Antiangiogenic and Antifibrotic Gene Therapy in a Chronic Infusion Model of Peritoneal Dialysis in Rats

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Abstract. To identify the relative importance of peritoneal fibrosis and angiogenesis in peritoneal membrane dysfunction, adenoviral mediated gene transfer of angiostatin, a recognized angiogenesis inhibitor, and decorin, a transforming growth factor–β–inhibiting proteoglycan, were used in a daily infusion model of peritoneal dialysis. A peritoneal catheter and subcutaneous port were inserted in rats. Five and fourteen d after insertion, adenovirus-expressing angiostatin, decorin, or AdDL70, a null control virus, were administered. Daily infusion of 4.25% Baxter Dianeal was initiated 7 d after catheter insertion and continued until day 35. Three initial doses of lipopolysaccharide were administered on days 8, 10, and 12 to promote an inflammatory response. Net ultrafiltration was used as a measure of membrane function, and peritoneum-associated vasculature and mesenteric collagen content was quantified. Ultrafiltration dysfunction, angiogenesis, and fibrosis were observed in daily infusion control animals. Animals treated with AdAngiostatin demonstrated an improvement in net ultrafiltration (-3.1 versus -7.8 ml for control animals; P = 0.0004) with a significant reduction in vessel density. Ad-Decorin-treated animals showed a reduction in mesenteric collagen content (1.8 versus 2.9 μg/mg; P = 0.04); however, AdDecorin treatment had no effect on net ultrafiltration. In a rodent model of peritoneal membrane failure, net ultrafiltration was significantly improved and peritoneal-associated blood vessels were significantly reduced by using adenovirus-mediated gene transfer of angiostatin. Decorin, a transforming growth factor–β–inhibiting proteoglycan, reduced collagen content but did not affect net ultrafiltration. Improvement in the function of the peritoneum as a dialysis membrane after treatment with angiostatin has implications for treatment of peritoneal membrane dysfunction seen in patients on long-term dialysis.

During long-term peritoneal dialysis, the peritoneum undergoes histologic changes that include increased submesothelial collagen deposition, increased vascularization with vasculopathy, and loss of mesothelial cells (1,2). These changes suggest that both fibrogenic and angiogenic processes are active. Peritoneal concentration of vascular endothelial growth factor (VEGF) (3) and transforming growth factor–β (TGF-β) (4), key angiogenic and fibrogenic growth factors, have also been shown to correlate with peritoneal membrane function. These features strongly suggest that fibrogenic and angiogenic processes in the peritoneum are responsible for the alteration in peritoneal membrane function that is seen in patients on long-term peritoneal dialysis.

In these experiments, we used a short-term daily infusion model of peritoneal dialysis in rats. This model is associated with increased expression of VEGF and TGF-β, thickening of the submesothelial zone of the parietal peritoneum, increased angiogenesis, ultrafiltration failure, and increased transport of solutes (5). Similar models have previously been used to demonstrate the effect of peritoneal membrane rest (6) and systemic angiotensin-converting enzyme inhibition (7) on peritoneal membrane function.

Adenovirus-mediated gene transfer has been shown to be an effective tool for studying changes induced by transient overexpression of cytokines or growth factors on the peritoneal membrane. We have demonstrated that the adenovirus vector, when administered to the peritoneum, is highly infective for mesothelial cells. The transferred gene product is produced in high quantities and is expressed from approximately day 4 to day 14 after infection. We have previously demonstrated that adenovirus-mediated gene transfer of the active form of TGF-β1 leads to fibrosis of the peritoneum with increased VEGF expression and vasculogenesis (8). This led to an associated change in function, including increased transport of glucose and decreased net ultrafiltration.

The antiangiogenic and antifibrotic approaches we used in the following experiments have been previously described. Angiostatin is a potent inhibitor of endothelial cell proliferation that was first described for its ability to inhibit cancer growth by inhibiting tumor neovascularization (9). The adenovirus carrying the gene product for angiostatin (AdAngiostatin) has been effective in several studies, including a direct tumor injection model (10).
Decorin is a proteoglycan that binds and inactivates TGF-β. Decorin has been shown to be effective in direct application to prevent lung fibrosis (11) and as systemic gene therapy to modify renal fibrosis (12). We have shown adenovirus-mediated gene transfer of decorin (AdDecorin) to be an effective treatment in preventing pulmonary fibrosis in a murine bleomycin model (13).

In these experiments, we introduced AdAngiostatin, AdDecorin, or a control adenovirus (AdDL70) into a 4-wk, daily dialysate exposure model in rats. We demonstrate that animals treated with AdAngiostatin showed reduction in the parietal peritoneal vasculature, decreased glucose transport, and improved ultrafiltration. Animals treated with AdDecorin did not demonstrate alteration in peritoneal vascular ultrafiltration, but they did show reduced peritoneal fibrosis.

Materials and Methods

Animals

All animal studies were carried out according to the Canadian Council on Animal Care Guidelines. Sprague-Dawley rats, 200 to 250 mg (Harlan, Indianapolis, IN) were anesthetized with ketamine/xylazine. A 7 French silicone catheter (Access Systems, Newark, NJ) was inserted into the peritoneum through a small incision in the abdominal wall. The catheter was tunneled subcutaneously to an implanted port (Access Systems) on the back.

Animals were administered adenovirus (2 × 10⁹ plaque-forming units [pfu] per animal) 5 d after catheter insertion with a repeat administration on day 14. Both administrations were given intraperitoneally via the dialysis catheter. Daily infusion of dialysate (4.25% Dianeal; Baxter Healthcare, McGaw Park, IL) was initiated 7 d after catheter insertion, beginning at 15 ml but quickly increasing to 25 ml daily. Animals also received intraperitoneal lipopolysaccharide (LPS–75 μg; Sigma, Oakville, ON) on days 8, 10, and 12. Animals were treated with rotating antibiotics consisting of intraperitoneal cefazolin, intraperitoneal ciprofloxacin, and oral sulfamethoxazole-trimethoprim.

Four groups of animals were studied, and all were killed after 5 wk. The first was a positive control group (n = 11) that received control virus (AdDL70). The second group (n = 10) received AdAngiostatin, and the third group (n = 7) received AdDecorin. For negative control, we used a group of animals (n = 5) with no catheter inserted and administered AdDL70 at initiation. At week 3 in all animals, a peritoneal fluid sample was taken 4 h after infusion of 20 ml of 2.5% Dianeal. At week 5, 20 ml of 2.5% Dianeal was infused, and 4 h later, the animal was killed, an accurate ultrafiltration volume was measured, and blood and tissue samples taken. In a subset of AdAngiostatin-treated animals, we took a peritoneal fluid sample 4 d after first administration of adenovirus to demonstrate effective gene transfer.

Whole blood was centrifuged at 5000 rpm for 10 min and the serum removed. Peritoneal fluid samples were centrifuged at 1500 rpm for 5 min. Samples were analyzed on a Hitachi 917 automated chemistry analyzer (Roche Diagnostics, Laval, Canada) for creatinine, glucose, and albumin. Mass transfer of glucose out of the peritoneum was calculated as (initial dialysate glucose – initial volume)/(final dialysate glucose × final volume). Creatinine and albumin clearances were calculated as mass transfer divided by the serum solute concentration. All values were corrected for animal weight at death.

To demonstrate the safety of adenovirus-mediated gene transfer of angiostatin, we studied 8 rats without peritoneal catheters. These animals were treated with 1 (n = 4) or 2 (n = 4) doses of AdAngiostatin (2 × 10⁹ pfu intraperitoneally). Groups of 2 animals were sacrificed 4 and 7 d after treatment, and tissue samples were taken, fixed in neutral buffered formalin, embedded in paraffin, sectioned, and stained for hematoxylin/eosin and Masson trichrome. These sections were examined for pathologic changes in the peritoneum, liver, spleen, kidney, bowel, and ovaries.

Adenovirus

The cDNA for murine angiostatin was constructed from the four kringle regions of plasminogen ligated to the endogenous signal sequence as described previously (14). The construction of the adenovirus for human decorin (15) and control virus (AdDL70) (16) have been previously described. Adenovirus preparations were purified by CsCl gradient centrifugation and PD-10 Sephadex chromatography (Amersham Pharmacia, Baie d’Urfe, Quebec), and plaque was sized on 293 cells as described previously (17).

Histology

In daily dialysis-treated animals, tissue samples were taken at sacrifice from the lower anterior abdominal wall distant from the site of catheter placement. Sections from both sides of the midline were taken and fixed in a sufficient amount of 4% phosphate-buffered formaldehyde for 24 h. The tissue samples were then paraffin processed, embedded and 5 μm sections cut. Cut sections were then stained for Masson trichrome, and immunohistochemistry was carried out with antibodies to von Willebrand factor–factor VIII–related antigen (Dako Corporation, Carpentry CA). Negative control sections were run in parallel. Sections were deparaffinized in xylene followed by 100% ethanol and then placed in a methanol H₂O₂ solution for 30 min to block endogenous peroxidase activity. After hydration to water with graded alcohols, the sections were placed in 0.05 M Tris-buffered saline (TBS), pH 7.6, digested with 0.05% Pronase (Sigma) in TBS with calcium chloride for 17 min at room temperature then blocked in 5% normal goat serum (NGS) followed by a 1 h incubation in the 1:500 rabbit anti-human factor VIII in 1% NGS. Sections were then incubated in a prediluted kit of a biotinylated goat anti-rabbit followed by a streptavidin/peroxidase conjugate (Zymed Labs, San Francisco, CA) as per manufacturer’s instructions. Incubations were carried out at room temperature, and sections were washed in between incubations 3 × 5 min with 0.05 M TBS, pH 7.6, except before the addition of the primary antibody. All sections were rinsed in 0.05 M acetate buffer, pH 5.0, before development in an aminoethylcarbazole (AEC) chromogen substrate for 15 min. All sections were counterstained in Mayer’s hematoxylin for 2 min before mounting with glycerin gelatin.

We stained for apoptosis with in situ labeling for free 3’ hydroxy-DNA using terminal deoxynucleotidyl transferase (Apotag, Intergen, Purchase NY) according to the manufacturer’s direction. Briefly, sections were deparaffinized in xylene, treated with proteinase K (20 μg/ml) for 15 min, quenched with 3.0% hydrogen peroxide, and then exposed for 1 h to terminal deoxynucleotidyl transferase. Sections were further labeled with antidigoxigenin peroxidase conjugate, washed, and then developed with peroxidase substrate. Sections were counterstained with methyl green.

Blood Vessel Analysis

Sections of the anterior abdominal wall, immunostained for factor VIII, were studied in blinded fashion using two methods. First, we used a standardized microscope grid to count peritoneal-associated blood vessels and to measure the thickness of the submesothelial zone. Slides contained four separate transverse tissue sections taken from
the lower anterior abdominal wall, and each slide was examined at 12 random high-power views. All measurements were carried out at the same magnification. Second, we took the same sections and digitized 12 fields of view containing peritoneal tissues using a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany). We then analyzed these images using Leica Qwin Image Processing Software (Leica Imaging Systems, Cambridge, England) with subroutines we created. In both methods, we limited the analysis to the submesothelial collagogenous zone. Results for both analyses were reported as number of vessels/mm² of peritoneal tissue. We then multiplied these values by the thickness of the submesothelial zone to arrive at an estimate of the total vasculature measured as vessels/mm. Finally, our image-processing subroutine was able to estimate the total vessel cross-section area in each digitized image, and we could therefore calculate an average cross-sectional area per vessel for each slide analyzed.

We compared these two methods of evaluating blood vessels in histologic sections using regression analysis. This showed a very close correlation between the two methods ($r = 0.87; P < 0.0001$).

Hydroxyproline Assay

A portion of mesentery was taken and frozen for a hydroxyproline assay, modified from Woessner method (18). Tissues were weighed, homogenized in water, and centrifuged at 1000 rpm for 5 min, and the superficial fatty material was removed by vacuum suction. Solid material was precipitated with TCA with centrifugation at 1500 rpm for 15 min at 4°C. Samples were hydrolyzed overnight in 6 N HCl at 110°C. Hydroxyproline content is quantified by Ehrlich’s reagent (Sigma) and assayed by measuring the optical density at 557 nm. A hydroxyproline standard sample (Sigma) was used to create a standard curve.

Western Blot Analysis

Peritoneal dialysis fluid taken at week 3 was analyzed for angiostatin and decorin. Equal volume of fluid was separated on a 15% sodium dodecyl sulfate–polyacrylamide gel under nonreducing conditions and transferred to Immobulon-P membranes (Millipore, Mississauga, ON). For angiostatin, the membrane was probed with a 1:500 dilution chicken anti-rabbit-plasminogen IgY (14) (generous gift of Dr. Mark Hatton, McMaster University, Hamilton, ON) followed by a secondary rabbit anti-chicken alkaline phosphatase–conjugated antibody (Zymed). Decorin was detected by using a 1:2000 dilution of anti-decorin rabbit antibody (generous gift from Dr. Larry Fisher, National Institutes of Health, Bethesda, MD), followed by a secondary anti-rabbit alkaline phosphatase–conjugated antibody (Sigma). The probed membranes were developed by using NDT/BCIP (Promega, Madison, WI) according to the manufacturer’s instructions. Standard molecular weight markers (Life Technologies, Burlington, ON) were used.

Statistical Analyses

Data is presented ± SD unless otherwise noted. Comparison between groups was made by $t$ test. Regression analysis was used to look for correlation between blood vessel number/mm, net ultrafiltration, and solute transport.

Results

Adenoviral Expression and Safety of Repeated Dose of AdAngiostatin

We have previously demonstrated that adenovirus is efficiently taken up and expressed by mesothelial cells in the peritoneum after intraperitoneal delivery (8). We have also previously shown that the control virus, AdDL70, had little effect on the peritoneum after a mild initial inflammation lasting approximately 48 h (19). In the experiments reported here, we investigated the adenoviral gene product expression by Western blot analysis of peritoneal dialysis fluid. We showed expression of angiostatin after both the first and second administration of adenovirus (Figure 1A). Likewise, Ad-Dcorin-treated animals showed increased presence of decorin in the peritoneal dialysate (Figure 1B), suggesting effective infection and adenoviral transgene expression in this model. We have previously shown the in vitro efficacy of both AdAngiostatin (14) and AdDecorin (13).

We used $2 \times 10^9$ pfu of intraperitoneally delivered adenovirus, as we have shown high levels of transgene product at this dose with little persisting adenovirus effect (8). In this model, we used two doses of adenovirus vector delivered 9 d apart. There is an immunologic response to the adenovirus that makes second administration less effective but still demonstrable (20). Others have demonstrated effective repeated intraperitoneal administration of adenovirus in a tumor model (21). In our model, we studied animals administered one or two doses of

![Figure 1.](image-url)
AdAngiostatin with no daily dialysis. Histologically, we could not detect any effects of angiostatin on kidneys, spleen, liver, or ovaries. After the second administration of angiostatin, there was a mild peritoneal response with occasional eosinophils and neutrophils present in the submesothelial tissue. The mesothelial cells appeared rounded up and activated (data not shown). The effect of this peritoneal inflammatory response to adenovirus in the setting of our chronic, inflammatory model is uncertain.

**Changes in Structure of the Peritoneum**

We compared the histology of control animals (AdDL70, no catheter) with animals treated with 4 wk of daily dialysis, initial LPS, and either AdDL70, AdAngiostatin, or AdDecorin. Daily dialysate infusion and LPS exposure significantly altered the structure of the parietal peritoneum. As we have previously demonstrated (5), daily dialysate infusion leads to thickening of the submesothelial collagenous zone with increased total number and density of blood vessels (Figure 2; Table 1). Hydroxyproline content of mesenteric tissue is also significantly increased in animals treated with daily dialysate.

Administration of AdDecorin had significant impact on fibrogenesis in this model. There was a significant decrease in the mesenteric hydroxyproline concentration (1.8 versus 2.8 \( \mu g/mg; P = 0.04 \)) (Figure 2; Table 1) compared with AdDL70, daily infusion animals. AdAngiostatin had an impact on the vascularity of the peritoneum with a significant decrease in the number (\( P = 0.03 \)) and density (\( P = 0.02 \)) of peritoneal vessels (Table 1). We also saw a significant decrease (\( P = 0.03 \)) in the average cross-sectional area of blood vessels after AdAngiostatin treatment. The submesothelial thickness was not significantly reduced, but the hydroxyproline concentration of the mesentery was unchanged after AdAngiostatin treatment (Table 1).

Histologic sections of AdAngiostatin-treated animals showed areas in which blood vessels appeared to have been lost from the parietal peritoneum, and residual cells demonstrated small, condensed nuclei, suggesting apoptosis or necrosis (Figure 3). We further carried out staining for fragmented DNA using terminal deoxynucleotidyl transferase labeling and could identify an number of cells undergoing apoptosis, including endothelial cells, in sections from animals treated with AdAngiostatin. Sections from animals treated with AdDL70 did not show significant apoptosis in the submesothelial tissue (Figure 3).

**Changes in Peritoneal Function**

Daily dialysate also had a significant effect on the function of the peritoneum as a dialysis membrane (Table 2). Specifically, we saw a significant impairment in ultrafiltration with associated increased mass transport of glucose out of the peritoneum. There was an increase in albumin clearance along with a decrease in clearance of creatinine. When we grouped all animals studied, we identified a significant inverse correlation between the number of vessels seen in the submesothelial tissue and net ultrafiltration (\( r = -0.61; P = 0.0002 \); Figure 4). We also saw a positive correlation between the number of

![Figure 2: Histology of the anterior abdominal wall. (A) Control animal treated with AdDL70, no catheter, and no daily dialysate. Section shows normal histology with thin submesothelial zone above the abdominal wall muscles stained in red. (B) Animal treated with AdDL70, catheter, and daily dialysate. Histology is remarkably altered with thick, dense submesothelial zone and increased vascularization. (C) Animal treated with AdDecorin and daily dialysate. Section shows increased submesothelial thickening but somewhat reduced collagen deposition but similar vascularization to daily dialysate control animals. Masson trichrome stain. Magnification, ×50.]
Structural changes in the peritoneum after treatment with daily dialysate and null, angiostatin, or decorin adenovirus-mediated gene transfer compared with control (no catheter) animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AdDL70, No Catheter (n = 5)</th>
<th>AdDL70 + Catheter (n = 11)</th>
<th>AdAngiostatin (n = 10)</th>
<th>AdDecorin (n = 7)</th>
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<tr>
<td>Thickness (µm)</td>
<td>30.8 ± 13.5</td>
<td>193 ± 134b</td>
<td>122 ± 38b</td>
<td>200 ± 88b</td>
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<tr>
<td>Vessels/mm</td>
<td>0.4 ± 0.3</td>
<td>12 ± 9b</td>
<td>5 ± 4b,c</td>
<td>11 ± 7b</td>
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<tr>
<td>Vessels/mm²</td>
<td>12 ± 10</td>
<td>58 ± 21b</td>
<td>36 ± 19c</td>
<td>43 ± 16</td>
</tr>
<tr>
<td>Area/vessel (µm²)</td>
<td>ND</td>
<td>277 ± 96</td>
<td>180 ± 86c</td>
<td>285 ± 128</td>
</tr>
<tr>
<td>Hydroxyproline (µg/mg)</td>
<td>1.4 ± 0.3</td>
<td>2.8 ± 1.0b</td>
<td>3.2 ± 1.9</td>
<td>1.8 ± 0.9c</td>
</tr>
</tbody>
</table>

a ND, no data.
b P < 0.05 compared with AdDL70, no catheter control.
c P < 0.05 compared with AdDL70 + catheter control.

submesothelial vessels and the glucose transport (r = 0.55; P = 0.0023) but no association with creatinine clearance (P = 0.3) or albumin clearance (P = 0.7).

AdAngiostatin treatment appeared to have a significant positive impact on peritoneal function. There was an improvement in the ultrafiltration dysfunction (P = 0.0004) with a decrease in glucose transport (P = 0.003). The creatinine and albumin clearances were unchanged compared with the daily infusion control animals (Table 2). AdAngiostatin treatment did not completely reverse the ultrafiltration dysfunction, and the ultrafiltration in these animals was still significantly reduced compared with no catheter control animals. AdDecorin treatment had little impact on peritoneal function (Table 2). Ultrafiltration dysfunction and glucose transport were unchanged. Albumin clearance was slightly increased, but this change was not statistically significant.

Discussion

These experiments have allowed us to investigate antifibrotic and antiangiogenic intervention in an acute fibroproliferative model of peritoneal dialysis. This model employed a daily infusion of high-concentration glucose dialysate over a 4-wk period with an initial exposure to LPS and resulted in changes in the peritoneum similar to those seen in patients on long-term peritoneal dialysis. The submesothelium becomes thickened, and there is increased collagen deposition with angiogenesis. We observed associated changes in peritoneal function, including ultrafiltration dysfunction and increased glucose transport. The processes involved in peritoneal changes in patients on chronic dialysis are obviously slower and more progressive. Certain features, such as loss of mesothelium and vasculopathy seen in patients on long-term dialysis, are not seen in our model. The extent to which this daily infusion model mimics what may happen in chronic peritoneal dialysis patients is yet to be determined.

Angiogenesis is an important element of fibrosing diseases (22). We have previously shown AdAngiostatin to effectively reduce angiogenesis in a matrigel model (14) and in a direct tumor injection model (10) and to reduce lung metastases in a murine breast cancer model (14). In this work, we demonstrated a reduction in peritoneal-associated vessels with an improvement in ultrafiltration dysfunction and decreased glucose absorption after administration of AdAngiostatin. Interestingly, we observed a reduction in submesothelial thickening that was not statistically significant, but no change in hydroxyproline concentration of the mesenteric tissue. These experiments were not designed to measure the effect of antiangiogenic therapy on fibrosis. Previous work has identified an inhibition of lung fibrosis by blocking proangiogenic CXC chemokines (23) or peritoneal adhesions by directly blocking angiogenesis (24). In both these experiments, the total amount of fibrotic tissue was assessed, whereas we measured the concentration of tissue collagen, which was not effected by angiostatin. The nonsignificant reduction of submesothelial thickness may be an indication of reduced total fibrotic tissue and therefore in agreement with previous work noted above. A larger sample size would be necessary to prove this hypothesis in our model.

The importance of angiogenesis in the alteration in peritoneal membrane function in patients on long-term dialysis has become increasingly clear (25). The interstitium of the peritoneum may have a role as a barrier to water reabsorption and larger molecule transport (26), but the capillary wall and the total vascular surface area likely have the greatest impact on transport of small solutes and ultrafiltration. In agreement with this hypothesis, our experiments demonstrated that inhibition of angiogenesis improved ultrafiltration and reduced small solute transport, but inhibition of fibrosis, through treatment with AdDecorin, did not alter peritoneal function. Also, in all animals treated with daily dialysis, we saw a strong inverse correlation between number of blood vessels and net ultrafiltration.

Decorin is a member of the small leucine-rich proteoglycan family (27). Previous work has demonstrated that decorin can alleviate organ fibrosis in several animal models (12,13). This is in agreement with the present experiments, where we show that treatment with AdDecorin in our model significantly reduced hydroxyproline concentration of mesenteric tissue, but did not significantly impact membrane-associated vessels or peritoneal function. We have previously demonstrated that the intraperitoneal delivery of AdTGF-β1 to the peritoneum increased VEGF expression and induced peritoneal angiogenesis.
Figure 3. Histology of the anterior abdominal wall. (A) Section from animal treated with AdDL70 and daily dialysate shows submesothelial thickening and collagen deposition with increased vascularization shown by factor VIII-stained section in panel B. (C) Section from animal treated with AdAngiostatin and daily dialysate shows increased thickness and hypercellularity but a significant loss of vascularity, especially noticeable in factor VIII-stained section in panel D. (E) Staining for DNA fragmentation in animals treated with AdAngiostatin shows apoptotic cells, including endothelial cells (arrows) with corresponding factor VIII-stained section shown in panel F. (G) DNA fragmentation in animals treated with AdDL70 and daily dialysis shows virtually no apoptotic cells. Panels A and C are Masson trichrome; panels B, D, F are factor VIII; panels E and G are labeled with terminal deoxynucleotidyl transferase. Magnifications: ×100 in A through D; ×200 in E through G.
Figure 4. Inverse correlation between peritoneal-associated blood vessels counted on factor VIII-stained sections and net ultrafiltration measured after 4 wk of daily dialysate infusion. $r = -0.61; P < 0.001$.

There are likely other mechanisms in our model that lead to VEGF expression and angiogenesis, such as indirect induction through inflammatory cytokines, or direct induction through exposure to components of dialysate. TGF-$\beta_1$ may have a relatively minor role to play in the induction of angiogenesis in our model. This suggests that fibrosis does not have a significant impact on peritoneal dysfunction and again indicates the importance of the vasculature as a cause of dysfunction in peritoneal dialysis. We did see a nonsignificant increase in albumin transport in animals treated with AdDecorin. It is possible that, through alteration in the composition of the interstitium of the peritoneum that large molecule transport was affected. Further work is required to explore this possibility.

We were unable to completely reverse the effects of peritoneal dysfunction with AdAngiostatin in this model. Clearly, even high and repeated doses of angiostatin were not sufficient to block a substantial component of angiogenesis. This suggests either that the angiogenic stimuli are very strong or that other factors are present which inhibit the ability of angiostatin to induce widespread endothelial apoptosis.

We believe that the results from these studies have direct therapeutic implications for patients on peritoneal dialysis. Our findings support the hypothesis that new blood vessel formation is a key component of peritoneal membrane dysfunction. Therapeutic strategies that reduce the peritoneal vasculature should be developed. We noted that creatinine clearance, although decreased in our model compared with untreated animals, was not changed after treatment with AdAngiostatin compared with AdDL70-positive control animals. This suggests that antiangiogenic therapy may not decrease the amount of dialysis provided by the peritoneum. The decreased vascular surface area and small solute transport were balanced by increased dialysate volume; therefore, creatinine clearance was preserved.

Previous work has demonstrated that anti-VEGF antibodies can prevent angiogenesis and peritoneal dysfunction in a hyperglycemic animal model (28). Other strategies targeting vascular growth factors and angiogenesis have been developed for the treatment of cancer and may be applicable to the problem of increased solute transport and ultrafiltration failure in the peritoneum (29). The most effective treatment will likely be prevention. Angiogenesis is likely driven by uremia, inflammation, and nonphysiologic dialysate. Optimizing dialysis treatment to avoid these elements may prevent peritoneal angiogenesis and best protect the peritoneum as a long-term dialysis membrane.

Acknowledgments

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References


Table 2. Functional properties of the peritoneal membrane in control animals (no catheter) and animals treated with daily dialysate and adenovirus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AdDL70, No Catheter (n = 5)</th>
<th>AdDL70 + Catheter (n = 11)</th>
<th>AdAngiostatin (n = 10)</th>
<th>AdDecorin (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net ultrafiltration (ml)</td>
<td>4.3 ± 1.8</td>
<td>-8.9 ± 3.3$^a$</td>
<td>-3.2 ± 2.7$^{ab}$</td>
<td>-9.0 ± 3.5$^a$</td>
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<td>Glucose transport (mmol/kg)</td>
<td>6.8 ± 0.4</td>
<td>8.0 ± 0.6$^a$</td>
<td>7.1 ± 0.4$^c$</td>
<td>7.7 ± 0.2$^a$</td>
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<tr>
<td>Creatinine clearance (L/g)</td>
<td>49.3 ± 6.8</td>
<td>22 ± 10$^a$</td>
<td>24 ± 8$^a$</td>
<td>22 ± 8$^a$</td>
</tr>
<tr>
<td>Albumin clearance (L/g)</td>
<td>1 ± 1.3</td>
<td>2.9 ± 2.0$^a$</td>
<td>4.3 ± 2$^a$</td>
<td>4.9 ± 2.3$^a$</td>
</tr>
</tbody>
</table>

$^a$ P < 0.01 compared with AdDL70, no catheter control.

$^b$ P = 0.0004 compared with AdDL70 + catheter control.

$^c$ P = 0.0025 compared with AdDL70 + catheter control.


