Peritoneal morphological and functional changes associated with platelet-derived growth factor B

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Abstract

Background. Morphological changes associated with long-term peritoneal dialysis (PD) include increased vascular surface area due to angiogenesis, submesothelial fibrosis and epithelial mesenchymal transition. Platelet-derived growth factor (PDGF) has been associated with all of these phenomena, and is a prototypical ‘response to injury’ growth factor.

Methods. Rats received an intraperitoneal injection of adenoviral vector expressing PDGF-B. At sacrifice, we analysed the structure and function of the peritoneal membrane. Gene expression in the peritoneal tissue was assessed for changes suggestive of epithelial mesenchymal transition.

Results. Over-expression of PDGF in the rat peritoneum led to significant angiogenesis, cellular proliferation and submesothelial thickening. Although PDGF induced expression of transforming growth factor β, there was a lack of activation of this growth factor, and we believe that this explains the lack of significant collagen accumulation observed by a hydroxyproline assay. Despite evidence of angiogenesis and subsequent increased solute transport, we observed only a transient, non-significant impact on ultrafiltration function. This suggests that increased vascular surface area is necessary, but not sufficient, to produce ultrafiltration dysfunction. There was no evidence of epithelial mesenchymal transition observed either in regulation of associated genes such as Snail or E-Cadherin or in the lack of dual-labelled epithelial and mesenchymal cells on immunofluorescence. Mesothelial cells exposed to PDGF-B demonstrated increased collagen gene expression.

Conclusions. PDGF-B induced angiogenesis without fibrosis in the peritoneum. The lack of significant ultrafiltration dysfunction and epithelial mesenchymal transition, as observed in patients on PD, suggests that PDGF-B may play a role, but is not the integral component, in response to peritoneal injury.

Keywords: adenovirus; angiogenesis; fibrosis; peritoneal dialysis; platelet-derived growth factor

Introduction

Peritoneal dialysis (PD) is an attractive therapeutic option for patients with end-stage renal disease. During long-term PD treatment, the peritoneal membrane undergoes structural and functional alterations [1] as a consequence of uraemia, inflammation, infection and exposure to bioincompatible dialysis solution components. The most common functional alteration during long-term PD is an increased peritoneal small-solute transport rate, resulting in impaired ultrafiltration efficiency [2]. The structural alterations correlating with ultrafiltration dysfunction are unknown, but are presumed to include fibrosis and angiogenesis in the peritoneal tissue. Increased vascularization of peritoneal tissue causes an increased small-solute transport and ultimately leads to ultrafiltration dysfunction [3]. Several studies, however, have suggested that an increase in vascularization is necessary, but not sufficient for ultrafiltration dysfunction [2,4].

Alterations in the submesothelial layer have been hypothesized to result in a progressive increase in solute transport and ultrafiltration dysfunction [5]. Long-term exposure to dialysis solutions results in epithelial-to-mesenchymal transition (EMT) of mesothelial cells [6], generation of submesothelial myofibroblasts and increased extracellular matrix (ECM) deposition in the submesothelial compact zone. This fibrotic layer may impact ultrafiltration through alteration of the hydrodynamic properties of the interstitium. The combined changes of fibrosis and angiogenesis result in increased solute transport with a loss of the osmotic gradient and these changes, combined with a decrease in the osmotic conductance of the peritoneal tissue, may lead to ultrafiltration dysfunction [7].

There is limited evidence regarding the involvement of platelet-derived growth factor (PDGF) in peritoneal membrane dysfunction. PDGF is a pleotropic cytokine and
exists in three active isoforms resulting from the homologous or heterologous dimeric combination of A and B chains. PDGF signals through two tyrosine kinase receptors: PDGF receptor-α (PDGF-Rα) and β (PDGF-Rβ) [8]. PDGF dimers bind to specific PDGF receptor subunits; PDGF-A binds to PDGF-Rα whereas PDGF-B binds to PDGF-Rβ with high affinity and can bind the other receptor subtypes [9].

In fibrogenesis, PDGF-B is generally regarded as a potent inducer of chemotaxis and is mitogenic for myofibroblasts, recruiting these cells to the site of tissue injury [10]. PDGF-B has been shown to be involved in fibrosis of lung, liver and kidney [11,12]. However, the role of PDGF-B in fibrosis is complex. For example, in a study by Isaka and colleagues, introduction of both transforming growth factor β1 (TGFβ1) and PDGF into the kidney induced glomerulosclerosis. However, TGFβ1 caused ECM accumulation whereas PDGF-B induced mesenchymal cell proliferation [13]. PDGF-B has also been shown to increase TGF-β1 mRNA expression in immortalized human proximal tubular epithelial cells [14].

Cell motility plays a critical role in many physiological and pathological processes such as wound healing, fibrosis and angiogenesis. PDGF-B is among the most potent stimuli for mesenchymal cell (fibroblast and vascular smooth muscle cell) migration [15]. PDGF-B knock-out mice develop a phenotypic defect involving a lack of vascular smooth muscle cell migration to the vasculature [16]. In addition to TGF-β, which is a potent stimulator of EMT [17], PDGF-B is also a major factor influencing transformation from the quiescent to activated phenotype of hepatic stellate cell, a process involved in pathogenesis of liver fibrosis [11]. However, direct evidence of involvement of PDGF-B in the EMT process is lacking.

Work by Beavis and colleagues suggests that PDGF is upregulated in peritoneal mesothelial cells exposed to human PD effluent [18]. Lai and colleagues measured PDGF in a group of stable PD patients and found that, unlike TGFβ, PDGF did not correlate with solute transport [19]. Furthermore, effluent TGFβ1 concentration did not correlate with the PDGF concentration.

Two further isomers of PDGF—PDGF-C and-D—have been identified. These isomers bind to the PDGF receptors. PDGF-C has been identified as having a role in renal and pulmonary fibrosis [20].

In the present study, we establish the role of PDGF-B in peritoneal membrane dysfunction by using adenovirus-mediated gene transfer of PDGF-B to the rat peritoneum. We show that PDGF-B induces sustained angiogenesis, cellular proliferation and submesothelial thickening. Despite significant angiogenesis and increased solute transport, ultrafiltration was minimally impacted. Over-expression of PDGF-B did not induce significant peritoneal fibrosis as measured by a hydroxyproline assay. There was also no evidence of EMT as observed by immunofluorescence and gene expression. It has been suggested that PDGF-B may play a role in peritoneal membrane dysfunction and could be a potential therapeutic target [21]. However, our study suggests that PDGF-B over-expression does not recreate aspects of peritoneal injury as seen in PD patients, including fibrosis, EMT and ultrafiltration dysfunction. Therefore, we suggest that PDGF-B is not an essential growth factor in peritoneal membrane injury.

Methods

Animal model

All animal studies were performed according to the Canadian Council on Animal Care guidelines (38). Sprague-Dawley rats (225–275 g; Harlan, Indianapolis, IN, USA) were given intraperitoneal injections of AdPDGF-B or AdDL70 (control). The animals received $1 \times 10^9$ plaque-forming units of adenovirus, diluted in 100 µl of PBS on Day 0. Five animals were included in the AdPDGF-B-treated group at each time point. As our previous observations suggest that AdDL70 had minimal impact on peritoneal structures, we included three animals in each group at each time point for control. Animals were euthanized on Days 4, 7, 14 and 28. Before euthanasia, a survival peritoneal equilibration test (PET) was performed. This test involved the intraperitoneal administration of 90 ml/kg body weight of 4.25% Dianeal PD solution (Baxter Healthcare, Round Lake, IL, USA). Four hours later, a blood sample was taken by cardiac puncture, and the total peritoneal fluid was recovered for accurate ultrafiltration measurement. Peritoneal effluent at time 0 and 4 h was assayed for glucose, and the 4-h effluent and serum were assayed for urea. Samples were analysed with a Hitachi 917 automated chemistry analyser (Roche Diagnostics, Laval, Canada). Solute transport was calculated by the effluent glucose at 4 h divided by the effluent glucose at time 0 ($D/D_0$ glucose) and by the 4-h effluent urea divided by the serum urea ($D/P$ urea).

Recombinant adenoviruses

In this experiment, we used AdPDGF-B and a null adenovirus, AdDL70 as control. PDGF-B sequence was isolated by PCR from mRNA extracted from rat lung tissue, cloned into a shuttle plasmid and co-transfected in 293 cells as has been previously described [22]. Purified AdPDGF-B or control adenovirus was infected in A549 cells and supernatants were taken and analysed by ELISA (R&D Systems, Minneapolis, MN, USA) to confirm transgene expression. Adenoviruses were purified by CsCl gradient centrifugation and PD-10 Sephadex chromatography (Amersham Pharmacia, Baie d’Urfe, PQ, Canada) and plaque titred on 293 cells.

Histology

Tissue samples were taken at sacrifice from the lower anterior abdominal wall and fixed in 4% phosphate-buffered formalin for 24 h. The samples were embedded and 5-µm sections were stained with Masson’s trichrome.

Immunohistochemistry was carried out with antibodies to von Willebrand Factor (vWF, Dako Corporation, Carpenteria, CA, USA) using 0.05% pronase (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) for antigen retrieval.
An antibody against Ki67 (Clone SP6, Medicorp, Montreal, QC, Canada) was used after boiling a 10 mM citrate buffer for antigen retrieval. For VEGF staining, an anti-VEGF antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) with proteinase K antigen retrieval was used.

Sections were also stained for α-smooth muscle actin (SMA) and cytokeratin using dual immunofluorescent labelling. Antigen retrieval was carried out by boiling a 10 mM citrate buffer. The primary antibody included a monoclonal mouse anti-human α-SMA antibody (Dako) with a Texas Red goat anti-mouse secondary antibody (Molecular Probes, Inc., OR, USA) followed by an FITC-labelled monoclonal mouse anti-cytokeratin (Sigma-Aldrich). Sections were mounted using Vectashield Dapi mounting media (Vector Laboratories Inc., Burlingame, CA, USA).

Quantitative histology

Sections of anterior abdominal wall, immunostained for vWF or Ki67, were studied in a blinded manner. The vWF antigen is highly expressed in endothelial cells, and we have used this staining previously as a robust vascular marker suitable for quantification [23]. Ki67 is a widely used marker for cellular proliferation [24]. Each slide containing parietal peritoneal tissue was examined at 12 random fields of view. The fields were digitized and analysed using Northern Eclipse software (Empix, Mississauga, ON, Canada). Results were reported as number of vessels per millimetre of cross section of the peritoneal tissue. We also evaluated the thickness of the submesothelial collagenous zone. The density of Ki67-positive cells in the submesothelial zone was also reported.

Cytokine analysis

Peritoneal fluid was analysed using an enzyme-linked immunosorbent assay (ELISA, R&D Systems) for rat PDGF, VEGF and total and active TGF-β1. To measure total TGF-β1 concentration, samples were first activated using 1 M HCl for 10 min and then normalized using 1 M NaOH to dissociate TGF-β1 from its latency-associated binding protein.

Quantitative PCR

Peritoneal mRNA was extracted from sacrificed animals by immersion of the parietal peritoneal surface in Trizol reagent (Invitrogen, Burlington, ON, Canada) for 20 min. RNA was extracted from Trizol according to the manufacturer’s instructions (Qiagen RNeasy Minikit, Mississauga, ON, Canada) and the concentration was determined by optical density (OD) using an ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, Canada). RNA (1 μg) was treated with DNase and reverse transcribed using a standard protocol and reagents (Invitrogen). Quantitative real-time PCR for E-cadherin, α-SMA, Collagen A2 (COL-1A2), Snail, 18s and GAPDH was performed using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and primers from Mobix (Hamilton, ON, USA) and probes from Applied Biosystems. Pooled mRNA from control animals was used to create a standard curve for comparative quantification. Negative controls included no-RNA or no-RT samples.

Hydroxyproline assay

A portion of mesentery was frozen and used for a hydroxyproline assay, modified from Woessner’s method [25]. Tissues were weighed, homogenized in water and centrifuged at 160×g for 5 min, and the superficial fatty material was removed by vacuum suction. Solid material was precipitated with trichloroacetic acid with centrifugation at 360×g for 15 min at 4°C. Samples were hydrolyzed overnight in 6 M HCl at 110°C. Hydroxyproline contents were quantified with Erlich’s reagent (Sigma-Aldrich) and were assayed by the measurement of the OD at 557 nm. Standard hydroxyproline samples (Sigma-Aldrich) were used to create a standard curve.

In vitro analysis

Mesothelial cells were isolated from rat peritoneal tissue and cultured as previously described [23]. The cells were plated in six-well plates and when 80% confluent, they were serum starved for 12 h. They were exposed to media alone or recombinant PDGF-B (R&D Systems) at a concentration of 100 ng/ml for 6, 24 or 48 h. The cells were washed and mRNA was extracted using Trizol reagent. Then mRNA was reverse transcribed and assessed using quantitative real-time PCR as outlined above. This experiment was repeated in triplicate.

In a separate experiment, cells were exposed to PD effluent from animals 7 days after exposure to AdDL70 or AdPDGF-B. For a positive control, recombinant TGFβ1 (R&D Systems) was used at a concentration of 2.5 ng/ml. PD effluent was diluted 10% in serum-free media. Cells were washed and protein was extracted using a standard lysis buffer with protease inhibitors. Protein was electrophoresed and transferred protein was identified with antibodies to phosphorylated Smad2 (pSmad2, Calbiochem, Mississauga, ON, Canada) or total Smad2 (Santa Cruz Biotechnology).

Statistical analyses

SPSS 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables are expressed as mean ± standard deviation (SD). Comparison between groups was made by the t-test or ANOVA as appropriate. ANOVA was carried out between the treated and control animals over the time course of the experiment. A P-value ≤ 0.05 was considered statistically significant.

Results

Intraperitoneal infection with AdPDGF-B induced obvious changes in the submesothelium of rats (Figure 1). We observed significant angiogenesis (P = 0.021, by ANOVA) and submesothelial thickening (P < 0.001, by ANOVA) measured by quantifying vWF-stained sections and cellular proliferation (P < 0.001, by ANOVA) from Ki67-stained...
sections (Figure 2). These effects were somewhat transient, with partial resolution in all these features observed by Day 28. Some of the proliferation is accounted for by the increase in vascular structures, along with an increase in interstitial myofibroblasts.

The function of the peritoneal membrane was assessed using a 4-h PET. We found a significant increase in small-solute transport, including urea and glucose (Figure 3). The temporal pattern of observed changes demonstrated an increased solute transport at Days 4 and 7 and resolution by Day 14. The increased vascularization (Figure 2) and small-solute transport (Figure 3A and B) did not correlate with ultrafiltration dysfunction (Figure 3C). At Day 4, there was a transient, non-significant impairment in ultrafiltration in both AdPDGF-B- and control adenovirus (AdDL70)-treated animals. This is suggestive of a transient effect due to viral-induced inflammation. The ultrafiltration function was fully normalized by Day 7, the same time point at which there was an increased vascularization of the peritoneal tissues and an increased solute transport.

Peritoneal effluent concentration of PDGF-B was elevated 4–14 days after infection with AdPDGF-B (Figure 4A, \( P = 0.006 \)). PDGF-B levels were elevated over 30-fold at Day 4, and 7-fold at Day 7, compared with AdDL70-treated animals. This increased PDGF-B production in the peritoneal cavity was associated with significant increases in peritoneal VEGF (Figure 4B, \( P = 0.029 \)) and total TGF\( \beta1 \) concentration (Figure 4C, \( P < 0.001 \)). Using immunohistochemistry (Figure 1E), strong VEGF expression was seen in interstitial fibroblasts and vascular structures. There was also increased staining observed in the mesothelial cell layer. In our previous work using AdTGF\( \beta1 \) [23], we...
observed fibrosis and angiogenesis in association with a significant expression of active TGFβ1.

In these experiments, despite high levels of total TGFβ1 induced in the peritoneum, we did not see a significant increase in active TGFβ1 in the peritoneal effluent (Figure 4D, P = 0.45). In order to confirm this lack of activation of TGFβ, peritoneal effluents were exposed to mesothelial cells in culture, and the levels of phosphorylated Smad2 were used as an indicator of TGFβ activity. Effluent from animals 7 days after treatment with AdPDGF-B did not induce phosphorylation of Smad2.

The thickened submesothelium was suggestive of a fibrogenic process, but we did not see a significant increase in collagen deposition as measured by the hydroxyproline assay of omental tissue (Figure 5, P = 0.8). In support of this observation, the gene expression of fibrosis-related genes measured in RNA extracted from the peritoneal surface of the parietal peritoneum did not demonstrate significantly...
Fig. 4. Peritoneal effluent growth factor concentration as measured by ELISA. Black bars—AdPDGF-B-treated animals, open bars—control AdDL70-treated animals. (A) PDGF-B concentration was significantly increased by AdPDGF-B treatment (*P = 0.006, ANOVA). (B) Peritoneal VEGF expression was increased after AdPDGF-B treatment (*P = 0.029 by ANOVA). (C) Total TGFβ1, was increased in AdPDGF-B-treated animals (*P < 0.001, ANOVA) but active TGFβ1 (D) concentration was not significantly different (P = 0.45 ANOVA). (E) Peritoneal effluent from PDGF-B-treated animals did not increase Smad2 phosphorylation confirming that active TGFβ was not present. Effluent from AdDL70-treated animal served as a negative control, and recombinant TGFβ1 was used as a positive control.

Fig. 5. Peritoneal fibrosis, as measured by hydroxyproline of omental tissue. Black bars—AdPDGF-B-treated animals, open bars—control AdDL70-treated animals. There was no statistically significant difference in collagen concentration between AdPDGF-B- and AdDL70-treated rats (P = 0.8 by ANOVA).

increased collagen 1A2 expression (Figure 6A, P = 0.105) although there was certainly a trend towards an induction of this gene by AdPDGF-B. PAI-1 (Figure 6B, P = 0.015) and TGFβ1 (Figure 6C, P = 0.002) were both significantly induced by PDGF-β over-expression.

In the setting of TGFβ1 over-expression in peritoneal tissue, we have observed evidence of an EMT process leading to the induction of submesothelial myofibroblasts. Because of the submesothelial thickening, proliferation and angiogenesis induced by the over-expression of PDGF-B, we sought evidence of EMT in this experiment. Interestingly, we did not observe gene expression changes characteristic of EMT: Snail, a zinc-finger regulatory protein, was not up-regulated (Figure 6D), and E-Cadherin, an epithelial adhesion marker, was not down-regulated (Figure 6E). There was a modest increase in the gene expression of α-SMA (Figure 6E) likely related to the increase in perivascular smooth muscle cells involved in the angiogenic response. The lack of significant EMT induced
Fig. 6. Peritoneal gene expression after AdPDGF-B infection. mRNA was extracted from the peritoneal surface of the anterior abdominal wall and analysed by quantitative PCR. Black bars—AdPDGF-B-treated animals, open bars—control AdDL70-treated animals. (A–C) Expression of fibrogenic-related genes collagen 1A2 (A), PAI-1 (B) and TGFβ1 (C). There is a trend towards an increase in the collagen gene expression ($P = 0.105$, ANOVA) with a significant increase in PAI-1 ($P = 0.015$, ANOVA) and TGFβ1 ($P = 0.002$, ANOVA). (D–F) Expressions of genes related to epithelial mesenchymal transition are not strongly regulated after exposure to PDGF-B. (D) α-SMA expression tends to increase ($P = 0.078$, ANOVA, $P = 0.045$ by t-test, Day 7) whereas Snail (E, $P = 0.44$, ANOVA) and E-Cadherin (F, $P = 0.44$) are not regulated.

by PDGF-B is also evidenced by our inability to identify dual-labelled (cytokeratin—epithelial marker and α-SMA—mesenchymal marker) cells in the peritoneal tissues (Figure 7). From previous work, these transitional cells were clearly evident after an over-expression of TGFβ1 [17].

We assessed the effects of recombinant PDGF-B on rat mesothelial cells in culture (Figure 8), and this generally supported our in vitro findings. Collagen gene expression was significantly up-regulated 6 h after exposure to PDGF-B and remained up-regulated till 48 h. TGFβ and VEGF gene expression was transiently increased at 6 h. Snail and E-cadherin were not significantly changed after exposure to PDGF-B; however, there was a non-significant increase in the E-Cadherin gene expression.

Discussion

PDGF-B is commonly associated with the wound-healing response, being mitogenic for myofibroblasts and involved in angiogenesis through the recruitment of perivascular cells [8]. Overall, it is not clear whether PDGF-B is directly
Fig. 7. Dual immunofluorescence staining of the peritoneal surface of the anterior abdominal wall for α-SMA (red) and pan-cytokeratin (green) with DAPI nuclear counterstaining (blue). (A) AdPDGF Day 4 (B) AdPDGF Day 7 (C) AdDL70 Day 4. All sections were taken at ×200 magnification.

PDGF and the peritoneum

responsible for the accumulation of ECM or acts indirectly through increased expression of TGFβ1. There are limited data suggesting PDGF-B is involved in peritoneal injury to the peritoneum [18,19]. The presumed fibrogenic and angiogenic properties of PDGF-B, along with preliminary in vitro data [18], suggest that PDGF-B may have an important role to play in peritoneal membrane injury in patients on long-term PD.

In earlier work, we over-expressed active TGFβ1 in the rodent peritoneum and identified an increase in peritoneal tissue vascularization and fibrosis with an associated increase in small-solute transport [23]. This led to an increase in glucose transport with a rapid loss of osmotic gradient and subsequent ultrafiltration failure. Rippe [4] and Davies [2] have argued on a theoretical and clinical basis, respectively, that increased vascularization and increased solute [26] transport is necessary, but not sufficient, for ultrafiltration dysfunction. The increased vascular surface area will not only lead to increased solute transport, but will also increase the surface area available for ultrafiltration that will partially offset the loss of osmotic gradient [4]. Along with vascularization, there needs to be a secondary impairment in hydraulic conductance of the peritoneal membrane. It is possible that this occurs through increased ECM deposition and fibrosis [7]. When we over-expressed TGFβ1 in the rat peritoneum, we induced both angiogenesis and fibrosis which led to a persistent ultrafiltration dysfunction [23]. In this present model, PDGF-B induced significant angiogenesis and increased solute transport without fibrosis. The ultrafiltration dysfunction we observed was transient, limited to the earliest time point, and may be related to an adenoviral effect as it is observed in both control adenovirus and AdPDGF-B groups. By Day 7, despite a persisting increase in small-solute transport, ultrafiltration was completely normalized.

This finding is somewhat contradictory to our previous work [26] where we used a chronic infusion model and tested adenovirus gene transfer of angiostatin or decorin. Angiostatin reduced the number of blood vessels in the peritoneal tissue and improved ultrafiltration. Decorin, a proteoglycan that binds and inhibits TGFβ, reduced ECM accumulation but did not affect ultrafiltration. TGFβ1 has multiple actions, and in this highly inflammatory model, we hypothesize that blocking TGFβ might have increased peritoneal inflammation and led to these contradictory results.

The observed submesothelial thickening and angiogenesis are likely supported by some ECM deposition and cellular proliferation. However, the hydroxyproline assay, which measures collagen concentration in tissue, showed no
overall increase in this respect. Contradicting this, we found that the gene expression of TGFβ1, and the total effluent TGFβ1 concentration, was significantly increased by AdPDGF-B treatment. Further analysis showed, however, that active TGFβ1 concentration in the peritoneal effluent was not elevated. We hypothesize that PDGF-B expression alone is not able to activate TGFβ1 from its latent state. Known activators of TGFβ1 include heat, low pH, thrombospondin-1, integrin αvβ6, or enzymes of the matrix metalloproteinase family and plasmin [27,28]. In our previous model, we used an adenovirus that expressed a mutated TGFβ1 with a single amino acid substitution that prevented binding of the expressed molecule to its latency-associated protein [29]. There are other possible explanations for the lack of ECM accumulation in these present experiments. For example, PDGF-B may induce a net collagenolytic environment through an increased expression of metalloproteinase and thus allow for increased ECM degradation.

We were surprised to find a lack of EMT in this model. EMT was readily apparent in our previous TGFβ1 over-expression model [17] and has been found in samples from patients undergoing PD [6]. There is evidence that suggests that PDGF alone is sufficient to induce fibrosis in the liver and lung, causing hepatic stellate cell proliferation with collagen synthesis and hyper-proliferation of normal lung fibroblasts [20,30]. However, there is no in vivo and little in vitro evidence that PDGF-B can independently induce EMT [31].

In summary, AdPDGF-B over-expression in the rat peritoneum led to significant functional and structural changes. Some of these changes, such as increased solute transport, associated with angiogenesis are reminiscent of changes seen in the peritoneum of patients on long-term PD [1]. An essential difference, however, was the lack of significant fibrotic response. This may be related to a lack of EMT or a lack of TGFβ1 activation. PDGF-B did lead to increased vascularization with increased solute transport—a common end-result of long-term PD. This suggests that PDGF-B may be an important cytokine involved in peritoneal membrane dysfunction of PD patients and deserves to be further explored in human studies.

This study used the isolated over-expression of a single growth factor. Although helpful in understanding the basic mechanisms involved, these results cannot be directly applied to patients on PD. In PD patients, many factors will be present both in the PD fluid and as a response to uremia and dialysis that can activate TGFβ. PDGF-B may have a different effect in the high-glucose environment in the peritoneum [14]. Finally, first-generation adenovirus gene transfer leads to a high-transient transgene expression. It is quite possible that lower, longer duration exposure to PDGF-B may yield very different results.

References

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