Alterations in proteoglycan components and histopathology of the peritoneum in uraemic and peritoneal dialysis (PD) patients

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Abstract

Background. Proteoglycans (PGs) are dominant extracellular matrices (ECMs) in the peritoneal tissues. Human peritoneal mesothelial cells synthesize small proteoglycans including decorin. Peritonitis and long-term peritoneal dialysis (PD) cause fibrotic changes in the peritoneum that result in ECM remodelling and PG synthesis.

Methods. Twenty-five peritoneal tissues from eight patients at initiation of PD, five long-term PD (>6 years) patients with severe peritonitis lasting for almost 1 month, nine patients after long-term PD (>6 years) without peritonitis and three normal subjects were included in the present study. Expressions of decorin, versican, hyaluronan, MMP-2, alfa smooth muscle actin (αSMA) and CD68 for macrophages in these specimens were examined by immunohistochemical staining.

Results. Although expression of decorin was detected in normal subjects, it was markedly decreased with long-term PD treatment. In long-term PD patients, the expression of versican was observed in their fibrotic-thickened peritoneum. Versican was present in fibrous regions, elastic lamina of the peritoneum, vascular walls and perivascular regions. Hyaluronan was observed in the whole thickened peritoneum, but its distribution differed in part from that of versican. MMP-2 was mainly observed around the blood vessels. Alfa SMA-positive cells, namely ‘myofibroblasts’ and CD68-positive cells, i.e. macrophages, were observed in the fibrotic—thickened peritoneum of long-term PD patients. Expressions of MMP-2, hyaluronan, SMA and CD68 in the peritoneum were marked in long-term PD patients’ samples, which were strongly immunostained by versican, and were especially high in peritonitis patients.

Conclusions. It appears that alterations in PGs, including marked induction of versican with peritonitis and disappearance of decorin, are involved in peritoneal remodelling in PD patients. Versican expression was closely related to the appearance of myofibroblasts and macrophages. These observations suggest that the alteration in PG components following PD therapy and severe inflammation contribute to fibrous thickening of the peritoneum.

Keywords: alfa SMA; decorin; hyaluronan; peritoneal dialysis; versican

Introduction

In long-term peritoneal dialysis (PD) patients, morphological findings of the peritoneum are loss of mesothelium and thickening of the submesothelial layer due to fibrosis including collagen deposition and vasculopathy [1]. The submesothelial layer is known to be the basic matrix of remesothelialization and has a charge barrier for passage of plasma molecules. Therefore, changes in the structural components of proteoglycans (PGs) may be involved in fibrotic changes in long-term PD patients. The PGs’ fibrotic—thickened peritoneum may be associated with inflammatory cell infiltration and changes in long-term PD, but this has not been investigated to date.
The dominant extracellular matrix (ECM) of the submesothelial layer consists of PGs, which have various biological roles [2]. Yung et al. [3] reported that cultured mesothelial cells synthesize small chondroitin/dermatan sulfate proteoglycans such as decorin, but not aggregated, large-size proteoglycans such as versican. Decorin, an anti-fibrogenic molecule, is reported to reduce collagen accumulation via inhibition of transforming growth factor beta (TGF-β) in vitro and in vivo [4,5].

Versican, often observed in atherosclerotic blood vessels, exhibits anti-adhesive activity for cells to the substratum, and affects cell proliferation and differentiation via binding to hyaluronan, which is increased in the peritoneum of peritonitis patients [6,7]. The versican–hyaluronan complex may facilitate cell proliferation and migration by decreasing cell–matrix adhesion [8].

Therefore, it is possible that the alterations of PG components in the submesothelial layer may be induced in patients with uraemia, peritonitis and repeated exposure of bioincompatible PD fluid, and contribute to cell infiltration, vasculopathy and expansion of fibrosis. To clarify these changes in the development of peritoneal fibrosis, the distribution of decorin, versican, MMP-2, one of the proteolytic enzymes with PG as the substrate, and hyaluronan in the peritoneal tissues of PD patients was evaluated using histochemical analyses. Furthermore, the appearance of macrophages and myofibroblasts as cell components involving inflammation and fibrosis was evaluated immunohistochemically and was compared with versican expression.

Materials and methods

Clinical characteristics of participants
Participating patients were divided into four groups including the control group, uraemic group and the long-term PD with or without peritonitis groups (Tables 1 and 2). Age and gender did not deviate in the four groups. The control group consisted of normal healthy individuals undergoing elective abdominal surgery. No patients were diabetic. Origins of renal failure included glomerulonephritis and nephrosclerosis. At initiation of PD, patients were in the uraemic state resulting from conservative therapy of chronic renal failure. The long-term PD without peritonitis group consists of patients with extraction of the PD catheter due to ultrafiltration failure, underdialysis or abdominal operations after long-term PD without peritonitis. The Ethics Committee of Juntendo University School of Medicine gave permission to evaluate morphologic findings in peritoneal samples.
Histochemical staining

Twenty-five anterior parietal peritoneal tissues were obtained from 25 patients including 3 normal subjects, 8 patients at initiation of PD, 9 patients on long-term PD (PD duration: >72 months) without peritonitis and 5 patients on long-term PD with peritonitis, from 1997 to 2004 in Juntendo University Hospital.

The specimens were fixed in 10% neutral formalin and embedded in paraffin. The 4-μm tissue sections were processed for immunohistochemical staining or hyaluronan staining using the hyaluronan binding protein. The monoclonal antibodies used in this study are shown in Table 3. All antibodies were classified as mouse monoclonal IgG.

Decorin (6B6). After treatment with proteinase K (DAKO, CA, USA) at 37°C for 10 min, Chondroitinase ABCR (Seikagaku-Kogyo, Tokyo, Japan) digestion was performed in 0.4 U/ml Tris–HCl at 37°C for 60 min. Endogenous peroxidase was blocked with 0.3% (v/v) H2O2 in methanol at room temperature for 15 min. After blockage of nonspecific binding sites with 10% non-immune horse serum, the sections were incubated with the 6B6 antibody (Seikagaku-Kogyo) in 0.01 M phosphate-buffered saline (PBS) at 4°C overnight, followed by addition of the biotin-labelled horse anti-mouse IgG secondary antibody, and then treated with avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA). Finally, they were treated with diaminobenzidine and hydrogen peroxide (DAKO) to obtain a colour reaction. The sections were then counter-stained with haematoxylin. The primary antibody was not applied to negative control sections.

Versican. Endogenous peroxidase was blocked with 3% (v/v) H2O2 in methanol at room temperature for 20 min. Chondroitin/dermatan sulfate chains were digested with 5 U/ml Chondroitinase ABC Protease Free (Seikagaku-Kogyo) in PBS at room temperature for 15 min. Then, immunohistochemical analysis using a 2B1 antibody (Seikagaku-Kogyo) was performed using the same procedure as in the immunostaining of decorin. The primary antibody was not applied to negative control sections. To evaluate elastic tissues, the same sections were prepared by Elastica–van Gieson (EVG) staining after immunostaining of versican.

MMP-2. Following blockage of endogenous peroxidase, the same staining as with versican and decorin was performed on MMP-2 using the F68 antibody (Daichi Fine Chemical, Toyama, Japan).

Hyaluronan. Following blockage with endogenous peroxidase using 0.3% (v/v) H2O2 in methanol, chondroitin/dermatan sulfate chains were digested with 5 U/ml Chondroitinase ABC in PBS at room temperature for 2 h. After blockage of nonspecific binding sites, the sections were incubated with 2 μg/ml Hyaluronic Acid Binding Protein-Biotin (Seikagaku-Kogyo) at room temperature for 2 h. This was followed by treatment with avidin–biotin–peroxidase complex, and diaminobenzidine containing hydrogen peroxide for visualization. Negative control sections were pre-incubated with 200 TRU/ml hyaluronidase at 37°C overnight.

αSMA. For heat-induced epitope retrieval, the sections were autolaved in a 10 mM citrate buffer (pH 6.0) at 121°C for 10 min. Then, immunohistochemical analysis using the anti-human SMA clone1A4 antibody (DAKO) was performed using Ventana automated NexES IHC (Ventana Medical Systems, Tucson, AZ, USA) with the Ventana iVIEW DAB detection kit. The primary antibody was not applied to negative control sections.

CD68. The sections were pretreated with 0.1% Trypsin (Sigma, St Louis, MO, USA) and 0.1% CaCl2 in 50 mM Tris–HCl (pH 7.6) at 37°C for 30 min. Then, immunohistochemical analysis using the anti-human CD68 clone PG-M1 antibody (DAKO) was performed using the same procedure as in the immunostaining of αSMA. The primary antibody was not applied to negative control sections.

Semi-quantification of histochemical staining of submesothelial interstitium

To semi-quantify the intensity of histochemical staining for decorin, versican, hyaluronan and MMP-2, the degree of staining was visually graded as negative, weak, moderate and strong, for each dimension and intensity, in Figure 1 [9].

Quantification of immunostaining for versican: planimetric analysis using an image analyser (Figure 4)

Quantification of the dimensions of versican immunostaining was performed using a computer-assisted colour image analysis device, KS400 (Zeiss, Jena, Germany). The submesothelial compact zone and versican-immunopositive dimensions of each sample in the immunostaining image on the computer display were measured. The ratio of versican-immunopositive dimensions to the submesothelial compact zone was also calculated. The area from the upper limit of the adipose tissue to the mesothelial surface was defined as the submesothelial compact zone [10]. The ratio of the versican staining dimensions to total peritoneal dimensions was also evaluated in each group. Two PhDs and one MD evaluated the grades on each preparation. Each examiner looked at the images independently. The mean size of the biopsy specimens was 15.3 mm2. The mean of the total areas of each field involving adipose tissues was 1 463 000 μm2 (√×100). The number of fields averaged by each examiner was 10.3.

Semi-quantification of versican expression related to the number of αSMA- or CD68-positive cells in long-term PD patients’ peritoneum with or without peritonitis (Table 4)

The relationship between semi-quantification of versican expression and the number of αSMA- or CD68-positive cells in the long-term PD patients’ peritoneum is shown in Table 4. The intensity of versican expression was graded by standards of semi-quantification of histochemical staining in Figure 1, and then distinguished into two groups by with or without peritonitis. The numbers of αSMA smooth muscle actin (SMA)- or CD68-positive cells were counted by one PhD and one MD on each preparation. The mean of the total areas of each field and the numbers of fields were same as antecedent paragraph. We compared the intensity of versican expression with the number of αSMA- or CD68-positive cells.

Statistical analysis

The results of planimetric analysis were expressed as mean ± SE. P < 0.05 denoted significance.

Results

The expressions of decorin and versican were semi-quantitatively evaluated in each stage including normal, initiation of PD and long-term PD stages, without reference of the location, as shown in Table 3. In normal peritoneum, decorin was detected but versican was not observed. In the fibrotic–thickened peritoneum of long-term PD patients, decorin was markedly decreased with or without peritonitis. On the other hand, versican was not detected in normal peritoneum but was detected in long-term PD peritoneum, and was especially intense in patients with peritonitis (Table 3 and Figure 4).

In the uraemic peritoneum of patients at initiation of PD, detection of versican and MMP-2 expression was variable. Hyaluronan was not detected in normal subjects, but observed in uraemic patients at both initiation of PD and on

Table 3. List of antibodies for immunostaining

<table>
<thead>
<tr>
<th>Name</th>
<th>Class</th>
<th>Antigen</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>6B6</td>
<td>Monoclonal, mouse IgG</td>
<td>Human dermatan sulfate PG (decorin)</td>
<td>1:500</td>
</tr>
<tr>
<td>2B1</td>
<td>Monoclonal, mouse IgG</td>
<td>Human large PG (versican)</td>
<td>1:2000</td>
</tr>
<tr>
<td>F68</td>
<td>Monoclonal, mouse IgG</td>
<td>Human MMP-2</td>
<td>1:100</td>
</tr>
<tr>
<td>1A4</td>
<td>Monoclonal, mouse IgG</td>
<td>Human SMA</td>
<td>1:400</td>
</tr>
<tr>
<td>PG-M1</td>
<td>Monoclonal, mouse IgG</td>
<td>Human CD68</td>
<td>1:50</td>
</tr>
</tbody>
</table>
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Fig. 1. Standards of semi-quantification of histochemical staining, ×100. (A) Negative; no expression. (B) Weak; low expression in the peritoneum. (C) Moderate; distinct and local expression in the peritoneum. (D) Strong; intense and diffuse expression in the peritoneum. Bars = 100 µm.

Table 4. Correlation between versican expression and the number of αSMA- or CD68-positive cells

<table>
<thead>
<tr>
<th>Semi-quantification of versican expression and in long-term PD peritoneum</th>
<th>Without peritonitis</th>
<th>With peritonitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Weak</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>αSMA-positive cell number/field (×100)</td>
<td>CD68-positive cell number/field (×100)</td>
<td></td>
</tr>
</tbody>
</table>

long-term PD. MMP-2 expression was weak in all normal subjects, but was marked in PD patients. The histochemical data were independent of age, gender, origin of renal failure and infected bacteria.

Locations of positive findings of versican, decorin, MMP-2 and hyaluronan are shown in Figures 2, 3, 5 and 6. Decorin was observed in the submesothelial layer of normal subjects (Figure 2A), and scattered in the uraemic peritoneum at initiation of PD (Figure 2B).

Versican was not observed in the submesothelial interstitium of normal subjects (Figure 3A). In long-term PD patients with peritonitis, versican was marked in the submesothelial compact zone (Figure 3B). The expression of versican in the submesothelial compact zone was diffuse and colocalized with collagen fibres interlaced with fibrin in long-term PD patients (Figure 3C and D). EVG staining showed the elastic fibre in black, the collagen fibre in pink and fibrin in yellow (Figure 3D). The surface of the peritoneum of long-term PD patients was covered by fibrin, which was interlaced with collagen fibres (Figure 3D). Versican expression on the split elastic lamina of the peritoneum was revealed by EVG staining using the same section of each sample, but expression of versican was not observed in the intimal elastic lamina of the small
Fig. 2. Immunostaining of decorin. (A) Decorin was detected in submesothelial and muscle layers of normal peritoneum, and (B) sporadically in the uraemic peritoneum at initiation of PD. Original magnification: ×200 Bars = 50 µm.

arteries (Figure 3D). The peritoneal elastic lamina was split in long-term PD patients, but the intimal elastic fibre was well preserved (Figure 3D and E).

Versican was detected in adventitia of the small arteries, perivascular fibrous tissues and venous walls (Figure 3C and E) in long-term PD patients. Versican was detected partially in the thickened intima and media of stenotic, hyalinized small arteries (Figure 3E).

In a quantitative evaluation with planimetric analysis, the ratio of versican expression in long-term PD patients with or without peritonitis was significantly increased compared with that in uraemic patients at initiation of PD \( (P < 0.01) \) (Figure 4). The ratio of versican expression in long-term PD patients with peritonitis was significantly higher than that in those without peritonitis \( (P < 0.05) \).

Hyaluronan was observed over the whole thickened submesothelial compact zone at initiation of PD. In long-term PD patients, hyaluronan was detected widely and homogeneously over the peritoneum (Figure 5A), while versican expression was marked in the shallow layer of the submesothelium (Figure 5B), perivascular regions and vascular walls. Although hyaluronan expression was similar to versican expression in fibrotic tissues all over the peritoneum, the distribution of hyaluronan was wider than that of versican. Hyaluronan did not exactly correspond to versican in the adjacent sections (Figure 5A and B). Versican was clearly localized in the elastic lamina, adventitia, vascular walls and shallow layers of the submesothelium with fibrin deposits.

MMP-2 was observed in the perivascular regions and scattered in the peritoneal interstitium (Figure 6A). In peritonitis patients, MMP-2 was strongly detected in the submesothelial compact zone, showing the intense immunoreactivity of versican (Figure 6).

Alfa SMA- or CD68-positive cell count increased with the increase in versican expression (Table 4). Expression of αSMA was observed in many blood vessels in the peritoneum of normal subjects and at initiation of PD. No immunostaining of αSMA was shown in other areas except blood vessels of normal subjects and patients at initiation of PD. In the peritoneum of long-term PD patients, the immunostaining for αSMA in the vascular sclerosis was decreased, and αSMA-positive cells outside the blood vessels were scatteredly observed in all specimens (Figure 7A). Alfa SMA-positive cells in the fibrotic–thickened ECMs were suggested to be myofibroblasts. In long-term PD with peritonitis, the number of αSMA-positive cells was 10–50/field in fibrotic–thickened peritoneum except for blood vessels of all five cases (Table 4). Alfa SMA and versican expressions in the peritoneum of long-term PD patients with peritonitis are shown in Figure 7. The αSMA-expressing area did not always correspond to the versican-expressing area except in the vascular area (Figure 7). In specimens with many αSMA-positive cells, versican was strongly observed (Figure 7).

CD68-positive cells were not detected in the peritoneum of normal patients and patients at initiation of PD (Table 4). In the peritoneum of long-term PD patients with or without peritonitis, many CD68-positive cells, i.e. macrophages, were detected (Table 4). Especially in patients with peritonitis, marked infiltration of CD68-positive cells was observed (Table 4). The CD68 and versican expressions in the peritoneum of long-term PD patients with peritonitis are shown in (Figure 8). Specimens with many macrophages showed intense expression of versican. The CD68-positive cell infiltrating area did not always correspond to the versican accumulating area (Figure 8). In specimens with many CD68-positive cells, versican was strongly observed (Figure 8).

**Discussion**

In this study, we evaluated alterations of PG components in the peritoneum during PD to find the triggers for the development of peritoneal sclerosis. Decorin is concerned with maintenance of the tissue structure in many connective tissues. Mesothelial cells have been reported to synthesize decorin [3]. Decorin gene therapy was effective for reduction of collagen accumulation in the rat PD model [4]. Decorin-expressing cells were reported to down-regulate the synthesis of the angiogenic factor [11]. The disappearance of decorin in the submesothelial layer in this study may
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Fig. 3. Immunostaining of versican. (A) Versican was not detected in the submesothelium of normal peritoneum. (B) In long-term PD patients with peritonitis, versican was detected in the diffuse thickened submesothelial compact zone. (C) In the submesothelial compact zone of long-term PD patients, versican was observed in the intima of the small arteries (arrow 1), adventitia and perivascular fibrous tissues (arrow 2), the split peritoneal elastic fibre (arrow 3) and interstitium intermingled with the collagen fibre (arrow 4). (D and E) Elastic fibre, collagen fibre and fibrin were shown by EVG staining after immunostaining of versican. (D) The closed arrowhead points to the split elastic lamina of peritoneum, and the open arrowhead points to the intimal elastic lamina of the small arteries. (D) Versican was not observed in the intimal elastic lamina of the small arteries. (E) Versican was intensely observed in venous walls (arrow 5), adventitia of the arteries and perivascular fibrous areas (arrow 6), and partially detected in the thickened intima (arrow 7) and media (arrow 8) of stenotic arteries. (E) EVG staining demonstrated that the intimal elastic fibre of the small arteries was well maintained, unlike the peritoneal elastic fibre. Original magnification: (A–D) ×200, bars = 50 µm; (E) ×400, bars = 20 µm.

be associated with the loss of mesothelium, vasculopathy and expansion of fibrotic change in sclerotic peritoneum. Versican, a large aggregating chondroitin sulfate PG, was first isolated from chick embryonic mesenchyme [12]. Versican has roles in a variety of fibroproliferative disorders or vascular diseases [7,13]. Versican expression at initiation of PD suggests that alteration of PG components has already started in the uraemic condition, independent of PD therapy. Another study showed that the versican-rich areas contain little mature collagen but do contain myofibroblasts stained for type I procollagen, suggesting that versican affects the early repair processes in pulmonary fibrosis [13]. In our study, prominent expression of versican in patients with peritonitis may indicate subsequent synthesis of collagen and developing fibrosis. The ability of PGs to interact with collagen contributes to regulation of their biosynthesis in the inflammatory environment [2]. Versican and hyaluronan affect the retention of inflammatory cells and contribute to macrophage adhesion [14]. Monocytes/macrophages contribute to ECM proliferation and alterations via the production of cytokines and growth factors. Thus, inflammatory migrating cells may be involved in fibrotic thickening of the peritoneum with an increase in versican. Immunoreactivity of versican was previously observed in the adventitia of
normal, small arteries, and also in the media of atherosclerotic small arteries [15]. In neointima after vascular injury, cell proliferation and migration were up-regulated by versican [16]. Versican cooperates with collagen to promote platelet aggregation [17]. In our study, versican was observed partially in the thickened intima and media of peritoneal small arteries of long-term PD patients, in addition to intense accumulation in the adventitia. These findings show the contribution of versican to vasculopathy involving intimal thickening and stenosis in peritoneal small arteries in patients with fibrotic–thickened peritoneum and peritonitis. Thus, disappearance of decorin and increase in versican might accelerate the progression of peritoneal fibrosis and vasculopathy. We assume that conversion of PGs is caused by an aggregation disorder of small PGs, and an imbalance in the synthesis or degradation of small and large PGs.

Elastic fibres inhibit intimal hyperplasia and subendothelial proliferation of arterial smooth muscle cells. Elastica interna of small arteries disappears in atherosclerotic and restenotic vascular diseases. Versican inhibits an elastic fibre assembly, via the chondroitin sulfate chain [7]. In patients on long-term PD, accumulation of versican was observed in the split elastic lamina of the peritoneum, but not in the well-conserved elastica interna of small arteries with intimal hyperplasia. These findings suggest that

Fig. 4. Quantification of immunostaining of versican. The ratio of versican-immunopositive dimensions to the submesothelial compact zone was significantly increased in long-term PD patients with or without peritonitis. (a) $P < 0.01$, (b) $P < 0.05$.

Fig. 5. Hyaluronan (A) and versican (B) expressions in long-term PD patients with peritonitis. (A) Hyaluronan was observed widely and homogenously in the submesothelial compact zone. (B) Versican was especially detected in the shallow layer of the submesothelium, perivascular sites and vascular wall on the adjacent section of (A). Original magnification: (A and B) $\times 100$, bars = 100 $\mu$m.

Fig. 6. Immunostaining of MMP-2 (A) and versican (B) in long-term PD patients with peritonitis. (A) MMP-2 expression was observed in perivascular sites and adventitia (arrowheads), and showed a patchy distribution in fibroblasts (arrows) in peritonitis patients. (B) Versican expression was mainly observed in perivascular sites and vascular walls on the adjacent section of (A). Original magnification: (A and B) $\times 100$, bars = 100 $\mu$m.
versican inhibits the normal peritoneal elastic fibre assembly and is involved in peritoneal ECM remodelling.

In our study, hyaluronan was distributed more diffusely than versican in the submesothelial compact zone of long-term PD and peritonitis patients. Changes in the binding sites of versican to hyaluronan in the pathological environment may affect the distribution of hyaluronan. In plaque erosion, versican and hyaluronan were increased, while decorin was decreased [18]. These changes of matrices may also contribute to endothelial loss and thrombotic events in the vasculopathy of the peritoneum. Versican binding to hyaluronan encourages cell proliferation and migration by anti-adhesive activity, and plays an important role in regulating cell shapes and epithelial–mesenchymal transformation [8,19]. Anti-adhesive activity of the versican–hyaluronan complex may be involved in the denuded mesothelium by decreasing cell–matrix adhesion and ECM remodelling related to PD. MMP-2, which is increased in peritoneal injury, was previously reported to cleave versican in hydraulic lung oedema of rabbits [20,21]. MMP-2 was immunohistochemically detected in macrophages, smooth muscle cells and fibroblasts in the normal lung, and was up-regulated in inflammatory disorders, in agreement with our study [22]. In our study, MMP-2 was colocalized with versican in the vascular walls and perivascular sites, especially in peritonitis patients. MMP-2 dissolving PGs may be involved in the breakdown and remodelling of PGs and vasculopathy.

Our findings showed a close relationship between infiltration of macrophages and αSMA-positive cells and alterations in proteoglycans. In the peritoneum of long-term PD patients, the infiltrated macrophages contributed to peritoneal fibrosis via the secretion of cytokines and growth factors. Alfa SMA was scatteredly observed in the pericytes and in cells of the fibrotic–thickened area, especially in patients with peritonitis. The numbers of αSMA-positive cells were correlated with versican expression in each sample. It was suggested that transformation to myofibroblasts is involved in fibrogenetic changes in peritoneal fibrosis, such as glomerulosclerosis, tubulointerstitial fibrosis, vascular sclerosis and liver fibrosis [23,24].

Fig. 7. Immunostaining of alfa SMA (A) and versican (B) in long-term PD patients with peritonitis. (A) Alfa SMA was observed in blood vessels, pericytes and myofibroblasts in the fibrous-thickened peritoneum. In specimens with many αSMA-positive cells, versican showed a high expression. The αSMA-expressing cells did not always correspond to the versican-expressing area on the adjacent section of (A), except the vascular area. Original magnification: (A and B) ×100, bars = 100 µm.

Fig. 8. Immunostaining of CD68 (A) and versican (B) in long-term PD patients with peritonitis. (A) CD68 was observed in the fibrous-thickened peritoneum. In specimens with many CD68-positive cells, versican showed a high expression level. Original magnification: (A and B) ×100, bars = 100 µm.
Our results showed that the alteration of PGs with the increase in hyaluronan, myofibroblasts and macrophages is induced in the peritoneum of patients on long-term PD, especially those with peritonitis. It appears that qualitative alterations in PGs in the submesothelium is involved in submesothelial thickening resulting from a loss of mesothelial cells, cell infiltration, disassembly of peritoneal elastic fibres and vasculopathy. It appears that infiltrating cells and vascular pericytes especially may be triggers for PG metabolism in the injured peritoneum of PD patients.

Conflict of interest statement. None declared.

(See related article by D. J. Fraser and N. Topley. Altering peritoneal membrane function: removing the GAG? Nephrol Dial Transplant 2009; 24: 3271–3273.)

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