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Received for publication: 7.3.2013; Accepted in revised form: 14.6.2013

Mesenchymal stem cell transplantation may provide a new therapy for ultrafiltration failure in chronic peritoneal dialysis

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Keywords: chronic peritoneal dialysis, mesenchymal stem cell transplantation, ultrafiltration failure

ABSTRACT

Background. The purpose of this study was to investigate possible healing effects of intraperitoneal (IP) mesenchymal stem cell (MSC) transplantation on ultrafiltration failure (UFF) in a chronic rat model of peritoneal dialysis (PD).

Methods. Rats were initially divided into two groups. The APUF group received once-daily IP injections of 20 mL of 3.86% glucose PD solution for 6 weeks to stimulate the development of UFF and a control group received no injections. The PUF group was sub-divided into three groups: a PUF-C group, an MSC group and a Placebo (P) group. Peritoneal equilibration tests (PETs) and peritoneal biopsies were performed in the control and PUF-C groups. MSCs were administered by IP injection in the MSC group and the PUF-C and P groups received IP injection of placebo. PETs and peritoneal biopsies were performed in the MSC and P groups at the first [P-1 (and MSC-1 groups] and second [P-2 and MSC-2 groups] week after receiving MSCs or placebo.
**Results.** When compared with the control group, ultrafiltration capacity significantly decreased and the submesothelial thickness increased in the PUF-C and P groups (P-1, P-2) (P < 0.05), but there were no differences between the control and MSC groups (MSC-1, MSC-2). The rate of glucose transport was high in the PUF-C and P-2 groups compared with the control group, and D/Pc rates in the PUF-C and P-2 groups were lower than in the control group (P < 0.05). However, D/D0 glucose was higher and D/Pc was lower in the MSC-2 group than in the PUF-C and P-2 groups (P < 0.05). Transforming growth factor-β (TGF-β) levels were lower in the MSC groups than in the P and PUF-C groups (P < 0.05).

**Conclusion.** The PUF-C group had a high permeability UFF. These results showed that MSC transplantation exerted positive effects on UFF in a chronic rat model of PD. MSC transplantation may provide new options for the renewal of the peritoneum in chronic PD patients with UFF.

**INTRODUCTION**

Peritoneal dialysis (PD) is a well-established replacement therapy for patients with end-stage renal disease, which is especially valuable for treatment during childhood and infancy. However, continuous exposure to biocompatible PD solutions and peritonitis can lead to marked functional and morphological alterations of the peritoneal membrane [1, 2]. These changes include loss of mesothelial cell mass, interstitial fibrosis and alterations in the structure and number of peritoneal blood vessels. Such alterations can eventually result in a gradual increase in peritoneal small solute transport rates, reflecting the effective peritoneal vascular surface area and resulting in ultrafiltration failure (UFF) [1–3]. While the underlying pathophysiological mechanisms of these changes are not clearly understood, accumulating evidence has implicated epithelial–mesenchymal transition as a potential mechanism for the development and progression of peritoneal fibrosis during the long term[4]. There is increasing evidence that a number of cytokines, growth factors and prostaglandins play key roles in regulating and sustaining the processes like PD, which leads to failure of peritoneal membrane function [5].

At present, transforming growth factor-β (TGF-β1) is the main target for therapeutic intervention against peritoneal fibrosis. In previous studies, intraperitoneal (IP) or systemic administration of various drugs has been used to alleviate TGF-β1-mediated fibrosis [6–14].

The mesothelial cell layer is important for the maintenance of peritoneal morphology and function. Because of this, recent studies have demonstrated the feasibility of mesothelial cell transplantation, although it is hampered by prolonged activation of the peritoneum [15–17].

Mesenchymal stem cells (MSCs) are a multipotent cell source, which initiate the remodelling process by replacing damaged tissues and cells. They appear to function through paracrine mechanisms that exert immunosuppressive, anti-inflammatory, anti-apoptotic, mitogenic and other organ-protective and repair stimulating actions [18, 19]. MSCs are presently being studied for prophylaxis and therapy for a variety of diseases, such as spinal cord injury, cardiac damage, bone degeneration, Crohn’s disease and organ rejection, as well as for the prevention of acute renal failure in high-risk situations [20–25].

In the current study, we evaluated whether MSCs can ameliorate functional and structural derangements of the peritoneal membrane caused by chronic PD fluid exposure. To our knowledge, these studies are the first to utilize MSCs for the treatment of peritoneal UFF.

**METHODS**

**Animals**

Fifty-nine non-uraemic male Wistar albino rats weighing 250–350 g were purchased from the Erciyes University Experimental and Clinical Research Centre. The animals were also housed in the same centre in a controlled environment at 22 ± 2°C with a 12 h light/dark cycle. Food and water were given *ad libitum*. They were allowed 1 week of acclimatization before the start of experiments. The experimental protocol was approved by the Animal Experiments Ethical Committee of Erciyes University.

**Peritoneal UFF development (PD rat model)**

Peritoneal UFF was induced by daily 20 mL IP injection of 3.86% glucose PD solution (Dianeal 3.86%; Eczacibasi-Baxter Healthcare, Istanbul, Turkey), which included 125 mg/L ceftazidime for prophylaxis against peritonitis, for 6 weeks. These injections were made using a 22-gauge needle through alternating right and left abdominal sides, as previously described [26].

**Experimental design**

Rats were randomly divided into two groups. The control group (C group, n = 10) did not receive any injection during the study, and the peritoneal UFF group (PUF group) received once-daily 20 mL IP injections of 3.86% glucose PD solution that included 125 mg/L ceftazidime for prophylaxis against peritonitis for 6 weeks. At the end of 6 weeks, two rats in the C group and seven rats in the PUF group were excluded from the study. At this time, the PUF group was then divided into three sub-groups: a peritoneal UFF control group (PUF-C group, n = 8), an MSC group (n = 17) and a placebo group (P group, n = 17). The MSC and P groups were further divided into two groups: MSC-1 and MSC-2, and P-1 and P-2.

A peritoneal equilibration test (PET) was applied to evaluate peritoneal permeability function in the C and PUF-C groups at the end of 6 weeks. Then, the rats were sacrificed and tissue samples from the abdominal peritoneum were taken to examine histological changes in the peritoneal membrane caused by the PD fluid. PETs and peritoneal biopsies were performed on the MSC and P groups at the first (n = 8) and second (n = 9) week after receiving MSCs and placebo to evaluate the effect of IP MSC transplantation on UFF and on peritoneal membrane histology. The study groups and design are shown in Figure 1.
MSC transplantation

Green fluorescent protein (GFP)-labelled rat bone marrow MSCs were purchased from the Centre for Stem Cells and Gene Therapies Research and Practice, Kocaeli, Turkey. These stem cells were stored at −80°C until they were used. The frozen vial of cells was taken from the refrigerator and rapidly thawed in a 37°C water bath for 1 min. The vial was promptly disinfected with 70% ethyl alcohol and opened. The cells were resuspended by gentle pipetting, and they were transferred to a sterile 15 mL conical tube. Five to six millilitres of medium (100 mL medium content = 88 mL RPMI medium, 10 mL foetal bovine serum, 1 mL l-glutamine and 1 mL penicillin–streptomycin solution) were added into the tube. The suspended cells were centrifuged at 800 rpm for 5 min. The supernatant was discarded. Three to four millilitres of medium was added and mixed using pipetting. The suspended cells were again centrifuged. The pellet was suspended in 2 mL of medium, then propagated in a culture flask. The flask was placed in a 37°C, 5% CO2 humidified incubator. The next day, fresh medium was changed by a washing process with phosphate-buffered saline (PBS). Then, the medium was changed every 3 days until the cells reached 70–80% confluency. The medium was discarded, the cultures were washed with PBS and the cells were harvested using 2 mL of 0.25% trypsin. After centrifugation and pipetting, cells were again inoculated in a flask. After trypsinization, the cells were washed twice with PBS, and a third passage was used. The cells were administered by IP injections of 1.5 × 10⁶ cells/kg rat.

PET

The ultrafiltration function of the peritoneal membrane was assessed by carrying out a 90-min PET using the following procedure. At time zero, a sample of 3.86% glucose PD fluid was taken for measurement of the glucose concentration (D₀ glucose) in the dialysis solution prior to infusion into the peritoneal cavity. At the same time, 35 mL of PD solution containing 3.86% glucose was slowly injected into the peritoneal cavities of rats using a 22-gauge needle. Then at 90th minute, the animals were anaesthetized with ketamine (60 mg/kg BW) and xylazine (40 mg/kg BW). An injector was inserted into the peritoneal cavity through a midline incision while preventing dialysate leakage from the peritoneal cavity. Immediately thereafter, all dialysate in the peritoneal cavity was collected and a blood sample was obtained by a direct cardiac puncture. Ultrafiltration capacity was calculated using the formula:

\[ \text{Ultrafiltration capacity} = \frac{\text{initial infusion volume (35 mL)}}{\text{collected dialysate volume}}. \]

After the collection of the dialysate and blood, the peritoneal cavity was opened and checked for residual fluid. After the PET procedure, the animals were sacrificed for pathological examination.

Blood and dialysate fluid sample analysis

Blood and dialysate fluid samples (2 mL) were collected for measurement of glucose at 0 and 90 min after initiation of PD. Within 1 h of collection, these samples were centrifuged at 1500g for 3 min prior to subsequent biochemical analysis. The serum was decanted and stored at 4°C within 1 h after collection for biochemical analysis. Serum and dialysate fluid levels of glucose were measured using the glucose oxidase method. Serum and dialysate urea nitrogen, sodium and creatinine levels were measured using the enzymatic kinetic method (Randox Laboratories, San Francisco, CA). Dialysate and serum protein levels were measured by the turbidimetric method.
Peritoneal solute transport analysis

Peritoneal membrane transport was quantified as the dialysate-to-plasma ratio (D/P) of urea nitrogen, creatinine, sodium and protein, and D/D0\textsubscript{glucose}, where D is the glucose concentration in the dialysate after 90-min dwell, and D0 is the glucose concentration in the dialysis solution prior to infusion into the peritoneal cavity. The D/P\textsubscript{protein} values were expressed as D/P multiplied by 1000. The rate of glucose transport was measured by calculating D/D0, where D is the glucose concentration in the dialysate and D0 is the glucose concentration in the dialysis solution prior to infusion into the peritoneal cavity. In addition, the dialysate-to-plasma water-sodium ratio (D/PNa\textsubscript{water}) was calculated to assess peritoneal water transport and aquaporin function. The mass transfer of glucose out of the peritoneum was calculated using the formula: (initial dialysate glucose × initial infusion volume) − (final dialysate glucose × final drain volume) [27].

High D/P and low D/D0\textsubscript{glucose} ratios indicate higher transport; low D/P and high D/D0\textsubscript{glucose} indicate low transport.

Histological assessment

Following the PETs, rats were sacrificed and the anterior parietal peritoneum was excised from sides opposite of the injection points. Parietal peritoneum tissue was divided into two samples. One sample was fixed with 10% formalin and embedded in paraffin. Tissue sections (5 μm) were then stained with haematoxylin and eosin and Masson trichrome. All sections were examined by light microscopy by the same pathologists who were blinded to the experimental groups. The presence of inflammation, submesothelial oedema, fibrosis and neovascularization was evaluated as previously described [6]. Submesothelial thickness was also measured from the inner surface of the muscle to the mesothelium. The thickness of the submesothelial area was randomly measured at 10 points by two independent observers. In brief, the specimens were equally divided into 10 areas, and the thickness of the peritoneum was measured at each area. Other peritoneal tissue taken from the parietal peritoneum was embedded in tissue freezing medium and was immediately frozen at −20°C. After freezing, tissues were transferred to lysine glasses by taking 0.7 μm frozen microtome sections. Preparations were evaluated directly by using blue light in a fluorescent microscope.

Immunofluorescence microscopy

To examine the survival of transplanted cells, GFP-positive cells were detected on serial sections using blue light in an immunofluorescence microscope. Preparations displaying green fluorescence along the mesothelial line were regarded as being positively marked, and images of the preparations were taken under the microscope.

Measurement of VEGF and TGF-β content in peritoneal tissues

Samples of parietal peritoneum without muscle tissue were used for the measurement of vascular endothelial growth factor (VEGF) and TGF-β levels. Wet peritoneal weight was recorded. Tissues were placed in PBS. The weight/volume ratio was 1 g wet tissue/7 mL of PBS. Tissues were then homogenized with a mechanical tipped homogenizer and centrifuged at 10 000 g for 30 min at 4°C. Supernatants were stored at −80°C until analysis. VEGF and TGF-β levels in the supernatants were assayed by an enzyme-linked immunosorbent assay (ELISA) using commercial kits [RayBio® ELISA kit (RayBio®, Norcross, GA) and Multispecies TGF-β1 kit (Biore-source, Nivelles, Belgium)] according to the manufacturer’s instructions. The results were expressed as pg/g wet weight of tissue for VEGF and pg/mg wet weight of tissue for TGF-β1.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 17.0. First, the distributions of all data were determined using the Shapiro–Wilk test. Data with normal distributions were expressed as means ± SD, and data with non-normal distributions were expressed as medians (min–max). Comparisons between groups having normally distributed data were done using analysis of variance with the post hoc Tukey procedure. Comparisons of data with abnormal distributions were done using the Kruskal–Wallis test. Then, when statistical significance was found using the Kruskal–Wallis test, differences between the two groups were tested by the Mann–Whitney U-test. Comparisons of the histopathological findings were performed by the chi-square test. All tests were considered as statistically significant if P-values were <0.05.

RESULTS

Histopathology of the peritoneum

The thickness of the peritoneum significantly increased with daily IP administration of PD fluid containing 3.86% glucose (P < 0.01, Table 1, Figure 2). Furthermore, the PUF-C group showed more inflammation and neovascularization in the peritoneal membrane than the C group (Table 1). The rats in the MSC-1 and MSC-2 groups had similar peritoneal membrane thicknesses to those in the C group. The peritoneal membrane thickness was also lower in the MSC-1 and MSC-2 groups than in the P-1 and P-2 and PUF-C groups (P < 0.05, Table 1, Figure 2). Compared with the PUF-C group, rats in the MSCs and P groups had fewer inflammatory cells in the peritoneal membrane (P < 0.05). Following IP injection of GFP-labelled MSCs, peritoneum sections taken from the MSC-1 and MSC-2 groups were examined directly via immunofluorescent microscopy. Fluorescence flashing was observed along the mesothelium line in the MSC-1 and MSC-2 groups, but was not observed in the placebo group (Figure 3).

Peritoneal function

UF capacity. UF capacity was significantly higher in the MSC-1 group than in the PUF-C and P-1 groups (P < 0.05). UF capacity of the MSC-2 group was also significantly higher than the PUF-C and P-2 groups (P < 0.05). In the P-2 group, UF capacity significantly decreased compared with the values in the C group (P < 0.05).
Table 1. Evaluation of peritoneal membrane histopathology and permeability of all groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Control</th>
<th>PUF-C</th>
<th>MSC-1</th>
<th>P-1</th>
<th>MSC-2</th>
<th>P-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submesothelial thickness (µm)</td>
<td>66.3 ± 24.8</td>
<td>132.9 ± 40.1*</td>
<td>84.9 ± 15.1** ***</td>
<td>153.7 ± 85.3*</td>
<td>69.7 ± 9.3*** ****</td>
<td>140.7 ± 30.1*</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>4*</td>
<td>0**</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>1</td>
<td>6*</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>UF (mL) median (min–max)</td>
<td>4 (−2−10)</td>
<td>−3 (−24−6)*</td>
<td>10.8 (8−13)* *** ***</td>
<td>1.5 (−11−9)*</td>
<td>4.1 (−10−14)* *** ****</td>
<td>−4.2 (−18−7)*</td>
</tr>
<tr>
<td>D/P urea (mean ± SD)</td>
<td>0.40 ± 0.05</td>
<td>0.59 ± 0.14*</td>
<td>0.49 ± 0.1</td>
<td>0.51 ± 0.4</td>
<td>0.53 ± 0.13*</td>
<td>0.49 ± 0.09*</td>
</tr>
<tr>
<td>D/P Cr (mean ± SD)</td>
<td>0.65 ± 0.07</td>
<td>0.88 ± 0.15*</td>
<td>0.77 ± 0.23</td>
<td>0.74 ± 0.2</td>
<td>0.63 ± 0.14***</td>
<td>0.83 ± 0.14*</td>
</tr>
<tr>
<td>D/P protein (mean ± SD)</td>
<td>114.8 ± 6.9</td>
<td>125.38 ± 37.5*</td>
<td>124.01 ± 10.7</td>
<td>114.97 ± 8</td>
<td>120.2 ± 13.1</td>
<td>125.1 ± 7.3*</td>
</tr>
<tr>
<td>D/P Na (mean ± SD)</td>
<td>0.78 ± 0.02</td>
<td>0.84 ± 0.03*</td>
<td>0.72 ± 0.26</td>
<td>0.73 ± 0.1</td>
<td>0.76 ± 0.05**</td>
<td>0.76 ± 0.06**</td>
</tr>
<tr>
<td>D/D0 glucose (mean ± SD)</td>
<td>1.45 ± 0.22</td>
<td>0.65 ± 0.35*</td>
<td>1.26 ± 0.31***</td>
<td>0.87 ± 0.2*</td>
<td>1.33 ± 0.33*** ****</td>
<td>0.64 ± 0.2*</td>
</tr>
<tr>
<td>Glucose mass transfer (mean ± SE)</td>
<td>−9649 ± 7985.6</td>
<td>12 005 ± 5911*</td>
<td>20 542 ± 6404****</td>
<td>7335 ± 3648</td>
<td>−5556 ± 2375******</td>
<td>17 724 ± 3650*</td>
</tr>
</tbody>
</table>

*P < 0.05 versus control group.
**P < 0.05 versus PUF-C group.
***P < 0.05 versus P-1.
****P < 0.05 versus P-2.
Glucose transport. The $D/D_{0_{\text{glucose}}}$ rate was significantly higher and the glucose mass transfer level was significantly lower in the MSC-1 and MSC-2 groups than the placebo and PUF-C groups ($P < 0.05$).

Solute permeability. Peritoneal permeabilities to sodium, urea, creatinine and protein in the MSC-1 group were similar to those of the PUF-C and P-1 groups. The $D/P_{Cr}$ level in the MSC-2 group was lower than in the PUF-C and P-2 groups ($P < 0.05$). However, the $D/P_{Na}$ level was lower in the MSC-2 group than in the PUF-C and P-2 groups ($P < 0.05$). The $D/P_{Na}$ level was decreased in the MSC-2 and P-2 groups compared with the PUF-C group ($P < 0.05$). These results indicated that the PUF-C and P groups had high peritoneal membrane permeability.

Effects of MSC treatment on cytokines

Although the groups had similar peritoneal tissue VEGF levels, the MSC-1 and MSC-2 groups had significantly
The results of our study show that administration of PD solution to rats caused peritoneal damage and UFF, which was reversed by IP injection of bone marrow-derived MSCs.

PD patients develop increased peritoneal thickness, fibrosis and angiogenesis that progress with the duration of PD therapy [2, 28]. Exposure to bioincompatible PD fluids and episodes of bacterial and fungal infection or hemoperitoneum induce acute and chronic inflammation that causes damage to the peritoneal tissue [4]. The most important component of PD solutions causing peritoneal deterioration appear to be glucose degradation products, which form advanced glycation end products to stimulate the production of extracellular matrix components as well as the synthesis of cytokines and growth factors, including TGF-β and VEGF [2, 29].

Peritoneal membrane dysfunction, manifested by UFF, is the major problem of the long-term PD. The incidence of UFF is high, with ~35% of all PD patients affected after 4 years of treatment [30, 31]. Honda et al. [31] reported that PD duration was positively correlated with peritoneal thickness and vascularity. Peritoneal thickness was also correlated with vascularity. In the group with impaired UF capacity, the peritoneum was thicker than in the group with maintained UF capacity. In an animal study, a PD solution with low-glucose degradation products effectively attenuated the peritoneal vascularization changes and fibrosis caused by conventional PD fluids [32].

The findings of our study also showed that chronic exposure of the rat peritoneal membrane to a high-glucose conventional PDF resulted in mesothelial damage, submesothelial and interstitial fibrosis, neoangiogenesis and UFF. These results clearly show that PD treatment, by itself, strongly impacts peritoneal injury.

The protection of the peritoneal membrane against the long-term toxic and metabolic effects of high GDP-containing conventional, glucose-based solutions is a prime objective for improving PD outcomes. The recent development of new, more biocompatible PD solutions should help to preserve peritoneal membrane function, promote UF, improve nutritional status, preserve the peritoneal membrane and improve overall PD outcomes. However, there are no currently available PD solutions that meet all requirements of an ideal solution [33].

Preservation of the peritoneal membrane or healing of a damaged membrane is of crucial importance for patients with end-stage renal disease having no alternatives for renal replacement therapies, except PD. A number of clinical and experimental studies have aimed to preserve peritoneal membrane function or reverse peritoneal injury caused by surgical intervention or bioincompatible PD fluids. Contrary to expectations, some of these studies reported poor outcomes [6–12, 15–17].

The mesothelium is an important component of the peritoneal membrane and is responsible for regulating inflammatory responses as well as microrcirculation and fibrin homeostasis [17, 34, 35]. Many studies have shown that chronic PD or surgical procedures were followed by denudation of the mesothelial cell layer [2, 4, 29, 36]. Mesothelial cell transplantation studies reported that transplanted mesothelial cells tend to collect in denuded areas of the peritoneum surface [15, 16, 37]. Most of these studies examined whether transplantation caused healing of mesothelial layer damage following surgical procedures and did not evaluate peritoneal permeability.

Mesothelial cell transplantation has not yet been accepted as a method for reversing peritoneum damage in patients on PD for several reasons. First, transplanted mesothelial cells produce a prolonged inflammatory response in the peritoneum. Second, there are difficulties in obtaining mesothelial cells from PD patients. Finally, the optimal time for mesothelial cell transplantation has not yet been precisely determined [38].

MSCs are reported to require transforming factors and growth factors in order to be transformed into certain cell types within an in vitro environment; however, they are able to transform into the closest cell type within an in vivo environment with the help of factors released from tissues [34, 35]. In vivo

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Control (n = 8)</th>
<th>PUF-C (n = 8)</th>
<th>MSC-1 (n = 9)</th>
<th>P-1 (n = 9)</th>
<th>MSC-2 (n = 9)</th>
<th>P-2 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/g wet weight)</td>
<td>260 ± 130</td>
<td>279 ± 184</td>
<td>289 ± 61</td>
<td>280 ± 145</td>
<td>355 ± 83</td>
<td>261 ± 95</td>
</tr>
<tr>
<td>TGF-β (pg/mg wet weight)</td>
<td>2.6 (0.9–9.6)</td>
<td>6.1 (0.6–14.3)</td>
<td>undetectable</td>
<td>7.02 (0.17–14.57)</td>
<td>1.7 (0.9–17.4)</td>
<td>8.8* (5.6–27.8)</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD or median (min–max). VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β.

**P < 0.05 versus C.
***P < 0.05 versus PUF-C groups.
****P < 0.05 versus P-1.
*****P < 0.05 versus P-2 group.
models have shown that MSCs transplanted to organs such as the liver, bone, cartilage, lung, heart, spinal cord and kidney adhered to those organs and became functional by transformation into the cells of the transplanted tissues [20, 21].

In this study, we examined whether structural and functional defects in the peritoneal membrane caused by PD fluid were altered by IP MSC administration. To our knowledge, this is the first study to examine this issue.

Our study showed that IP PD fluid exposure caused histopathological damage, including increased submesothelial thickness, number of inflammatory cells, neovascularization and high permeability in the peritoneal membrane.

Following MSC transplantation injection into the damaged peritoneum, the submesothelial thickness in the MSC groups clearly decreased compared with the PUF-C and P groups. Again, while inflammation in the MSC groups clearly decreased compared with the PUF-C group, the non-significant differences between the P groups and PUF-C groups indicate that MSCs may have reduced submesothelial oedema and inflammation. Togel et al. [39] reported that MSCs increased the production of anti-inflammatory cytokines and decreased the expression of proinflammatory cytokines and nitric oxide, indicating that they had an anti-inflammatory effect. Recently, Tülparch et al. [40] showed that high inflammatory cytokine levels (TGF-β, TNF-α and IL-6) in peritoneal damaged rats decreased after MSC transplantation injection into the damaged peritoneum. Therefore, our finding of a significant decrease in TGF-β levels in peritoneal tissue after IP MSC transplantation was similar to previous studies.

We examined histopathological changes and peritoneum permeability at 1 week following MSC administration. We found that UF in the MSC group clearly increased compared with the P-1 and PUF-C groups. Also, the glucose transfer rate in the MSC-1 group was significantly lower than in the PUF-C and P-1 groups. However, D/P$_{Cr}$, D/P$_{urea}$ and D/P$_{protein}$ rates were not different between the MSC-1 and PUF-C groups. These findings indicate that MSCs at 1 week after transplantation may have decreased glucose transfer while increasing UF capacity, but had no effect on urea, Cr, protein or Na permeability. In summary, we believe that at 1 week following MSC administration, high permeability in the P-1 group was maintained, peritoneum permeability in the MSC-1 group was similar to the control group, which together indicated that MSCs exerted healing effects on peritoneum permeability.

In the second week, the submesothelial thickness clearly decreased in the MSC-2 group compared with the PUF-C and P-2 groups. In addition, the lack of inflammatory or neovascular differences between the MSC-2 and P-2 groups indicated that inflammation decreased over time with a resting of the peritoneum; however, submesothelial oedema continued to occur.

While examining PD in rats, Kim et al. [41] reported that 3 weeks of dialysis increased peritoneal thickness and caused a highly permeable peritoneum membrane deficiency. Resting the peritoneum by ceasing PD for 4 weeks produced histopathological and functional healing of the peritoneum membrane.

When we evaluated peritoneum permeability at the second week, we found that UF in the MSC-2 group increased compared with the PUF-C and P-2 groups; the increased Cr clearance and glucose transport in the PUF-C group were normalized in the MSC2 group and preserved in the P-2 group. These findings suggest that the high peritoneal permeability following exposure to PD fluids was almost normalized by MSC transplantation.

In our study, we showed that MSCs adhered to the damaged peritoneum membrane along the mesothelial line. We believe that MSCs adhering to the mesothelial line may be replacing mesothelial cells, which were displaced during exposure to PD fluid; therefore, healing of the peritoneum may be secondary to anti-inflammatory effects and to the transforming of mesothelial cells.

According to our results, similar UF capacities were observed in the MSC and control groups, and significant positive results were obtained for both the PUF-C and placebo groups.

This is the first study to examine the effect of IP MSC transplantation on UF efficiency. Our results suggest that MSC transplantation may provide hope for a new treatment that renews the peritoneum and restores function in chronic PD patients with UF deficiency and damaged peritoneum, who currently have no treatment options for PD.

The current study had some limitations. First, evidence for MSC engraftment was weak. Western blot analysis of peritoneal tissues can be used to confirm the presence of GFP-positive cells in the peritoneum. Nevertheless, Duffield et al. [42] demonstrated that infusion of MSCs led to repair after acute kidney injury without requirement for MSC engraftment. Also in our study, the immune-histochemical method could not be used to assess the number of blood vessels. Therefore, a semi-quantitative evaluation of neovascularization was not possible.

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Received for publication: 27.8.2012; Accepted in revised form: 26.2.2013