Technical Note

An experimental sclerosing encapsulating peritonitis model in mice

Yasuo Ishii¹, Tokihiko Sawada¹, Akira Shimizu², Tamotsu Tojimbara¹, Ichiro Nakajima¹, Shohei Fuchinoue¹ and Satoshi Teraoka¹

¹Tokyo Women’s Medical University, Kidney Center, Department of Surgery and ²Nippon Medical School, Department of Pathology, Tokyo, Japan

Abstract
Background. Sclerosing encapsulating peritonitis (SEP) is a life-threatening complication of continuous ambulatory peritoneal dialysis. To elucidate the mechanism and develop treatments for this condition, an experimental SEP model in mice was constructed.

Methods. C57BL/6 mice were administered 0.3 ml of 0.1% chlorhexidine gluconate and 15% ethanol dissolved in saline, intraperitoneally, on a daily basis for 56 days (group 1, n = 15). A control group of C57BL/6 mice were administered 0.3 ml of phosphate-buffered saline only in the same manner (group 2, n = 15). The mice were sacrificed on days 3, 7, 21, 56 and were prepared for histological analysis.

Results. In group 1, all mice had developed macroscopic evidence of SEP 56 days after the injection. Microscopically we observed peritoneal fibrosis and an increase in infiltrates of mononuclear cells over time. The peritoneal fibrosis reached the chronic inflammatory stage by 56 days after the injection.

Conclusion. We have developed a convenient experimental model of SEP in mice, which may be useful in elucidating the pathogenesis of SEP and in establishing possible treatments.

Keywords: chlorhexidine gluconate; ethanol; murine model; peritoneal dialysis; sclerosing encapsulating peritonitis

Although it is believed to be induced by several causes, including bacterial peritonitis [2,3] and chemical stimuli [4,5], the precise mechanism leading to SEP remains unknown. The lack of a suitable in vivo model of SEP has hampered the clarification of the mechanism and the establishment of treatments for SEP. To elucidate the mechanism of SEP, it would be useful to have a convenient and reproducible experimental SEP murine model. We have previously reported the creation of an experimental SEP model in rats [6]. However, mice may provide a more useful experimental model for studies examining immune system involvement in disease progression because of the availability of histopathological and cytological antibodies, therapeutic agents, and genetically engineered mice, such as the SCID mouse. In the present study, we report the development of an experimental SEP model in mice.

Subjects and methods

Mice

C57BL/6 (H-2b) mice, aged 4–6 weeks, 20–30 g body weight, were purchased from Sankyo Labo Service (Tokyo, Japan). All mice were housed in antiviral and antibody-free microisolator conditions.

Construction of SEP in a murine model

To construct an SEP murine model, a solution termed SHS was employed [2]. SHS consists of 0.1% chlorhexidine gluconate and 15% ethanol dissolved in saline. In a previous study, 2 ml of SHS was administered intraperitoneally to rats weighing 200–250 g [2]. In pilot studies examining dose responses, all mice given 1 ml SHS died within 1 week, and mice administered 0.5 ml SHS died around 4–5 weeks after injection. Although mice administered 0.2 ml SHS lived beyond 8 weeks, changes in the peritoneum were mild and fibrosis remained weak. Since no mice given 0.3 ml of SHS died before 8 weeks after injection, this dose of SHS was chosen as the basic protocol. SHS was administered to mice intraperitoneally on a daily basis.
**Experimental design**

The mice were divided into two groups. Group 1 mice received 0.3 ml of SHS intraperitoneally; group 2 mice received 0.3 ml of phosphate-buffered saline (PBS) intraperitoneally.

**Histology**

Fifteen mice in each group were sacrificed by cervical dislocation on days 3 (n=3), 7 (n=3), 21 (n=4) and 56 (n=5) after the injection of SHS or PBS. After careful macroscopic inspection, the parietal peritoneum was harvested and 5–10-mm tissue sections were fixed in 10% formalin. Tissue sections were then stained with haematoxylin and eosin (H&E) and Masson trichrome stains.

**Evaluation of fibrotic changes**

Macroscopic and histological fibrotic scores have been described in a previous study [6]. Briefly, macroscopic fibrotic scores were determined by the characterization of 13 alterations in the abdominal cavity, such as dullness of the liver margin and thickness of the liver serosa. Scores ranged from 0 points, indicating absence of fibrosis, to a maximum of 39 points in the most severe fibrosis. Histological fibrotic scores were determined by the sum of three parameters, including the amount of subserosal fibrotic matrix, the amount of subserosal large collagen fibres, and degree of subserosal fibroblast proliferation. Scores ranged from 1 to 5 according to the amount or degree of changes. The scores were calculated as averages ± SD at each time point. The increase in the number of the histological fibrotic scores indicated that histological findings changed from oedema to fibrosis.

Because measuring thickness at the thickest point of the parietal peritoneum may result in increasing variance in individual experiments, we measured the actual thickness of the subserosal tissue, as previously described [6]. We measured the thickest point of the subserosal tissue (A0), the sites that were 100 μm apart from the thickest point (A1 and A2), the thinnest point (B0), and sites that were 100 μm apart from B0 (B1, B2). The cross-sectional areas of the thickest (Area\(_{\text{max}}\)) and the thinnest (Area\(_{\text{min}}\)) portions were calculated as follows:

\[
\text{Area}_{\text{max}} = 50 \times (2A0 + 1 + A2), \quad \text{Area}_{\text{min}} = 50 \times (2B0 + B1 + B2)
\]

Histological findings were evaluated using the diagnostic criteria for SEP from the Japanese SEP study group (Table 1) [7].

**Statistical analyses**

Statistical analyses were performed using the Kruskal–Wallis test. P values <0.05 were accepted as statistically significant.

**Results**

In group 1, all mice developed SEP 56 days after injection of SHS. Figure 1 shows the macroscopic findings from a representative mouse, 56 days after injection of SHS. The parietal peritoneum was thickened and the digestive tract covered with thin fibrous

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**Table 1. Diagnostic criteria of SEP from the Japanese SEP Study Group**

<table>
<thead>
<tr>
<th></th>
<th>Detachment of the mesentry</th>
<th>Infiltration of inflammatory cells</th>
<th>Luminal narrowing of arterioles</th>
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<tbody>
<tr>
<td><strong>Early phase</strong></td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td><strong>Median phase</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Late phase</strong></td>
<td>+</td>
<td>–</td>
<td>+</td>
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**Table 2. Macroscopic fibrotic scores**

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 21</th>
<th>Day 56</th>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td>1.7±0.5</td>
<td>3.3±0.4</td>
<td>10.2±1.8</td>
<td>18±3.2</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
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</tbody>
</table>

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**Fig. 1.** Macroscopic findings of SEP. C57BL/6 mice were given 0.3 ml of SHS intraperitoneally. Mice were sacrificed on day 56, and observed macroscopically. A representative mouse is shown. The parietal peritoneum is thickened and the gastrointestinal loops show adhesion. The liver colour is dark, and the edge is dull.
Fig. 2. Histological findings of SEP. Parts (A), (C), (E), (G), and (I) were stained with H&E. Parts (B), (D), (F), (H), and (J) were produced with Masson trichrome staining. Mice given 0.3 ml of PBS were sacrificed on day 56. Mice administered 0.3 ml of SHS were sacrificed on days 3 (n = 3), 7 (n = 3), 21 (n = 4), and 56 (n = 5).
tissue. The liver edge was dull and the liver colour was dark with whitish patches. The gastrointestinal loops adhered to each other along their entirety. The mesentery was contracted and thickened. The mice in group 2 showed no apparent fibrotic changes at 56 days after injection of PBS (data not shown). The macroscopic fibrotic scores in group 1 were higher than those in group 2 (Table 2), and the macroscopic changes in group 1 were uniform by day 56.

To gain an insight into the process leading to SEP, mice were sacrificed at days 3, 7, 21 and 56 after the injection of SHS, and the parietal peritoneum was analysed histologically (Figure 2). Figures 2A and 2B show histological findings of the peritoneum of mice in group 2, 56 days after injection of PBS. By day 3, group 1 mice showed mild oedema and mild infiltration of mononuclear cells (Figure 2C, D). At day 7, the parietal peritoneum was thickened, mainly due to oedema under the mesothelium (Figure 2E, F). At day 21, the fibrosis under the mesothelium progressed and moderate infiltration of mononuclear cells was observed (Figure 2G, H). At day 56, there was extensive fibrosis under the mesothelium and very few infiltrated mononuclear cells were observed (Figure 2I, J). These histological alterations were observed in all mice from group 1. The histological fibrotic scores, shown in Table 3, were higher in group 1 than in group 2. The scores increased in group 1 over time. The actual thickness of the subserosal tissue was significantly greater in group 1 than in group 2 (Table 4). These findings are in agreement with the diagnostic criteria of SEP from the Japanese SEP Study Group.

**Discussion**

Although SEP may be induced by various causes, some studies have indicated that the host immune system might be involved in the mechanism leading to SEP [8,9]. Indeed, the administration of steroids or immunosuppressants is known to have a positive effect in the treatment of SEP [10,11].

The importance of chlorhexidine gluconate and alcohol in the pathogenesis of clinical SEP was first described by Junor et al. in 1985 [12]. They reported that chlorhexidine gluconate in alcohol, which was used to sterilize the catheter exchange, may have contributed to the development of clinical SEP. In the present study, we used intraperitoneal administration of a solution containing 0.1% chlorhexidine gluconate and 15% ethanol to produce experimental SEP. All mice that were given 0.3 ml of the solution on a daily basis developed SEP.

In our mice with experimental SEP, the macroscopic findings corresponded to those of clinical SEP. The parietal peritoneum was thickened and had adhered to the intra-abdominal organs. The entire gastrointestinal tract was covered with thin fibrous tissue and the mesentery was thickened and contracted.

The fibrosis started with oedema and infiltration of a small number of mononuclear cells in the submesothelial space, as well as the superficial layer of muscle layer. Within 2–3 weeks, both oedema and cell infiltrates increased. By 8 weeks after injection of SHS, thick fibrosis had developed and a few cell infiltrates under the mesothelium were observed. These findings suggested that the peritoneal fibrosis had reached the chronic inflammatory stage.

Many studies have suggested that fibrin deposition plays an important role in tissue fibrosis [13,14]. Fibrin deposition in the tissue matrix is a chemoattractant factor and induces recruitment of inflammatory cells, macrophages, and fibroblasts, and leads to the synthesis of extracellular matrix. We stained our experimental SEP model in mice by the Phospho-tangistic acid–haematoxylin-stain method to detect the deposition of the fibrin. Although fibrin deposition was observed along the injured mesothelia by the injured mesothelium method to detect the deposition of the fibrin. Although fibrin deposition was observed along the injured mesothelium in group 1, deposition was not observed in the thickened area of the parietal peritoneum (data not shown).

The experimental SEP model in mice described in the present study may contribute to elucidating the mechanism of clinical SEP, and may help to establish treatments using immunological or immunohistochemical methods.

**Table 3.** Histological fibrotic scores

<table>
<thead>
<tr>
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<th>Day 3</th>
<th>Day 7</th>
<th>Day 21</th>
<th>Day 56</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>0.8 ± 0.2</td>
<td>2.7 ± 1.3</td>
<td>10.2 ± 3.4</td>
<td>23 ± 3.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
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**Table 4.** Actual thickness of the subserosal tissue

<table>
<thead>
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<th>Day 3</th>
<th>Day 7</th>
<th>Day 21</th>
<th>Day 56</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>Area&lt;sub&gt;max&lt;/sub&gt; 23.3 ± 10.2*</td>
<td>80.6 ± 20.6*</td>
<td>164.6 ± 37.6*</td>
<td>174.5 ± 29.8</td>
</tr>
<tr>
<td></td>
<td>Area&lt;sub&gt;min&lt;/sub&gt; 2.8 ± 2.5</td>
<td>4.8 ± 3.7</td>
<td>24.7 ± 12.8*</td>
<td>22.9 ± 18.6*</td>
</tr>
<tr>
<td>Group 2</td>
<td>Area&lt;sub&gt;max&lt;/sub&gt; 2.4 ± 2.2</td>
<td>1.9 ± 1.5</td>
<td>2.2 ± 1.9</td>
<td>2.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>Area&lt;sub&gt;min&lt;/sub&gt; 2.1 ± 1.9</td>
<td>2.1 ± 1.8</td>
<td>1.9 ± 0.7</td>
<td>2.3 ± 1.2</td>
</tr>
</tbody>
</table>

Area<sub>max</sub> and Area<sub>min</sub> are presented as means ± SD (×1000 μm). Area<sub>max</sub> and Area<sub>min</sub> in group 1 and group 2, observed on the same day, were compared. *P < 0.05.
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References


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