vascular wall seems to be increased after exposure to the combination of dialysate and cyclosporin. The larger number of vessels was not accompanied by faster peritoneal transport characteristics. This may have been caused by the increased thickness of the vascular walls, although the difference between the two experimental groups was only significant for the vessels with a diameter exceeding 20 μm. The luminal area did not decrease after exposure to cyclosporin, so no morphological indications for vasoconstriction were found in the cyclosporin exposed animals.

The morphologic alterations in the cyclosporin-treated animals were accompanied by a higher relative mRNA expression of the growth factors TGFβ1, CTGF and VEGF-A in the peritoneal tissues compared to those of the animals that were exposed to dialysate only. We reported previously that TGFβ1 in effluent is only present in its inactive form and does not reflect the increase of active TGFβ1 in the tissue [10], which could indicate that structural changes in the peritoneal membrane are not necessarily accompanied by changes in peritoneal effluent. Plasma levels of VEGF were higher in the cyclosporin treated animals compared to the infusion only group. To our knowledge effects of cyclosporin on circulating plasma VEGF levels have never been described. In agreement with increasing evidence for a role of CTGF in the development of fibrosis in diabetic conditions [8, reviewed in 18], we here show a strongly increased CTGF mRNA expression at the peritoneal tissue level in association with peritoneal
fibrosis induced by high glucose containing dialysate and further enhanced by exposure to cyclosporine A.

It is well known that chronic cyclosporin nephrotoxicity may progress to striped interstitial nephrosis, a process in which TGF-β plays an important role [18,19]. In addition, cyclosporin-A is able to increase the TGF-β transcription rate in human T lymphocytes [20]. Cyclosporin-A is able to modulate expression of TGF-β, as well as that of several other growth factors. In a model of chronic cyclosporin nephropathy, Shihab et al. demonstrated an upregulated expression of VEGF mRNA and protein along with an increased expression of VEGF-receptors [21]. This may explain why cyclosporin-A can enhance peritoneal angiogenesis.

Long-term peritoneal dialysis can lead to functional and morphological alterations in peritoneal tissues. The morphologic abnormalities consist of increased peritoneal fibrosis, diabetiform neoangiogenesis and submesothelial hyalinosis of arterioles and venules [22-24]. In some patients these may progress to encapsulated peritoneal sclerosis. Growth factors like TGFβ-1, VEGF [24] and CTGF [25] have been reported to be of importance in these processes.

It follows from the above that both cyclosporin and long-term peritoneal dialysis induce the expression of TGFβ1, CTGF and VEGF-A. This suggests that the combination may enhance the development of fibrosis and angiogenesis, which was indeed found in the present study. We could not induce encapsulating peritoneal sclerosis in our model. This may be due to the relatively short duration of exposure or to the model itself. It is noteworthy that the peritoneal lesions induced after 20 weeks exposure to conventional dialysis fluids caused marked neoangiogenesis and fibrosis, but no encapsulated peritoneal sclerosis [26].

The peritoneal sclerosis models in rats that have been developed are rather incomparable to the clinical situation, because they mostly make use of irritants, as chlorhexidine or fluids with an extremely low pH. Nevertheless, fibrosis and neoangiogenesis were also observed in a rat model of experimental peritoneal sclerosis [24], which confirms our previous observations in PD patients [9] and is also in accordance with our findings on angiogenesis and fibrosis in the present study.

It can be concluded that the known profibrotic and angiogenic effects of cyclosporin-A augment the morphological peritoneal abnormalities induced by dialysate, when applied in a chronic peritoneal exposure model in the rat. To evaluate the implications for the use of cyclosporin-A for immunosuppression after a renal transplantation in long-term PD patients requires further study. At present, nephrologists should be aware that the conventional peritoneal dialysis solutions and cyclosporin-A induce similar growth factors that may enhance development of peritoneal neoangiogenesis and fibrosis, possibly leading to sclerosis.

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