PART THREE

Host Defenses and Infection
To establish histologic criteria for a diagnosis of encapsulating peritoneal sclerosis (EPS), we investigated 69 peritoneal biopsy specimens histologically and immunohistochemically. The specimens included cases of EPS (n = 12), suspected cases of EPS without later manifestation (n = 5), cases of infectious peritonitis (n = 20), cases of ultrafiltration failure (n = 25), and peritoneum at the start of peritoneal dialysis (n = 7). For each specimen, we evaluated these histologic parameters: fibrin deposition, mesothelial denudation, interstitial fibrosis, peritoneal fibroblast swelling, perivascular bleeding, capillary angiogenesis, microvascular sclerosis, and interstitial mononuclear cell infiltration. We also evaluated these immunohistochemical markers: macrophage migration inhibitory factor (MIF), fibroblast growth factor (FGF), FGF receptor 2 (FGFR2), α-smooth muscle actin (αSMA), MIB1, and BCL2.

The most characteristic histologic findings for EPS were fibrin deposition and fibroblast swelling. The presence of capillary angiogenesis and mononuclear cell infiltration were also associated with EPS. Expression of FGF, FGFR2, MIF, MIB1, and BCL2 in peritoneal fibroblasts was frequently observed in EPS. Our results suggest that fibrin deposition and peritoneal fibroblast activation or proliferation (or both) are useful findings for the early diagnosis of EPS. Careful histologic observation of the peritoneal biopsy after withdrawal of peritoneal dialysis is required for the early diagnosis and prevention of EPS.

Key words
Fibrin, peritoneal fibroblast, MIF, FGF, MIB1, BCL2, peritonitis

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Materials and methods

Peritoneal biopsy and autopsy specimens
From August 1981 to September 2002, 149 peritoneal biopsies of CAPD patients were performed at the Kidney Center, Tokyo Women’s Medical University. Among them, we selected 69 specimens (including 3 autopsy specimens) that could be classified into these clinical categories: EPS cases (n = 12), suspected EPS cases without later clinical onset (n = 5), cases of infectious peritonitis (n = 20), cases of ultrafiltration failure (n = 25), and peritoneum at the start of peritoneal dialysis (n = 7). The 69 specimens came from 52 male and 17 female patients with the mean age of 43.0 ± 17.5 years and a mean peritoneal dialysis duration of 68.0 ± 47.7 months. Table I summarizes the clinical background of the five biopsy categories.

Histologic and immunohistochemical methods
Parietal peritoneal specimens were obtained during surgery for catheter removal or insertion. The tissues were fixed with 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, and cut into 4 µm sections. Hematoxylin and eosin and Mallory-Azan staining were performed for light microscopy. For the immunohistochemical study, paraffin sections were stained using a peroxidase-labeled streptavidin–biotin staining kit (Dako Corporation, Glostrup, Denmark). The primary antibodies were monoclonal anti–macrophage migration inhibitory factor (MIF) antibody (R&D Systems, Minneapolis, MN, U.S.A.), rabbit polyclonal anti–fibroblast growth factor 2 (FGF2) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), rabbit polyclonal anti–FGF receptor 2 (FGFR2) antibody (Santa Cruz Biotechnology), monoclonal anti–α smooth muscle actin (αSMA) antibody (Dako Corporation), monoclonal anti-MIB1 antibody (Ki-67: Dako Corporation), and monoclonal anti-BCL2 antibody (Dako Corporation).

Histologic and immunohistochemical evaluation
Frequency and grade of the following histologic parameters were evaluated: fibrin deposition, mesothelial denudation, interstitial fibrosis, peritoneal fibroblast swelling, perivascular bleeding, capillary angiogenesis, microvascular sclerosis, and interstitial mononuclear cell infiltration. The extent of the parameters was determined by a semiquantitative grading system: grade 0, no lesion; grade 1, mild; grade 2, moderate; and grade 3, severe. The frequency and grade of the parameters were compared for the five biopsy categories.

In the immunohistochemical study, the frequencies of MIF, FGF, FGFR2, αSMA, MIB1, and BCL2 expression in peritoneal fibroblasts were evaluated in 42 of the biopsy specimens, from the EPS (n = 12) and non EPS (n = 30) categories. The non EPS category included specimens from the suspected EPS cases (n = 5), the peritonitis cases (n = 9), the UFF cases (n = 11), and peritoneum at the start of PD (n = 5).

Statistical analysis
We used the chi-square test to evaluate the relationship between each histologic or immunohistochemical finding and the biopsy categories (EPS or non EPS). The Mann–Whitney U-test was used to compare the differences in the grades of the histologic parameters between two biopsy categories (for example, EPS vs. peritonitis). Significance was accepted as p < 0.05 in all analyses.

Results

Histologic findings in EPS
The histologic changes frequently observed in peritoneum with EPS (Figure 1) were fibrinous capsule formation, fibrin deposition, perivascular bleeding, fibroblast swelling and proliferation, interstitial fibrosis, and granulation tissue with vascular proliferation (capillary angiogenesis).
Figure 2 shows the frequencies of those histologic parameters in the five peritoneal biopsy categories. In the EPS category \((n = 12)\), the frequencies of all histologic parameters were higher than those in the non EPS categories \((n = 57)\). Among all histologic parameters observed, the frequencies of the following four parameters were significantly higher in the EPS category than in the non EPS categories: fibrin deposition \([9/12 (75\%) \text{ in EPS vs. } 15/57 (26\%) \text{ in non EPS}, p = 0.002]\), fibroblast swelling \([12/12 (100\%) \text{ in EPS vs. } 19/57 (33\%) \text{ in non EPS}, p = 0.0001]\), capillary angiogenesis \([10/12 (83\%) \text{ in EPS vs. } 16/57 (28\%) \text{ in non EPS}, p = 0.0006]\), and mononuclear cell infiltration \([9/12 (75\%) \text{ in EPS vs. } 21/37 (57\%) \text{ in non EPS}, p = 0.02]\). [Values are expressed as \(n\) positive specimens / \(n\) total specimens (%), with the \(p\) value obtained by chi-square test.]

We then compared the grade of those four histologic parameters for the biopsy categories EPS and peritonitis, and EPS and suspected EPS (Figure 3). In the comparison between the EPS \((n = 12)\) and peritonitis \((n = 20)\) categories, the grades of fibrin deposition \((1.25 \pm 0.04 \text{ in EPS vs. } 0.50 \pm 0.19 \text{ in peritonitis}, p = 0.025)\) and of peritoneal fibroblast swelling \((1.75 \pm 0.22 \text{ in EPS vs. } 0.55 \pm 0.19 \text{ in peritonitis}, p = 0.004)\) were significantly higher in the EPS category than in the peritonitis category. (Values are expressed as mean ± standard error of the mean and \(p\) value.) The
grades of the capillary angiogenesis and the mononuclear cell infiltration tended to be higher in the EPS category than in the peritonitis category, but the differences were not statistically significant. In the comparison between the EPS \((n = 12)\) and suspected EPS \((n = 5)\) categories, the grade of fibrin deposition \((1.25 \pm 0.04\) in EPS vs. \(0.20 \pm 0.20\) in suspected EPS, \(p = 0.038\)) and the grade of peritoneal fibroblast swelling \((1.75 \pm 0.22\) in EPS vs. \(0.80 \pm 0.38\) in suspected EPS, \(p = 0.05\)) were also significantly higher in the EPS category than in the suspected EPS category.

**Immunohistochemical findings in EPS**

Figure 4 shows the frequency of immunohistochemical detection of MIF, FGF, FGFR2, \(\alpha\)SMA, MIB1 (Ki-67), and BCL2 in peritoneal fibroblasts in the various biopsy categories. In peritoneum with EPS, \(\alpha\)SMA, MIF, FGF, and FGFR2 were all frequently expressed in peritoneal fibroblasts. In some EPS peritoneal biopsies, MIB1 and BCL2 were also expressed. Comparing the EPS \((n = 12)\) and non EPS \((n = 30)\) categories, the frequencies of MIF [10/12 (83%) in EPS vs. 11/30 (37%) in non EPS, \(p = 0.006\)], FGF [11/12 (92%) in EPS vs. 17/30 (57%) in non EPS, \(p = 0.04\)], FGFR2 [12/12 (100%) in EPS vs. 19/30 (63%) in non EPS, \(p = 0.02\)], MIB1 [7/12 (58%) in EPS vs. 5/30 (17%) in non EPS, \(p = 0.02\)], and BCL2 [6/12 (50%) in EPS vs. 3/30 (10%) in non EPS, \(p = 0.01\)] were significantly higher in EPS than in non EPS biopsies. [Values are expressed as \(n\) positive specimens / \(n\) total specimens (%), and the \(p\) value was obtained by chi-square test.]

Expression of those markers was also frequently detected in peritoneal biopsies from cases of infectious peritonitis. In particular, FGF, FGFR2, and \(\alpha\)SMA were frequently detected, even in peritoneum at the start of PD and peritoneum from ultrafiltration failure cases, although the staining intensities were weak, and the rates of positive fibroblasts were low. No expression of MIB1 or BCL2 was observed in peritoneum from the ultrafiltration failure and start of PD biopsy categories.
Encapsulating peritoneal sclerosis usually occurs after withdrawal of CAPD treatment. Prediction of the risk for EPS at the end of peritoneal dialysis is necessary for early diagnosis, treatment, and prevention of EPS. Peritoneal biopsy should be performed to evaluate the peritoneal inflammatory state at the end of peritoneal dialysis. Criteria for a diagnostic histology of EPS are required for interpreting the peritoneal biopsy.

The present study revealed some specific histologic findings for EPS: fibrin deposition, peritoneal fibroblast swelling, capillary angiogenesis, and mononuclear cell infiltration. Among those findings, the frequency and grade of fibrin deposition and peritoneal fibroblast swelling were significantly elevated in the EPS category as compared with the non EPS categories. The presence of capillary angiogenesis and mononuclear cell infiltration were also associated with EPS, but their grades were not statistically different from those in the non EPS categories.

Fibrin deposition is considered to be associated with the essential histologic phenomenon of EPS: encapsulation of the intestines by a thin membrane probably derived from fibrin. Morphology changes in peritoneal fibroblasts are also considered to be associated with progressive peritoneal fibrosis leading to adhesion of the intestines. We therefore considered that detection of fibrin deposition and fibroblast swelling were important to early recognition of EPS in the peritoneal biopsy, even though those histologic parameters are not specific for EPS and are frequently encountered in other clinical situations, especially infectious peritonitis.

We next performed an immunohistochemical examination to obtain more precise markers for fibroblast activation and proliferation. For peritoneal fibroblast activation markers, we used αSMA (4), MIF...
For peritoneal fibroblast proliferation markers, we used MIB1 (6) and BCL2 (7,8). Among those markers, MIF, FGF, and FGFR2 were useful for recognizing the activated state of peritoneal fibroblasts in EPS. However, those particular markers were also frequently expressed in peritoneal fibroblasts during infectious peritonitis and were not specific for EPS. Therefore, we consider that the differentiation of EPS from other inflammatory conditions such as infectious peritonitis remains difficult by immunohistochemical analyses. Nevertheless, detection of those markers provides useful information for evaluating the severity of the inflammatory state and determining a treatment strategy for EPS.

Conclusions
We propose the following histologic criteria for EPS: (A) fibrin deposition, (B) fibroblast swelling, (C) capillary angiogenesis, (D) mononuclear cell infiltration, and (E) expression of several immunohistochemical markers for peritoneal fibroblast activation and proliferation. Although those histologic criteria are not entirely specific for EPS at present, they are useful for the early detection of inflammatory changes leading to EPS. Careful histologic observation of peritoneal biopsy specimens after withdrawal from PD should be performed for the prevention and early treatment of EPS.

References
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Encapsulating peritoneal sclerosis (EPS) is recognized as a serious complication of peritoneal dialysis (PD). Involvement of inflammation is indispensable as a cause of EPS.

Here, we present a case of EPS that occurred in a 46-year-old man with alcoholic liver cirrhosis and ascites who was treated solely by hemodialysis (HD). We managed this patient surgically, with total intestinal enterolysis. Thick capsules had formed surrounding the ascites. Capsules had also covered the parietal peritoneum and intestinal surface and compressed the intestine. Examination of the ascites suggested the involvement of inflammation and the coagulation–fibrinolysis system.

Inflammation of a deteriorated peritoneum causes EPS, resulting in encapsulation subsequent to an accumulation of inflammatory products such as fibrin. Even without deterioration of the peritoneum, chronic inflammation and continual irritation caused EPS-like findings with encapsulation.

Key words
Encapsulating peritoneal sclerosis, hemodialysis, liver cirrhosis, surgical treatment

Introduction
Encapsulating peritoneal sclerosis (EPS) is the most serious and fatal complication of long-term peritoneal dialysis (PD), markedly reducing the effectiveness of the treatment. However, a recent retrospective study of clinical cases has elucidated the causes and pathologic features of EPS, and promoted the establishment of therapeutic guidelines for management (1,2). Previously, EPS-like symptoms have been reported as zuckergussdarm (3), regardless of the presence or absence of PD. Chronic intra-abdominal inflammation may therefore be involved in EPS-like symptoms.

Here, we report an encounter with a patient undergoing hemodialysis (HD), who developed EPS-like symptoms without a history of PD, and who was successfully treated by surgery. We also discuss the causes of EPS.

Patients and methods

Case report
The patient was a 46-year-old man with chronic glomerular nephritis who was introduced onto HD in November 1996. The patient’s condition was complicated by long-standing alcoholic cirrhosis, and chronic ascites was observed even after introduction of HD. However, the patient had no history of intra-abdominal inflammation such as peritonitis.

In July 2001, the patient was admitted to our hospital with ileus symptoms accompanied by abdominal pain and vomiting. An abdominal computed tomography scan showed that the small intestine was compressed by encapsulated ascites (Figure 1). Because decompression by ascites puncture improved the symptoms of ileus, the patient was discharged within a few days. However, the ileus symptoms recurred repeatedly (November 2001, and January, February, and April 2002). On each occasion, the patient was treated by decompression by ascites puncture. Surgical laparotomy was eventually scheduled because the recurrence interval had gradually decreased.

Preoperative biochemical blood examinations showed no abnormal findings suggestive of liver cirrhosis, but C-reactive protein (CRP) levels were at the upper limit of normal (0.5 mg/dL). The CRP levels increased only when ileus symptoms were ob-
served; during steady state, they varied within the upper limit of normal (0.3 – 0.5 mg/dL).

Laparotomy was performed on May 27, 2002. When the abdominal wall was opened by median incision, white capsules in contact with the parietal peritoneum were detected, and a large quantity of yellowish-brown ascites was observed when the white capsules were incised. In addition, after aspiration of the ascites, thick, glossy capsules were observed covering the surface of the intestines and liver below (Figure 2). The capsules adhered strongly to the visceral peritoneum, and fibrous adhesion of the intestines was observed within the capsules.

The entire small intestine was isolated by completely dissecting the visceral capsules from the surface of the small intestine (Figure 3). Although the transverse colon, greater omentum, and liver surfaces were also covered with capsules, these areas were not dissected, because the capsules were not involved in the development of the EPS-like symptoms.

Table I shows the analysis of the ascites collected intraoperatively. Although negative results were obtained from a bacterial culture and a measurement of endotoxin activity, leukocytosis with lymphocyte predominance, increased levels of interleukin 6 (IL-6), fibrin/fibrinogen degradation products (FDPs), thrombin–antithrombin III complex (TAT), and D-dimers were observed, suggesting inflammation and increased activity of the coagulation–fibrinolysis system.

Pathology examination of the specimens of parietal peritoneum demonstrated disappearance of the mesothelial cells, growth of hyaline connective tissues, fibrinoid precipitation, and mild inflammatory cell infiltration. In addition, pathology examination of capsule specimens demonstrated growth of fibrous connective tissues and infiltration by inflammatory cells.

The postoperative course of this patient was favorable, and oral food intake was initiated from the 5th postoperative day. The patient was discharged from our hospital on the 18th postoperative day. At the time of writing, 6 months have passed since surgery, and
the patient is undergoing stable HD without ileus symptoms.

Discussion
In EPS associated with PD, inflammation combined with peritoneal deterioration (attributable to prolonged exposure of the peritoneum to peritoneal dialysate) induces formation of intestinal adhesions and capsules, resulting in development of symptoms of ileus. Therefore, peritoneal deterioration and inflammation seem to be essential to the development of EPS.

However, EPS-like symptoms have been reported even in patients with abdominal disorders not associated with PD (4,5). At the start of the 20th century, EPS-like symptoms—referred to as zuckergussdarm or peritonitis cronica fibrosa incapsulata (3), and marked by intestinal adhesion and capsule formation or encapsulation resulting from chronic intra-abdominal inflammation—were thought to cause symptoms of ileus. Various abdominal disorders such as tuberculous peritonitis (6), intra-abdominal hemorrhage (7), chronic ascites (8), and cancerous peritonitis (9) have been reported to cause such chronic intra-abdominal inflammation. A retrospective study of clinical cases at 18 surgical institutions in France (10) showed that 32 patients with EPS-like symptoms were surgically treated during the past 16 years. Of those cases, only 3 were associated with PD. The rest probably arose from these causes: surgical invasion (19 cases), treatment with beta-blockers (4 cases), liver cirrhosis (4 cases), general peritonitis (3 cases), and iatrogenic causes (5 cases).

Thus, in patients with EPS-like symptoms, capsule formation on the intestinal surface may induce ileus symptoms—for which findings of peritoneal deterioration are not essential. Because the duration of PD and the severity of peritoneal deterioration both vary, even in patients with PD-associated EPS, the severity and duration of inflammation—in addition to the severity of peritoneal deterioration—are important factors in the development of EPS.

In our patient, EPS was caused by cirrhotic ascites, and the properties of the ascites played an important role in capsule formation. Increased levels of IL-6, FDPs, and TAT in the ascites demonstrated inflammation and were associated with an increase in the activity of the coagulation–fibrinolysis system, probably resulting in fibrin precipitation and fibrous capsule formation. Although the cause of the chronic inflammation remains unclear, reduced function of the reticuloendothelial system because of liver cirrhosis may have been involved. In our patient, the EPS-like symptoms were completely eliminated after surgery; however, capsule formation may recur because the liver cirrhosis persists. Strict observation of this patient is therefore needed in the future.

Conclusions
Inflammation combined with peritoneal deterioration causes EPS; however, long-term chronic stimulation of the peritoneum may result in capsule formation and the eventual development of EPS-like symptoms even in patients without peritoneal deterioration—as observed in our patient. Thus, the pathologic condition of encapsulating ileus not associated with peritoneal deterioration should be called “encapsulating peritonitis” (EP).

References

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Role of Adhesion Molecules in the Progression of Peritoneal Sclerosis

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To investigate the role of adhesion molecules [intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and integrin \(\alpha_5\beta_1\)] in the progression of encapsulating peritoneal sclerosis (EPS) under peritoneal dialysis, we examined changes in the expression of those adhesion molecules in Wistar–Kyoto (WKY) rats treated with acidic dialysis solution with or without angiotensin II type 1a receptor blocker (ARB).

We divided 48 WKY rats into 4 groups and dialyzed them with various solutions as follows: (1) pH 7 1.5% glucose dialysis solution (control group, \(n = 12\)); (2) pH 3.5 1.5% glucose dialysis solution (EPS group, \(n = 12\)); (3) pH 3.5 1.5% glucose dialysis solution, plus oral administration of CS866 5 mg/kg daily (ARB group, \(n = 12\)); and (4) pH 3.5 1.5% glucose dialysis solution, plus oral administration of amlodipine (CA group, \(n = 12\)). We injected the dialysis solutions into the abdominal cavity and administered the ARB and CA daily for 42 days. On days 3, 7, 14, and 42, three rats in each group were humanely killed by decapitation, and we studied the expression of adhesion molecules in peritoneum by the immunofluorescence method.

In the EPS rats, expression of adhesion molecules was observed in peritoneum on day 3 after start of acidic solution treatment, in conjunction with an increment of interleukin 6 (IL-6) in the dialysate. The peritoneum of EPS rats showed peritoneal fibrosis with interstitial cell infiltration. Treatment with ARB significantly suppressed expression of adhesion molecules in the peritoneum and suppressed peritoneal fibrosis. Treatment with a neutral solution induced no peritoneal fibrosis nor expression of adhesion molecules in the peritoneum.

Our results suggest that adhesion molecules play an important role in the progression of peritoneal fibrosis and resultant EPS. Treatment with ARB prevents the progression of peritoneal fibrosis and suppresses expression of adhesion molecules in the peritoneum.

Key words
Continuous ambulatory peritoneal dialysis, encapsulating peritoneal sclerosis, adhesion molecules

Introduction
Encapsulating peritoneal sclerosis (EPS) is one of the most serious complications for patients on continuous ambulatory peritoneal dialysis (CAPD) because it causes high morbidity and mortality rates from bowel obstruction (1–6). Several factors may induce EPS—among them, peritonitis, acetate buffer, chlorhexidine, high glucose, beta-blockers, plasticizers, etc.—but the mechanisms and etiopathogenesis remain unclear (3–5). Some investigators have reported that acidic dialysis solution may induce peritoneal fibrosis (6). Activation of the renin–angiotensin system has been reported to aggravate the progression of renal fibrosis and cardiac hypertrophy (7,8).

Angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II type 1a receptor blocker (ARB) belong to one class of drugs that has been shown to be of benefit in the treatment of renal fibrosis (9,10). Both ACEI and ARB have been shown to slow the progression of nephropathy in patients with diabetes and in patients with nephropathies from other causes.

Recently, many cases of EPS in CAPD patients have been reported; however, the mechanism of the progression of EPS is not known. The peritoneum is an anatomic site in which the immune response is vigorous, and it can be used for in vivo immunization. In the present study, to investigate the role of adhesion molecules [intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and integrin \(\alpha_5\beta_1\)] in the progression of EPS under peritoneal dialysis, we followed the expression of adhesion molecules in the peritoneum of Wistar–Kyoto (WKY) rats.
**Materials and methods**

Our study was performed in 48 male WKY rats weighing between 200 g and 350 g. Protocols were carried out in strict accord with the guideline for the use of laboratory animals published by the U.S. National Institutes of Health (12) and were approved by the Institutional Animal Care and Use Committee of Saitama Medical School. Peritoneal catheter (PE-50) insertion was carried out according to a previously described method (11).

For the first 2 days after catheter insertion, the peritoneal cavity was rinsed with 10 mL saline. The 48 rats were then divided into 4 groups and dialyzed with various solutions as follows: (A) pH 7 1.5% glucose dialysis solution (control group, \( n = 12 \)); (B) pH 3.5 1.5% glucose dialysis solution (EPS group, \( n = 12 \)); (C) pH 3.5 1.5% glucose dialysis solution, plus oral administration of CS866 (an angiotensin II type 1a receptor antagonist) 5 mg/kg daily (ARB group, \( n = 12 \)); and (D) pH 3.5 1.5% glucose dialysis solution, plus oral administration of amlodipine (a Ca antagonist; CA group, \( n = 12 \)).

We injected the dialysis solutions into the abdominal cavities of the rats daily for 42 days. On days 3, 7, 14, and 42, after peritoneal equilibration test (PET) measurements, 3 rats from each group were humanely killed by decapitation, and expression of ICAM-1, VCAM-1, and integrin \( \alpha 5\beta 1 \) in peritoneum were studied by the immunofluorescence method.

**Peritoneal equilibration test**

The PET in each rat was performed under pentobarbital anesthesia (50 mg/kg). The animal was placed on a heating pad to maintain a temperature of 37°C. Another intra-abdominal catheter (PE-50) was placed for the purpose of the PET, and a 20-mL dose of 10% glucose solution was injected into the abdominal cavity. Two hours later, the solution in the abdominal cavity was measured as for a PET.

**Immunofluorescence microscopy**

Tissues from the rats were perfused with phosphate-buffered saline (PBS). Organs were removed, sliced, and postfixed for 4 hours in the same solution. Sliced tissues were then cryoprotected overnight in PBS containing 20% (weight/volume) sucrose, embedded in OCT compound (Miles Scientific, Naperville, IL, U.S.A.), and frozen in liquid N\(_2\).

Cryostat sections (3 – 6 \( \mu m \)) were incubated for 30 minutes in PBS containing 1% bovine serum albumin and then with purified rat ICAM-1 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), rat VCAM-1 antibodies (Santa Cruz Biotechnology), and rat integrin \( \alpha 5\beta 1 \) antibodies (Santa Cruz Biotechnology), 2 – 10 \( \mu g/mL \) for 4 hours at room temperature. Control experiments were performed using PBS. For immunofluorescence, slides were incubated for 30 minutes with anti-goat immunoglobulin G conjugated with fluorescein and were then washed in PBS. Slides were photographed on Fujichrome Sensia II 100 daylight film using an Olympus AX80 ProVis fluorescence microscope (Olympus Optical, Tokyo, Japan).

**Statistical analysis**

All results are expressed as mean ± standard error of the mean of the experiments. Statistical analysis was performed using StatView software, version 5.0, on a Macintosh computer. Two-way analysis of variance followed by the Scheffé \( F \)-test for paired data were used for statistical analyses of blood pressure. Comparisons between groups were made using the Student \( t \)-test for unpaired data. Statistical significance was assumed at a \( p \) value of < 0.05.

**Results**

**Necropsy findings**

In rats treated with pH 3.5 dialysis solution, necropsy findings showed evidence of EPS. The typical appearance was multiple surfaces covered with granulation tissue or fibrotic tissue or both. Peritoneal dullness or sclerosis was interpreted as evidence of gross thickening of the peritoneum and was seen in all necropsies of rats injected with pH 3.5 dialysis solution. Adhesive peritonitis with multiple adhesions or complete encapsulation was seen in all necropsies of animals injected with pH 3.5 solution. No evidence of encapsulating adhesions was seen in animals treated with pH 7.0 dialysis solution. Partial adhesive peritonitis was seen in CS866- and amlodipine-treated rats; however, CS866 and amlodipine treatment prevented the progression of peritoneal fibrosis and adhesions of the peritoneum.

**Light microscopy findings**

After 42 days of intraperitoneal injections of dialysis solution, we found that subserosal tissue thickening
became more significant in parallel with the acidity of the solution (Figure 1). Among the solutions used, pH 3.5 dialysis solution induced loss of the mesothelial layer, resulting in typical peritoneal fibrosis. The subserosal fibrous tissue contained spindle-shaped and rounded mononuclear cells (for example, fibroblasts and monocytes) and dense amorphous substances (for example, collagen and fibronectin). In addition, remarkable vascular sclerosis was observed in the subserosal tissue of the rats injected with pH 3.5 solution. On the other hand, peritoneum from rats injected with pH 7.0 dialysis solution showed no subserosal tissue thickening. Subserosal tissue thickening was significantly reduced by oral administration of amlodipine and CS866. In CS866-treated rats, subserosal tissue thickening was significantly reduced as compared with the reduction seen in amlodipine-treated rats.

Changes in expression of adhesion molecules
Figure 2 shows the expression of ICAM-1 in the peritoneum of rats treated with pH 3.5 dialysis solution. On day 3 after dialysate injection, ICAM-1, VCAM-1, and integrin α5β1 were somewhat expressed in peritoneum. On day 7, ICAM-1, VCAM-1, and integrin α5β1 were significantly expressed. On day 42, expression of those adhesion molecules was diminished. Figure 3 shows the expression of ICAM-1 in the peritoneum of CS866-treated rats. Treatment with CS866 significantly suppressed expression of ICAM-1, VCAM-1, and integrin α5β1 in peritoneum. Treatment with neutral solution (pH 7.0) induced no expression of adhesion molecules in peritoneum.

Changes in peritoneal function measured by PET
The ultrafiltration volume in rats treated with pH 7.0 dialysis solution was 15.6 ± 1.8 mL. Treatment with pH 3.5 dialysis solution induced a significant reduction in ultrafiltration volume (3.1 ± 0.4 mL, p < 0.01). On the other hand, treatment with CS866 induced significant increases in ultrafiltration volume (8.9 ± 1.1 mL), and treatment with CA induced no significant change in ultrafiltration volume (4.8 ± 0.5 mL).

Discussion
In the present study, long-term intraperitoneal administration of acidic dialysis solution induced severe peritoneal adhesions. The rats treated with acidic dialysis solution had a disease process and pathologic conditions similar to those seen in human EPS, with weight loss, peritoneal sclerosis, and adhesions resulting in full-bowel EPS and cooon formation.

In the rats of the EPS group, expression of adhesion molecules was observed in the peritoneum on day 3 of treatment with acidic solution, in conjunction with an increment of IL-6 in the dialysate. The peritoneum of rats in the EPS group showed peritoneal fibrosis with interstitial cell infiltration. Treatment with ARB significantly suppressed the expression of adhesion molecules in the peritoneum and suppressed peritoneal fibrosis. Treatment with neutral solution induced no peritoneal fibrosis nor expression of adhesion molecules in the peritoneum.

The renin–angiotensin system is well known as an important regulator of renal function. Angiotensin II has several important endocrine, autocrine, and paracrine functions within the kidney. That peptide is integrally involved in the autoregulation of glomerular filtration rate in response to changes in renal perfusion pressure. Previously, we demonstrated that the renin–angiotensin system plays a key role in the regulation of peritoneal function in rats on peritoneal dialysis.
FIGURE 2  Expression of intracellular adhesion molecule 1 (ICAM-1) in peritoneum by immunofluorescence in a rat model of encapsulating peritoneal sclerosis (EPS).

FIGURE 3  Expression of intracellular adhesion molecule 1 (ICAM-1) in peritoneum by immunofluorescence in a rat model of encapsulating peritoneal sclerosis (EPS) treated with acidic solution plus CS866 [angiotensin II type 1a receptor blocker (ARB)].

(7). However, the long-term effects of activation and inhibition of the renin–angiotensin system on peritoneal function remain unclear.

The present study was carried out to investigate the role of the renin–angiotensin system in the progression of peritoneal fibrosis and adhesive peritonei-
Angiotensin II may contribute to the initiation and progression of nephropathy by several non hemodynamic mechanisms (8–11). In cultured human mesangial cells, angiotensin II increases the production of cytokines, including IL-6, tumor necrosis factor \( \alpha \) (TNF\( \alpha \)) and transforming growth factor \( \beta \) (TGF\( \beta \)) (13–15). It has been reported that TGF\( \beta \) is pivotal in the hypertrophy of mesangial cells and tubular cells, inducing the progression of glomerulosclerosis and interstitial fibrosis. Other cytokines, such as TNF\( \alpha \), also contribute to the progression of chronic renal disease. In addition, angiotensin II induces expression of certain adhesion molecules in mesangial cells in rat kidney. Those angiotensin-II induced responses can be inhibited by treatment with ACEI or ARB (8–11).

Angiotensin II is recognized as an important factor in the pathogenesis of hypertension and atherosclerosis. Angiotensin II–induced monocyte binding was not associated with induction of E-selectin, VCAM-1, or ICAM-1. Angiotensin II can accelerate the rate of atherosclerosis by increasing monocyte binding to endothelium (4). The adhesion molecules E-selectin, VCAM-1, and ICAM-1 participate in the mediation of interactions between leukocytes and endothelial cells, and they have been found to be expressed in atherosclerotic plaques. Angiotensin II, as an effector of the renin–angiotensin system, influences endothelial expression of E-selectin, VCAM-1, and ICAM-1 (5). Angiotensin II type 1a receptor antagonist reduces baseline soluble ICAM-1 levels and angiotensin II–related ICAM-1 increments. Angiotensin II upregulates ICAM-1 expression by human umbilical vein endothelial cells (HUVECs) and stimulates \textit{in vitro} and \textit{in vivo} soluble ICAM-1 release. Angiotensin II type 1a receptor blockade inhibits those endothelial effects of angiotensin II (6).

In the present study, interstitial fibrosis of the peritoneum induced by acidic solution was prevented by oral administration of ARB. Those changes in the peritoneum are completely compatible with the changes observed in the kidney.

Encapsulating peritoneal sclerosis is a serious complication induced by long-term peritoneal dialysis. We previously reported that acidic solution induced peritoneal sclerosis, peritoneal hypertrophy, and EPS (16). Recently, expression of adhesion molecules on lymphocytes, macrophages, and peritoneum have been studied in patients on CAPD. Fall et al. (17) reported the relative expression of adhesion molecules on monocytes and peritoneal macrophages obtained from patients on CAPD, the level of expression of various adhesion molecules having been analyzed by flow cytometry using receptor-specific monoclonal antibodies (17). Elsner et al. (18) reported that, during bacterial peritonitis in patients on CAPD, leukocytes—particularly polymorphonuclear neutrophilic granulocytes (PMNs)—migrated into the peritoneal cavity, and that adhesion molecules such as ICAM-1 (CD54) were involved in the interaction between endothelial cells and PMNs, leading to the accumulation of PMNs at the site of inflammation. Peritoneal PMNs from CAPD peritonitis patients showed enhanced expression of ICAM-1 mRNA; ICAM-1 is therefore newly synthesized when PMNs invade the peritoneal cavity (18). Honda et al. (19) studied the expression of ICAM-1 and lymphocyte function–associated antigen 1 (LAF-1) in lymphocytes to assess the immunologic aspects of CAPD. They concluded that upregulation of intercellular adhesion molecules is also observed in lymphocytes in dialysis solution and peripheral blood when a patient uses high-osmolar dialysate frequently. From those reports, we can speculate that adhesion molecules play an important role in the progression of inflammation during CAPD.

In the present study, we investigated the expression of adhesion molecules (ICAM-1, VCAM-1, and integrin \( \alpha 5 \beta 1 \)) in the rat peritoneum in the early stages of an EPS model. After 42 days, expression of adhesion molecules was diminished. In a group of rats treated with neutral solution (pH 7.0), no expression of adhesion molecules was induced. In a group treated with acidic dialysis solution and oral ARB, expression of adhesion molecules was significantly suppressed, and ultrafiltration volume was increased.

There is a possibility that adhesion molecules play an important role in the progression of peritoneal sclerosis, peritoneal hypertrophy, and peritoneal function.
failure. We consider that adhesion molecules are related to the start of sclerosis of the peritoneum. However, the role that adhesion molecules play in the pathogenesis of EPS is unknown. Further experiments are required.

Conclusions
Our results suggest that adhesion molecules play an important role in the progression of peritoneal fibrosis and resultant EPS. Treatment with ARB prevents progression of peritoneal fibrosis and suppresses expression of adhesion molecules in the peritoneum.

References

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