Effects of liposome-encapsulated clodronate on chlorhexidine gluconate-induced peritoneal fibrosis in rats

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Abstract

Background. Long-term peritoneal dialysis (PD) causes morphologic and functional changes in the peritoneum that hamper the continuation of PD therapy. Because macrophages play important roles in the development of peritoneal fibrosis and liposome-encapsulated clodronate (LC) induces macrophage apoptosis, we examined the effect of LC on chlorhexidine gluconate (CG)-induced peritoneal fibrosis in rats.

Methods. Fifty Sprague–Dawley rats were randomly allocated into five groups of 10 receiving intraperitoneal (i.p.) injections (1.5 mL/100 g) of either 0.1% CG (four groups) or vehicle (one group) three times a week. Three of the CG-treated groups also received intravenous injections of clodronate twice a week: 10 mg of LC, 20 mg of LC or 20 mg of unencapsulated clodronate (UC20). Twenty-one days after the first i.p. injection, the rats were sacrificed and the parietal peritoneum was harvested.

Results. The number of peritoneal macrophages in the rats given clodronate was significantly smaller than that in rats not given clodronate (92.0 ± 4.6 cells per field). It was 54.1 ± 3.2 cells per field in the group given 20 mg UC, 43.2 ± 5.2 cells per field in the group given 10 mg LC and 27.2 ± 2.8 cells per field in the group given 20 mg LC. This decrease in macrophage number was paralleled by decreases in peritoneal thickening, in the number of mesothelial cells staining positive for cytokeratin and α-smooth muscle actin and in messenger RNA expression for transforming growth factor-β1 and collagen types I and III.

Conclusions. These data suggest that macrophages play a critical role in the development of peritoneal fibrosis and that LC may be useful for treating peritoneal fibrosis in PD patients.

Keywords: continuous ambulatory peritoneal dialysis; epithelial-mesenchymal transition; liposome-encapsulated clodronate; macrophages; peritoneal fibrosis

Introduction

Peritoneal dialysis (PD) is often used to treat patients in end-stage renal failure (ESRF), but long-term PD therapy
causes morphologic and functional changes in the peritoneum [1] that can progress to encapsulating peritoneal sclerosis [2]. The peritoneum of patients undergoing long-term PD therapy typically shows severe submesothelial thickening with massive accumulation of collagen, loss of mesothelial cells and vasculopathy (neangiogenesis): a condition called peritoneal fibrosis [3, 4]. The mechanism of peritoneal fibrosis in PD patients remains poorly understood, and no effective therapy is available for now. Suga et al. [5] and Ishii et al. [6] developed experimental models for peritoneal fibrosis in rats and encapsulating peritoneal sclerosis in mice by injecting chlorhexidine gluconate (CG) into the peritoneum, and these animal models show many pathological findings in the peritoneum of long-term PD patients such as increases in the expression of type III collagen and α-smooth muscle actin (α-SMA)-positive cells [7].

Infiltrating inflammatory cells, especially macrophages and activated mesothelial cells, produce a wide variety of cytokines, growth factors and chemokines that cause membrane deterioration [8]. Inflammatory mediators such as interleukins (IL)-1 and -8 contribute to peritoneal fibrosis by stimulating resident fibroblast proliferation and extracellular matrix (ECM) deposition [9], and transforming growth factor-β (TGF-β) induces the epithelial–mesenchymal transition (EMT) of mesothelial cells [10–15]. The EMT of mesothelial cells enhances the production of vascular endothelial growth factor (VEGF) [11, 12], an angiogenic growth factor eliciting peritoneal fibrosis via neangiogenesis of submesothelial tissues [2, 10]. Thus, EMT has been suspected to induce peritoneal fibrosis associated with decreased ultrafiltration and increased solute transport [16].

Liposome-encapsulated clodronate (LC), a bisphosphonate encapsulated by liposomes, is known to systemically deplete macrophages in vivo [17]. Intravenously injected LC is rapidly taken up by macrophages. After the liposomes are digested by lysosomal phospholipases, the free clodronate released into the cells induces rapid apoptosis of the macrophages. Intravenously administered LC has been shown to cause the depletion of macrophages but not neutrophils or lymphocytes [18, 19].

In this study, we analyzed the effects of LC on CG-induced peritoneal fibrosis in rats in order to better understand the role of initial infiltrating macrophages in the process of progressive peritoneal fibrosis and to find an effective treatment of peritoneal fibrosis.

Materials and methods

Preparation of LC and unencapsulated clodronate

Clodronate was purchased from Sigma–Aldrich (St Louis, MO), and LC was prepared by using the liposome formulation kit COATSOME-EL-01-N (Nihonbush, Tokyo, Japan) according to the manufacturer’s instructions. Briefly, 100 mg of clodronate was dissolved in 2 mL of distilled water and the solution mixed with the component lipids by shaking. Unencapsulated clodronate (UC) was prepared by dissolving 100 mg of clodronate in 2 mL of distilled water.

Animals

All animal experiments were performed according to the National Defense Medical College guidelines for the care and use of laboratory animals in research. The study protocols were approved by the animal ethical committee of the National Defense Medical College. Male Sprague–Dawley (SD) rats, 8 weeks old, were obtained from CLEA Japan, Inc. (Tokyo, Japan) and were given water and standard chow ad libitum. Fifty SD rats were divided into five groups of 10. The CG group was given intraperitoneal (i.p.) injections of 1.5 mL/100 g body weight of 0.1% CG in 15% ethanol dissolved in saline three times a week, and the control group given the same dose of 15% ethanol dissolved in saline but without CG three times a week. The other three groups were given i.p. injections of 1.5 mL/100 g body weight of 0.1% CG in 15% ethanol dissolved in saline three times a week and also received intravenous injections of clodronate twice a week: the UC20 group was given 20 mg of UC, the LC10 group was given 10 mg of LC and the LC20 group was given 20 mg of LC. When the rats were sacrificed 21 days after the first i.p. injection, they did not show any bacterial infection in their ascites.

Peritoneal samples were immediately cut into several pieces that were either fixed in 10% formalin and embedded in paraffin for histological analysis or stored at −80°C for later studies.

Histological assessment and quantification of the submesothelial compact zone

Three micrometer-thick sections of formalin-fixed paraffin-embedded tissues from the anterior abdominal wall were stained with hematoxylin and eosin (H&E) and with Masson’s trichrome. Parietal peritoneal surfaces were evaluated by morphometry. Thickening of the submesothelial compact zone (the region from the abdominal muscle surface to the peritoneal cavity) was regarded as interstitial fibrosis [2]. Quantification of the submesothelial compact zone was performed by image analysis. For each of the rats, five images (×200) that contained the submesothelial compact zone were captured by using a digital camera. The thickness of the submesothelial compact zone in each image was measured by using image analysis software (Lumina Vision Ver. 2.04; Mitani Corp., Fukui, Japan) at the five randomly selected positions of each field and the results were averaged in each group [2, 20].

Immunoperoxidase staining

Three micrometer-thick sections of formalin-fixed paraffin-embedded tissues were evaluated by immunoperoxidase staining for the myofibroblasts with a mouse anti-human α-SMA antibody (1A4; DAKO Corp., Carpintia, CA), macrophages with a mouse anti-rat macrophage antibody (ED-1; Millipore, Massachusetts, MA), VEGF with a mouse anti-rat VEGF antibody (VG-1; Abcam, Tokyo, Japan) and platelet endothelial-cell adhesion molecule-1 (PECAM-1) with a goat anti-rat PECAM-1 antibody (M20; Santa Cruz Biotechnology, Santa Cruz, CA). The usual two-step indirect immunoperoxidase staining was performed as described previously [21, 22]. Images of five non-overlapping submesothelial compact zones, at ×200 magnification, from each section were obtained with a digital camera. The numbers of positive cells by each staining were counted by using Lumina Vision Ver. 2.04 (Mitani Corp.) and were averaged in each group.

Double staining for cytokeratin and α-SMA

Double staining for cytokeratin and α-SMA was performed on 3-μm-thick sections of formalin-fixed paraffin-embedded tissues. After being blocked with a blocking buffer (StainingBlock™, PIERCE, Rockford, IL), sections were incubated with a mouse anti-human cytokeratin antibody (AE1/AE3; DAKO Corp.) overnight at 4°C. Then, the sections were incubated with Alexa-Fluor-594-conjugated donkey anti-mouse IgG as the secondary antibody and washed with phosphate-buffered saline (PBS). After being blocked with normal mouse serum (DAKO Corp.), the sections were incubated with fluorescein isothiocyanate-conjugated mouse anti-human α-SMA antibody (Sigma–Aldrich), washed with PBS and mounted. Photomicrographs of five random peritoneal fields (×200) from each animal were obtained using a digital camera.
camera, and the picrosirius red-positive area of the submesothelial zone was measured by using Lumina Vision Ver. 2.04 (Mitani Corp.).

**Chloroesterase staining for neutrophils**

We used enzyme histochemistry, that is, chloroesterase staining to detect neutrophils on 3-μm-thick sections of formalin-fixed paraffin-embedded tissues. Procedures for this technique were described previously [23, 24]. As substrate, naphthol AS-D chloroacetate and Fast Blue BB salt were used (blue reactive products).

**Real-time reverse transcription–polymerase chain reaction**

Total RNA was extracted from the peritoneal tissues by using the RNaseasy fibrous tissue mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. RNA was quantified by absorbance at 260 nm. A 1-μg aliquot of total RNA was reverse-transcribed into first-strand complementary DNA (cDNA) with random primers by using the PrimeScript RT reagent kit (total volume 20 μl) (Takara, Shiga, Japan). The constructed cDNA served as a template for real-time polymerase chain reaction (PCR). We used TaqMan Gene Expression Assays with primer/probe sets for rat TGF-β1, type I collagen, type III collagen, VEGF, snail2; a transcription factor which mediates EMT, forkhead box C2 (FOXC2); a transcription factor that acts as an inducer of EMT [25] and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Applied Biosystems, Foster City, CA). PCR was performed in triplicate, using the ABI Prism 7900 sequence detection system (Applied Biosystems), in 96-well microtiter plates in a final volume of 20 μL, 10 μL of which was TaqMan Universal PCR Master Mix (Applied Biosystems), 1 μL of which was the primer/probe mix and 9 μL of which was cDNA. Reaction conditions consisted of pre-incubation for 2 min at 50°C and for 10 min at 95°C and then 40 cycles of 15 s at 95°C and 1 min at 60°C. The relative amount of messenger RNA (mRNA) was calculated using the comparative CΔCt method. All specific amplification products were normalized against GAPDH mRNA, which for internal control was amplified in the same reaction.

**Statistical analysis**

Data are shown as mean ± SE. Statistical analyses were performed using one-way analysis of variance and individual comparisons were made by Tukey’s post hoc analysis. A P-value < 0.05 was considered significant.

**Fig. 1.** Representative photomicrographs (original magnification: ×200) of Masson’s trichrome staining in peritoneum slices in (a) the control group, (b) the CG group, (c) the UC20 group, (d) the 10 mg of LC10 group and (e) the 20 mg of LC20 group. (f) The thickness for Masson’s trichrome staining in the submesothelial compact zone (mean ± SE, n = 10). In the LC10 and LC20 groups, the thickness was significantly less than that in the CG group. In the LC20 group, the thickness was significantly less than thicknesses in the UC20 group. **P < 0.01, ###P < 0.0001 versus CG group. †P < 0.05, †††P < 0.001 versus UC20 group. §§§P < 0.001 versus LC10 group. ‡P < 0.05 versus LC20 group.**
All statistical analyses were performed with JMP ver. 8.0.2 software (SAS Institute Inc., Cary, NC).

Results

Peritoneal fibrosis levels

The thickness of the submesothelial compact zone in the CG group (147.2 ± 9.7 μm) was significantly (P < 0.0001) greater than that in the control group (10.0 ± 0.6 μm), significantly less in the LC10 group (93.1 ± 17.0 μm, P < 0.01 versus the CG group) and less in the LC20 group (59.6 ± 5.4 μm, P < 0.0001 versus the CG group). The thickness of the submesothelial compact zone in the LC20 group was significantly less than that in the UC20 group (109.3 ± 12.2 μm, P < 0.05) (Figure 1).

The sirius red-positive area in the submesothelial compact zone of the CG group was significantly larger than that in the control group (P < 0.0001), significantly less in the UC20 group (P < 0.001 versus the CG group), less in the LC10 group (P < 0.0001 versus the CG group) and less in the LC20 group (P < 0.0001 versus the CG group) (Figure 2).

Macrophage levels

The number of macrophages (ED-1-positive cells) in the submesothelial compact zone of the CG group (92.0 ± 4.6 cells per field, Figure 3b) was markedly larger than that in the submesothelial compact zone of the control group (1.7...
The number of macrophages in the UC20 group (54.1 ± 3.2 cells per field) was significantly less than that in the CG group (P < 0.0001). The numbers of macrophages in the LC20 group (27.2 ± 2.8 cells per field) were less than that in the UC20 group (P < 0.001), and the numbers of macrophages differed significantly between the LC10 and the LC20 groups (P < 0.001) (Figure 3f).

Myofibroblast levels

The number of myofibroblasts (α-SMA-positive cells) in the submesothelial compact zone of the CG group was markedly greater than that in the submesothelial compact zone of the control group (P < 0.0001). The numbers of myofibroblasts in the UC20, LC10 and LC20 groups were less than that in the CG group (P < 0.001, P < 0.0001 and P < 0.0001, respectively) (Figure 4).

Levels of VEGF and PECAM-1

The number of VEGF-positive cells in the submesothelial compact zone of the CG group (73.4 ± 4.3 cells per field) was markedly larger than that in the submesothelial compact zone of the control group (11.1 ± 0.2 cells per field, P < 0.0001, Figure 5). The number of VEGF-positive cells in the UC20 group (45.7 ± 3.8 cells per field) was significantly less than that in the CG group (P < 0.0001). The number of VEGF-positive cells in the LC20 group (21.4 ± 2.3 cells per field) was much less than that in the UC20 group (P < 0.001).
The number of PECAM-1-positive vessels in the submesothelial compact zone of the CG group (20.6 ± 3.2 vessels per field) was markedly larger than that in the submesothelial compact zone of the control group (0 ± 0, P < 0.0001, Figure 6). The increased number of PECAM-1-positive vessels in the UC20 group (9.0 ± 0.8 vessels per field) was significantly smaller than that in the CG group (P < 0.0001), and the increased number of PECAM-1-positive vessels in the LC20 group (2.7 ± 0.3 vessels per field) was significantly less than that in the UC20 group (P < 0.05).

Double staining for cytokeratin and α-SMA

We performed cytokeratin staining as a mesothelial cell marker and double staining for cytokeratin and α-SMA as a marker of the EMT of mesothelial cells. In the control group, cytokeratin-positive mesothelial cells formed a monolayer at the surface of the peritoneum (Figure 7a). A layer of cytokeratin-positive mesothelial cells was not seen in the CG group (Figure 7b), and the number of cells positive for both cytokeratin and α-SMA in the submesothelial compact zone of the CG group (40.0 ± 2.6 cells per field, Figure 7h) was markedly larger than that in the submesothelial compact zone of the control group (1.9 ± 0.2 cells per field, P < 0.0001). The number of double-positive cells in the UC20 group (24.6 ± 2.0 cells per field) was significantly less than that in the CG group (P < 0.0001) and the numbers of double-positive cells in the LC10 and LC20 groups (15.8 ± 2.6 and 8.7 ± 1.0 cell per field, respectively) were significantly less than the number of
double-positive cells in the UC20 group (P < 0.05 and P < 0.001, respectively). Furthermore, we can see a layer of cytokeratin-positive mesothelial cells at the surface of the peritoneum in the LC20 group (Figure 7c).

**Gene expression of TGF-β1, type I collagen, type III collagen, snail2, FOXC2 and VEGF**

The mRNA expressions for TGF-β1, type I collagen and type III collagen in the CG group were significantly larger than those in the control group. TGF-β1 mRNA expressions in the LC10 group (2.1 ± 0.5-fold) and LC20 group (2.9 ± 0.4-fold) were significantly less than that in the CG group (7.4 ± 1.8-fold; P < 0.01 and P < 0.05, respectively). Type I and III collagen mRNA expressions in the LC groups were significantly less than those in the CG group (P < 0.05).

The mRNA expressions for snail2 and FOXC2, markers of EMT [25], in the CG group were significantly larger than those in the control group. Snail2 mRNA expression in LC20 group (1.6 ± 0.7-fold) was significantly less than that in the CG group (6.4 ± 2.2-fold; P < 0.05). FOXC2 mRNA expressions in the LC10 group (1.0 ± 0.2-fold) and LC20 group (1.1 ± 0.1-fold) were significantly less than that in the CG group (2.2 ± 0.3-fold; P < 0.05). The mRNA expression for VEGF in the CG group was significantly larger than that in the control group. VEGF mRNA expressions in the LC10 group (2.1 ± 0.5-fold) and LC20 group (2.9 ± 0.4-fold) were significantly less than that in the CG group (7.4 ± 1.8-fold; P < 0.01 and P < 0.05, respectively). Type I and III collagen mRNA expressions in the LC groups were significantly less than those in the CG group (P < 0.05).
Histological changes in long-term PD patients are characterized by a decrease or loss of mesothelial cells, submesothelial compact zone enlargement due to massive accumulation of macrophages, degeneration of collagens and vasculopathy [2]. Uremia is thought to cause peritoneal fibrosis because even before initiation of dialysis, the peritoneal membranes of ESRF patients are thicker than those of healthy control subjects [4]. Inflammatory cytokines, which are induced in the peritoneal cavity during peritonitis, may further promote chronic inflammation and fibrosis because they enhance the production of fibrogenic and angiogenic mediators in the mesothelial cells, infiltrating cells and vascular cells.

As far as we know, there is no animal model, which completely mimics human peritoneal fibrosis for now.
CG is not used clinically in PD but is a non-specific inducer of peritoneal fibrosis. However, CG model in rats resembles human disease in many points, such as loss of mesothelial cells, prominent macrophage infiltration, marked neoangiogenesis and thickening of the mesothelial compact zone with interstitial fibrosis [2, 6, 7]. Thus, although rats with CG do not perfectly follow the human condition, they are widely used as the model for peritoneal fibrosis. Important roles of macrophages in the development of peritoneal fibrosis have been widely reported [26]. Although the exact mechanism by which macrophages infiltrate the submesothelial compact zone in CG model has not been elucidated, infiltrated macrophages are thought to produce a wide range of mediators that lead to tissue fibrosis.

Because LC has been shown to induce macrophage apoptosis [19, 27–29], we investigated its effect on peritoneal fibrosis in a rat model and found that the administration of LC markedly decreased the number of macrophages in the submesothelial compact zone. Although we did not directly examine the apoptosis of macrophages in this study, the LC administration was suspected to induce macrophage apoptosis efficiently in situ or in circulation, thereby markedly depleting peritoneal macrophages. This decrement in macrophage number was paralleled by actual attenuation in peritoneal fibrosis. One of the mediators released from macrophages, TGF-β is considered the master molecule in the genesis of peritoneal fibrosis. It facilitates the production of ECM proteins, inhibits the degradation of

Fig. 7. Representative photomicrographs (original magnification: ×200) of immunofluorescence staining for cytokeratin in (a) the control group, (b) the CG group, (c) the LC20 group and for α-SMA in (d) the control group, (e) the CG group and (f) the LC20 group. Merged images for (g) the control group, (h) the CG group and (i) the LC20 group. (j) The number of cells in the submesothelial compact zone double positive for cytokeratin and α-SMA staining (mean ± SE). In the UC20, LC10 and LC20 groups, the numbers of cells double positive for cytokeratin and α-SMA staining were significantly less than that in the CG group. In the LC20 group, the number of cells double positive for cytokeratin and α-SMA staining was significantly smaller than that in the UC20 group. ##P < 0.0001 versus the CG group. †P < 0.05, †††P < 0.001 versus the UC20 group. §§§P < 0.001 versus the LC10 group.
ECM and induces the EMT of mesothelial cells [10–14]. In our study, the smaller numbers of macrophages in the LC groups (compared with the CG group) were accompanied by significant decreases in the expression of TGF-β1 mRNA. EMT of mesothelial cells enhances the production of VEGF [11, 12], which is also produced by macrophages. VEGF is angiogenic growth factor reported to elicit peritoneal fibrosis by stimulating the neoangiogenesis of submesothelial tissues [2, 10]. Angiogenesis is a process by which new blood vessels are formed from preexisting vessels. New blood vessel formation by angiogenesis involves the degradation of ECM combined with sprouting and migration of endothelial cells from preexisting capillaries. In this study, VEGF production and mRNA expression in the submesothelial compact zone was significantly smaller in the LC groups than it was in the CG group. In addition, there were significantly fewer PECAM-1-positive vessels, a hallmark of angiogenesis, in the LC groups than there were in the CG group.

It has been demonstrated that during the EMT mesothelial cells show a progressive loss of the epithelial phenotype and acquire myofibroblast-like characteristics [13]. The EMT of mesothelial cells may induce peritoneal thickening in PD patients [11]. It has been found to induce peritoneal thickening with ultrafiltration loss and increased solute transport [16]. Mesothelial cells that have undergone an EMT acquire higher migratory and invasive capacities, enabling them to invade the submesothelial stroma, where they contribute to peritoneal fibrosis and angiogenesis and

Fig. 8. Levels of mRNA expression for (a) TGF-β1, (b) type I collagen, (c) type III collagen, (d) snail2, (e) forkhead box C2 (FOXC2) and (f) VEGF (all values normalized to GAPDH mRNA expression). Levels of mRNA in the control group were considered as 1. In the LC20 group, the levels of mRNA expression for TGF-β1, type I collagen, type III collagen, snail2, FOXC2 and VEGF were smaller than those in the CG group. *P < 0.05, **P < 0.01, #P < 0.001, ##P < 0.0001 versus the CG group. †††P < 0.001 versus the UC20 group. §§§P < 0.0001 versus the LC10 group. ‡‡‡P < 0.01 versus the LC20 group.
ultimately lead to peritoneal membrane failure [13]. The myofibroblastic conversion of mesothelial cells has been confirmed in the TGF-β1-induced peritoneal fibrosis model [15]. In our experiment, we saw a layer of cytokeratin-positive mesothelial cells at the surface of the peritoneum in the control group (Figure 7a). In the CG group, this layer was not evident and cells positively stained for both cytokeratin and α-SMA, a hallmark of EMT, were significantly larger than they were in the control group. We also saw that the administration of LC significantly decreased the number of double-positive cells in a dose-dependent manner (Figure 7j) and regenerated the layer of cytokeratin-positive mesothelial cells (Figure 7c). Furthermore, mRNA expressions for snail2 and FOXC2, transcription factors related with EMT [25], were significantly greater in CG group than those in the control group and were significantly less in the LC20 group as compared with the CG group. These data suggest that LC may have interfered with the EMT in the peritoneum.

Thus, we suspect the mechanism of protection against peritoneal fibrosis by LC as follows. At first, peritoneal macrophages were depleted by LC via apoptosis. Decrease in macrophages suppressed the TGF-β1 and VEGF, both of which were produced by macrophages. Reduction in TGF-β1 prevented peritoneal fibrosis directly by modulating both ECM production and degradation and indirectly by suppressing EMT. VEGF was reduced both by macrophage depletion and by decrease in EMT, thereby resulting in angiogenesis and inhibited vasculopathy. Although we did not check in this study, macrophages were known to produce chemokines related with fibrosis, such as IL-8. So, decreased chemokine level by macrophage depletion may also contribute to the protection.

Questions regarding the safety of LC, particularly when administered chronically, have not yet been addressed. Van Rooijen et al. [30] have found that LC has no apparent direct effects on cells other than macrophages. No alternations in circulating lymphocytes or neutrophils have been reported [31]. Furthermore, we examined chloroesterase staining for neutrophils in this study. Neither CG nor LC administration altered the infiltration of neutrophils in the submesothelial compact zone. However, an important unanswered question is whether macrophage depletion with LC predisposes one to infection. Some investigators have examined LC in a variety of short-term experimental models of infection [32, 33]. In some cases, it actually protected from mortality [34, 35]. In most, however, macrophage depletion worsens mortality or other infection-related end points. Although we did not find any symptom of infections in any of the rats treated with LC in this study, we need to examine more about the toxicity of LC, especially long-term effect of this agent before using this agent clinically.

In summary, reducing the number of macrophages by giving LC attenuated the peritoneal thickening and suppressed the TGF-β1 expression, EMT (number of α-SMA-positive mesothelial cells), VEGF expression and vasculopathy (number of PECAM-1-positive-cells) in the CG model in the present study. These results, consistent with the idea that macrophages play a critical role in the development of peritoneal fibrosis, indicate that LC may have therapeutic potential in the treatment of peritoneal fibrosis in PD patients.

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