Tissue-type plasminogen activator deficiency attenuates peritoneal fibrosis in mice

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PERITONEAL DIALYSIS (PD) is an alternative therapy to hemodialysis for patients with end-stage renal disease. However, long-term PD therapy causes chronic injury to the peritoneum, which progressively undergoes marked submesothelial thickening and massive accumulation of collagen (13, 33). This structural alteration, or peritoneal fibrosis (PF), is known to be associated with vasculopathy and neangiogenesis, which is considered to be a principal cause of ultrafiltration loss, leading to discontinuation of PD therapy (6, 9, 10). Moreover, among a certain number of patients on long-term PD, peritoneal injury leads to a development of encapsulating peritoneal sclerosis (EPS), a serious complication associated with high mortality (7). To date, the mechanisms of PF and EPS are not fully understood, and no proven therapy has been established.

Tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are serine proteases that catalyze the conversion of plasminogen to plasmin, a broad-spectrum enzyme responsible for fibrinolysis, direct degradation of extracellular matrix, and activation of metalloproteinases (MMPs) (29, 32). Activation of the PA/plasmin/MMP system may prevent PF by limiting the accumulation of collagen and other extracellular matrices directly or indirectly via activated MMP. Yet the PA/plasmin/MMP system can also be profibrogenic by activating MMP and transforming growth factor-β1 (TGF-β1), a potent fibrogenic cytokine (11, 32, 39). The expression of tPA is reported to promote interstitial fibrosis of the kidney (36).

Activation of MMP-2 is also shown to induce renal fibrosis by promoting epithelial-to-mesenchymal transformation (EMT) (4). The pathological roles of the PA/plasmin/MMP system on the process of renal fibrosis are complicated (39), and even less is known about the process of fibrosis of the peritoneal membranes. In dialysate of PD patients, the levels of coagulation and fibrinolysis-related proteins, including PA, are known to be high (14). Moreover, the enhanced activation of MMP-2 was noted in the dialysate of patients with EPS as well as in rat PF models (16, 21), and a recent study shows that inhibition of MMP-2 prevents the development of PF in rats (27). The PA/plasmin/MMP system may play an important role in the pathogenesis of PF.

The present study was performed to examine the mechanisms of PF in view of the plasminogen activator (PA)/plasmin/matrix metalloproteinase (MMP) cascade. PF was induced in tissue-type PA (tPA) deficient mice and wild-type mice by intraperitoneal injection of chlorhexidine gluconate. Mice were killed on day 21, and tissue samples were taken. Histopathological studies were performed. Plasmin activity, gelatinases activity, and the levels of tPA, transforming growth factor-β1 (TGF-β1), and MMP-2 mRNA were determined. Protein levels of MMP-3, tissue inhibitor of metalloproteinases (TIMP)-1, -2, and -3, phospho-Smad3, membrane-type 1 (MT1)-MMP, and MT3-MMP were also studied. On day 21, tPA+/+ mice showed severe PF, whereas tPA−/− mice showed milder change. Submesothelial basement membranes were dissolved in tPA+/+ mice while they were relatively preserved in tPA−/− mice. The levels of macrophage infiltration, staining for α-smooth muscle actin (α-SMA) and collagen type III, and vascular density were all significantly lower in tPA−/− mice than in tPA+/+ mice. The levels of plasmin activity, pro- and active-MMP-2, mRNA expression of tPA and TGF-β1, and phospho-Smad3 protein were also lower in tPA−/− mice. No difference was observed between the two groups concerning the protein levels of MMP-3, TIMP-1, TIMP-2, TIMP-3, MT1-MMP, or MT3-MMP. These results indicate that the presence of tPA enhances inflammation, angiogenesis, and fibrogenesis in the peritoneum of the PF model mice. Activation of the PA/plasmin/MMP cascade may play a pivotal role in the pathogenesis of PF.

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MATERIALS AND METHODS

Animals. t-PA deficient (tPA−/−) mice on a C57BL/6 background and wild-type C57BL/6 (tPA+/+) mice were purchased from the Jackson Laboratory and were housed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocols were approved by the Animal Care and Use Committee of Nagoya University.

Mice PF model. PF model was induced in mice by the protocol reported previously (18) with a minor modification. Briefly, 9- to 10-wk-old female tPA+/+ mice (n = 6) and tPA−/− mice (n = 6) weighing ~20 g were administered 0.015 mg/g body wt of 0.1% CG dissolved in 15% ethanol intraperitoneally every 3 days. After 21 days, mice were killed, and peritoneal tissues were obtained for the study. Tissue samples obtained from tPA+/+ mice (n = 6) and tPA−/− mice (n = 6) without...
CG injection (day 0) served as controls. An additional experiment was performed to obtain the cells in the submesothelial zone from PA +/+ mice (n = 5) and tPA +/− mice (n = 5) with PF on day 21.

Macroscopic pathological studies. Median laparotomy was performed on day 21, and the macroscopic change in the visceral peritoneum was evaluated semiquantitatively, as described previously (31). Briefly, macroscopic fibrosis/adhesion scores were determined by four parameters, including dullness of the liver margin, area of adhesions, magnitude of adhesions, and ileus appearance. Scores ranging from 0 to 3 points were given to each parameter. Sums ranged from 0 points in the absence of fibrotic/adhesive change to a maximum of 12 points in the severest PF.

Histology and immunohistochemistry. Parietal peritoneal membranes were obtained for the histological studies. One part of the tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4-μm sections. They were deparaffinized and stained with Masson trichrome (28). Sections were also stained for muscle actin (α-SMA), collagen type III, laminin, and monocytes/macrophages as described (25, 28). In brief, they were incubated with rabbit antibodies against mouse type III collagen (CosmoBio: LSL, Tokyo, Japan), or laminin (abcam, Cambridge, UK), or rat antibodies against mouse F4/80 antigen (Serotec, Oxford, UK) followed by a conjugate of polyclonal goat anti-rabbit IgG antibodies or rabbit anti-IgG antibodies (Cappel, ICN Pharmaceuticals, Aurora, OH). They were also stained with a horseradish peroxidase (HRP) conjugate of monoclonal antibodies (Ab) against human α-SMA (Dako, Carpinteria, CA). The sections were visualized using an avidin-biotin complex kit (Vectastain Elite ABC KIT; Vector Laboratories, Burlingame, CA).

Another part of the tissue was frozen in optimal cutting temperature (OCT) compound (Miles, Elkhart, IN) and kept frozen at −80°C until use. Sections (2 μm thick) were cut with a cryostat, fixed in acetone, and incubated with polyclonal rabbit anti-rat thrombomodulin Ab (25) followed by fluorescein-conjugated goat anti-rabbit IgG Ab (Cappel). Negative controls were performed by the replacement of primary antibodies with isotype-matched or species-matched antibodies.

Morphometric analysis of histology and immunohistochemistry. Morphometric analyses were performed using an AXIOVISION 3.0 system microscope (Zeiss, Oberkochen, Germany). Fibrotic change was evaluated by measuring the full thickness of the submesothelium on Masson’s trichrome-stained sections oriented perpendicularly from the top of the muscle layer to the serosal surface as described (23). Inflammatory cell infiltration was assessed by counting the number of F4/80 positive cells (monocytes/macrophages) in four randomly selected high-power fields (×400). The results are shown as number per 0.144 mm². Neovascularization was evaluated by counting the number of vessels stained with anti-thrombomodulin Ab in 12 randomly selected high-power fields (×400). The results are shown as number per 0.432 mm². Four high-power fields (×100) from each section were photographed, and areas positively stained for α-SMA or type III collagen were measured in the submesothelial area of the peritoneum (above the muscle layer) were measured by computer-aided planimetry using Mac Scope (Mitani, Fukui, Japan) (22, 28).

Chromogenic assay of plasmin activity. Plasmin activity of the peritoneum was measured using a plasmin-specific chromogenic substrate, Chromozym PL (Roche Applied Science, Indianapolis, IN), as described (24). This substance is specifically cleaved by plasmin into a residual peptide and 4-nitroaniline, which can be detected spectrophotometrically. Samples (166 μl) containing 400 μg of peritoneal tissue protein, 667 μl of 50 mmol/l Tris (pH 8.2), and 167 μl of chromogenic substrate were mixed in a cuvette. The absorbance was measured at 405 nm twice over a 2-h interval. The increase in absorbance, corresponding to plasmin activity, was calculated. Results were expressed as ratios to a mean value in tPA +/+ mice without PF (day 0).

Real-time RT-PCR. The expression levels of tPA, MMP-2, and TGF-β1 mRNA were determined by real-time RT-PCR. Whole parietal peritoneal membranes were taken from tPA +/+ mice (n = 6) and tPA +/− mice (n = 6) without PF (day 0) and from tPA +/+ mice (n = 6) and tPA +/− mice (n = 6) with PF (day 21). Total RNA was prepared with TRIZol reagent (Life Technologies BRL, Rockville, MD), followed by further purification using Micro-to-Midi Total RNA Purification System (Invitrogen Life Technologies, Carlsbad, CA). The cells in the submesothelial zone were taken from PA +/+ mice (n = 5) and tPA +/− mice (n = 5) with PF (day 21) as described (20). Briefly, the peritoneal surfaces of the abdominal wall sections were immersed for 15 min in TRIZol reagent. In the submesothelium were gently scraped using a disposable blade, and total RNA was prepared using TRIZol reagent. One microgram of total RNA was reverse-transcribed using Takara RT kit (TaKaRa Biomedicals, Osaka, Japan). Real-time RT-PCR was performed using a SYBR green dye (Applied Biosystems, Foster City, CA) with the ABI PRISM 7500 equipment as described (38). DNA polymerase was first activated at 95°C for 10 min, denatured at 95°C for 15 s, and annealed/extended at 60°C for 1 min, for 35 cycles according to the manufacturer’s protocol. The sequences used are as follows: mouse tPA, forward primer 5’-CTGAGGTCACATGTC-CAAGCA-3’ and reverse primer 5’-ACAGATGCTGGTAGTGTCAG-3’; mouse MMP-2, forward primer 5’-GGCTGGAACACTCTCAG-GAC-3’ and reverse primer 5’-CGATGCCATCAAGAAGAACATG-3’; mouse TGF-β1, forward primer 5’-TGTTCTCAGGCCTCAAGAAGA-3’ and reverse primer 5’-TGTTTGTAGGAGGCAGAACC-3’; mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward primer 5’-TGCGAATCAAGCAACTC-3’ and reverse primer 5’-ATGAGGGCCATGGTGCCAC-3’. Relative quantitation of gene expression was carried out using the standard curve method (ABI PRISM 7500 Sequence Detection System; Applied Biosystems). Amplitons were run as triplicates in separate tubes to permit quantification of the target gene normalized to GAPDH as a control. Results are reported as relative ratios to the mean value of the tPA +/+ mice without CG injection (day 0) (whole peritoneal tissue), or to the mean values of the tPA +/+ mice with CG injection (day 21) (cells from submesothelium).

Detection of MMP-2 and MMP-9. Gelatin gel zymography was prepared using the extract of peritoneal membrane according to the method described previously (5). In brief, peritoneum lysates were prepared by homogenizing peritoneum with 100 mmol/l Tris-HCl (pH 7.4) containing 1% sodium dodecyl sulfate (SDS), 20 mmol/l NaCl, and 2 mmol/l phenylmethylsulfonyl fluoride. Protein concentration was determined using a Coomassie protein assay. Samples (30 μg protein/well) were loaded onto 10% SDS-polyacrylamide gels containing 1 mg/ml gelatin (Sigma-Aldrich) as substrate. After electrophoresis, SDS was removed from the gel by incubation in 2.5% Triton X-100 at room temperature for 30 min 3 times with gentle shaking. The gel was washed well with distilled water to remove detergent and incubated at 37°C for 48 h in a developing buffer containing 50 mmol/l Tris-HCl at pH 7.6, 0.15 mol/l NaCl, 10 mmol/l CaCl₂, and 0.02% NaNs. The gel was then stained with a solution of 50% methanol, 10% acetic acid, and 0.125% Coomassie brilliant blue, followed by destaining in the same solution without dye. Proteinase activity was detected as unstained bands on a blue background representing areas of gelatin digestion. The bands were quantified using National Institutes of Health Image software. Results are reported as relative ratios to the mean value of pro-MMP-2 in the tPA +/+ mice without PF (day 0). Markers for MMP-2 and MMP-9 were purchased from CosmoBio (Tokyo, Japan).

Western blot analysis. Western blot analysis was performed as described previously (12, 28) with minor modification. Briefly, peritoneal tissue obtained from tPA +/+ mice and tPA +/− mice with PF were homogenized in RIPA buffer using TisseLyser (Qiagen, Hilden, Germany). Fifty micrograms of proteins was separated by 10% SDS-PAGE, electroblotted onto PVDF membranes (Immobilon-Psq; Millipore), and blocked with 5% nonfat milk. The membranes were incubated with
Fig. 1. Levels of tissue-type plasminogen activator (tPA) mRNA expression and plasmin activity. A: left bar graph shows the levels of tPA mRNA in the parietal peritoneum determined by real-time RT-PCR. B: right bar graph shows relative plasmin activity of the peritoneal tissues. tPA +/+ and tPA −/− refer to wild-type mice and tPA deficient mice, respectively; d0, day 0 control mice; d21, mice on day 21 after chlorhexidine gluconate injection was started to induce peritoneal fibrosis (PF). *P < 0.05, **P < 0.01. NS, not significant.

antibodies against one of the following molecules: MMP-3, tissue inhibitor of metalloproteinases (TIMP)-1, TIMP-2, TIMP-3, membrane type 1 (MT1)-MMP (Santa Cruz Biotechnology, Santa Cruz, CA), MT3-MMP (AnaSpec, San Jose, CA), or phospho-Smad3 (Rockland Immunochemicals, Gibertsville, PA). Subsequently, the membranes were incubated with HRP conjugate goat anti-rabbit IgG or rabbit anti-mouse IgG (AnaSpec, San Jose, CA), or phospho-Smad3 (Rockland Immunochemicals, Gibertsville, PA). Subsequently, the membranes were incubated with HRP conjugate goat anti-rabbit IgG or rabbit anti-mouse IgG (AnaSpec, San Jose, CA), or phospho-Smad3 (Rockland Immunochemicals, Gibertsville, PA). Subsequently, the membranes were incubated with HRP conjugate goat anti-rabbit IgG or rabbit anti-mouse IgG (AnaSpec, San Jose, CA), or phospho-Smad3 (Rockland Immunochemicals, Gibertsville, PA).

RESULTS

Levels of tPA mRNA expression and plasmin activity. Quantified RT-PCR revealed that the level of tPA mRNA expression significantly increased in the parietal peritoneum after the induction of PF on day 21 (Fig. 1A). Chromozyme assay demonstrated that plasmin activity also increased in tPA +/+ mice on day 21 while it did not in tPA −/− mice (Fig. 1B).

Macroscopic findings. On day 21, tPA +/+ mice showed a severe macroscopic change with dullness of the liver margin and adhesion of the peritoneal membranes, while tPA −/− mice had only a mild change (Fig. 2). Semiquantitative analysis of the peritoneum showed that the macroscopic fibrosis/adhesion score was significantly higher in tPA +/+ mice than in tPA −/− mice (tPA +/+ : 5.42 ± 1.19; tPA −/− : 3.79 ± 0.82; P < 0.05) (Fig. 2).

Morphometric analysis of histology and immunohistochemistry. Histology showed that the submesothelium in tPA +/+ mice was significantly thicker than in tPA −/− mice (tPA +/+ : 178.2 ± 70.6 µm; tPA −/− : 60.7 ± 43.5 µm; P < 0.05) (Fig. 3).

Immunohistochemistry showed that α-SMA was strongly localized in the submesothelial sclerotic zone of the tPA +/+ mice. In contrast, only weak staining was observed in tPA −/− mice (Fig. 4A). A computer-aided quantitative analysis showed that the area positively stained for α-SMA was significantly smaller in tPA −/− mice than in tPA +/+ mice (P < 0.05). Fibrotic change was also evaluated by immunostaining for collagen type III. Positive staining was observed in the fibrotic area of the submesothelial sclerotic zone (arrows; Fig. 4B). The area positively stained for collagen type III was significantly smaller in tPA −/− mice than in tPA +/+ mice (P < 0.05) (Fig. 4B).

Immunostaining for laminin was performed to study the pathological change of basement membranes in the submesothelial tissue. Normal mice showed clear linear staining for laminin beneath a single layer of mesothelial cells (Fig. 5A). In tPA +/+ mice with PF (day 21), the basement membranes were distorted and mostly dissolved (Fig. 5B). In contrast, they were relatively preserved in tPA −/− mice (Fig. 5C).

Inflammatory reaction was evaluated by immunostaining for F4/80 on day 21. The results revealed that the number of monocytes/macrophages in the peritoneum was significantly less in tPA −/− mice than in tPA +/+ mice (tPA −/− : 248 ± 107; tPA +/+ : 434 ± 192; P < 0.05) (Fig. 6A).
the blood vessels in the peritoneal tissues was studied by staining with anti-thrombomodulin antibodies. The vessels in tPA+/+ mice had a distorted and narrowed lumen while those in tPA−/− were relatively preserved. When the number of blood vessels was counted, the vascular density was significantly less in tPA−/− mice than in tPA+/+ mice (tPA−/−: 65.0 ± 19.7; tPA+/+: 95.9 ± 32.7; P < 0.05) (Fig. 6B).

Fig. 3. Histology of the parietal peritoneum. Representative photographs show Masson trichrome (MT)-stained sections from tPA+/+ mice with PF (top left) and tPA−/− mice (top right). Arrows indicate the thickness of the submesothelium. Submesothelium was significantly thinner in tPA−/− mice. *P < 0.05. Original magnification, ×200.

Fig. 4. Immunochemistry for fibrogenic proteins. A: representative photographs show the staining for α-smooth muscle actin (α-SMA) in tPA+/+ mice (A, top left) and in tPA−/− mice (A, bottom left) on day 21. The graph demonstrates that the area positively stained for α-SMA was significantly smaller in tPA−/− mice than in tPA+/+ mice. B: representative photographs show the deposition of collagen type III in tPA+/+ mice (B, top left) and tPA−/− mice (B, bottom left) on day 21. Arrows indicate the area positively stained for collagen type III in the submesothelial sclerotic zone. The graph demonstrates that the expression level was significantly less in tPA−/− mice than in tPA+/+ mice. *P < 0.05. Original magnification, ×100.
tPA/H9252 MMP-2 and TGF-1 mRNA levels were significantly lower in the zone was also studied after the induction of PF (Fig. 8B). The gene expression in the cells of submesothelial mice without PF (day 0), while the level of pro-MMP-2 was not different between the two groups (day 21).

Expression of MMP-2 and TGF-1 mRNA. The levels of MMP-2 and TGF-1 mRNA in the whole peritoneum were studied by quantified RT-PCR. In tPA +/+ mice, the level of MMP-2 mRNA increased after the induction of PF (day 21) compared with the basal level (day 0). In contrast, no significant increase was observed in tPA −/− mice by the induction of PF (Fig. 8A). The expression of TGF-1 mRNA increased in tPA +/+ mice with PF (day 21) compared with tPA +/+ mice without PF (day 0) or tPA −/− mice with PF (day 21) (Fig. 8B). The gene expression in the cells of submesothelial zone was also studied after the induction of PF (day 21). Both MMP-2 and TGF-1 mRNA levels were significantly lower in tPA −/− mice than in tPA +/+ mice (Fig. 8, C and D).

Phosphorylated Smad3 in the peritoneum. Western blotting showed that the levels of phospho-Smad3 protein increased in the peritoneal tissue of tPA +/+ mice with PF (day 21) but not in tPA −/− mice with PF (day 21), suggesting enhanced levels of active TGF-1 in the former (Fig. 9). MMP-3, TIMPs, and MT-MMPs in the peritoneum. No difference was observed between the two groups concerning the protein levels of MMP-3, TIMP-1, TIMP-2, TIMP-3, MT1-MMP, or MT3-MMP in the peritoneal tissue (Fig. 10).

DISCUSSION

The present study clearly demonstrates that tPA expression when enhanced by the repeated injection of CG exacerbates peritoneal inflammation, angiogenesis, and fibrosis in a mouse model of PF. These data suggest for the first time that tPA is not protective but rather pathogenic to the peritoneal membrane.

Gelatin gel zymography of the peritoneal tissue. Gelatin gel zymography was performed on the peritoneal tissues to study the levels of MMPs. Pro-MMP-9 was not detectable in any group of mice. Active MMP-9 had increased on day 21 after the induction of PF, and no difference was observed between the two groups (Fig. 7, A and B). Likewise, no difference was observed in the basal levels (day 0) of pro- or active MMP-2 between the two groups (Fig. 7, A and C). In PF model mice (day 21), pro- and active MMP-2 had significantly increased in the peritoneum of tPA +/+ mice. The level of active MMP-2 on day 21 was significantly lower in tPA −/− mice than tPA −/−, while the level of pro-MMP-2 was not different between the two groups (day 21).

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MMPs is controlled by the MMP inhibitors or MMP inducers. Prevents PF in rats (27). It is known that the activation level of patients with EPS (21) and that the inhibition of MMP-2 clearly detectable in the peritoneum at a basal condition, and induction of PF. In contrast, the expression of MMP-2 was only weak expression was observed after the peritoneum. MMP-9 was not detected in the normal peritoneal tissue. MMP-9 was not detected in the normal peritoneum of tPA knockout (KO) mice with PF (day 21). A: representative photograph of gelatin gel zymography of the peritoneal tissues. Arrow, active matrix metalloproteinase (MMP)-9; open arrowhead, pro-MMP-2; closed arrowhead, active MMP-2. B: graph demonstrates the levels of MMP-2 mRNA in the whole peritoneum determined by 10.220.33.5 on November 6, 2017 http://ajprenal.physiology.org/Downloaded from

We found that none of the levels of MMP inhibitors, including TIMP-1, -2, and -3, were different between tPA knockout (KO) mice and wild-type mice. No difference was observed in the levels of MMP inducers (MT1-MMP and MT3-MMP) between these two groups. We also demonstrated that MMP-3 levels were not different between the two. Taken together, the data suggest that the net activity of MMP-2 was enhanced, which may play a role in the process of PF development.

Activation of a PA/plasmin/MMP cascade could enhance PF in several ways. First, MMP-2 in the peritoneal membrane is considered to accelerate migration of the fibroblasts and/or inflammatory cells into the submesothelial area (3, 8). We found that the number of macrophages increased in the peritoneum of tPA +/- mice compared with that of tPA -/- mice. The results suggest that the function of tPA to promote PF may be mediated by the enhanced migration of inflammatory cells. Second, MMP-2 may proteolytically generate active TGF-β1 peptide (4). In our study, the protein level of phospho-Smad3 was higher in the tPA +/- mice than in the tPA -/- mice on day 21, indicating that the level of active TGF-β1 had increased in the tPA +/- mice with PF. Third, MMP-2 may have the ability to stimulate angiogenesis (3, 26), providing another explanation for the morphological change in PF model mice. We found that the number of vessels in the peritoneum of the tPA -/- mice with PF had significantly decreased. It is not clear from our results whether angiogenesis is a cause of the PF or merely a result. Margetts et al. (19) clearly

To study the mechanisms of the fibrogenic process in the present PF model, we investigated the activity of plasmin, the natural substrate of tPA. We found that plasmin activity in the peritoneal tissue was enhanced by the presence of tPA after the induction of PF. Since plasmin is known to activate MMPs, we then studied the levels of MMP-2 and MMP-9 in the peritoneal tissue. MMP-9 was not detected in the normal peritoneum, and only weak expression was observed after the induction of PF. In contrast, the expression of MMP-2 was clearly detectable in the peritoneum at a basal condition, and both active MMP-2 and pro-MMP-2 had increased significantly in the peritoneal tissue obtained from PF model mice. These results were consistent with previous findings that MMP-2 is elevated in the dialysate of rats with PF (16) and patients with EPS (21) and that the inhibition of MMP-2 prevents PF in rats (27). It is known that the activation level of MMPs is controlled by the MMP inhibitors or MMP inducers.
demonstrated using an adenoviral vector system that overexpression of TGF-β/H9252, a fibrogenic cytokine, induced the expression of VEGF, a key molecule for angiogenesis. It is also reported that inhibition of angiogenesis suppressed PF. These results suggest that angiogenesis and fibrosis enhance each other, causing the further acceleration of PF. Last, MMP-2, activated by tPA and plasmin, can accelerate fibrosis by promoting EMT. Recent studies have elucidated the contribution of the gelatinases, MMP-2 and MMP-9, to the process of EMT (2, 4, 30, 35, 36). The significance of EMT was also shown in the peritoneum of PD patients (34). In our study, enhanced staining of α-SMA and collagen III was observed in the thickened submesothelial area of the PF model mice. Moreover, staining for laminin showed that the basement membrane was compromised and partially destroyed in the fibrotic peritoneum. These results suggest that EMT may actually have been taking place in our study as well. To determine the key mechanism in the process of PF, further studies using genetically modified mice, such as MMP-2 deficient mice, will be useful.

Originally, tPA was considered to attenuate tissue fibrosis due to its potential to degenerate the extracellular matrix (ECM). It was generally accepted that ECM degradation in glomeruli is mediated by a PA/plasmin/MMP cascade (1). It has also been demonstrated that urokinase receptor deficiency accelerates renal fibrosis in the interstitium (37). However, more recent studies have shown that tPA may act as a profibrogenic molecule. In a mouse unilateral urethral obstruction model, tPA was shown to enhance interstitial fibrosis of the kidney (36). In addition, Hertig et al. (15) reported that the deficiency of type I plasminogen activator inhibitor (PAI-1) aggravates the course of experimental glomerulonephritis through overactivation of TGF-β1. In the present study, we demonstrated that tPA accelerated the pathological alterations of the peritoneum in a mouse model of PF. Whether the PA/plasmin/MMP system is deleterious or beneficial seems to depend on the specific situation.

One of the limitations of the present study is the model employed. Although a PF model induced by the repeated injection of CG has been used to study the pathogenesis of peritoneal injury (16, 18), the processes could differ significantly from those taking place in actual patients. A PF model induced by the repeated injection of high-glucose solution may better reflect the pathogenesis of peritoneal damage in PD patients (17). However, the relative thickening of the peritoneal membrane in this model is not significant enough to facilitate a study of PF. Further studies analyzing the samples from PD patients will be necessary to clarify the actual role of the PA/plasmin/MMP system.

In summary, the present study demonstrated that mice deficient in tPA showed significantly milder peritoneal change compared with wild-type mice when PF was induced by CG. The results suggest that the presence of tPA enhances inflammation, angiogenesis, and fibrogenesis in the peritoneum of the PF model mice. Activation of the PA/
plasmin/MMP cascade may play an important role in these pathological processes.

DISCLOSURES

No conflicts of interest are declared by the authors.

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