Original Article
Rapamycin inhibits peritoneal fibrosis by modifying lipid homeostasis in the peritoneum

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Abstract: Peritoneal fibrosis (PF) is characterized by progressive accumulation of extracellular matrix (ECM) components in the peritoneum under high glucose conditions. Rapamycin has previously been shown to inhibit ECM accumulation of peritoneal mesothelial cells (PMCs) and prevent PF. Here we explored the undefined mechanisms by which rapamycin inhibits ECM accumulation of PMCs. We used high-glucose peritoneal dialysis solution (PDS) in a mouse peritoneal dialysis model to induce in vivo PF and in human PMCs in vitro to stimulate ECM accumulation. The mice that received chronic PDS infusions showed typical features of PF, including markedly increased peritoneal thickness, excessive matrix deposition, increased peritoneal permeability, and higher expressions of α-smooth muscle actin and collagen I. Rapamycin significantly ameliorated these pathological changes. There was a parallel decrease in lipid accumulation in the peritoneum of rapamycin-treated mice. Rapamycin significantly inhibited high-glucose PDS-induced ECM accumulation and reduced the lipid droplet in human PMCs in the presence of PDS. The effects of rapamycin on intracellular lipid metabolism correlated with a series of steps in lipid homeostasis; namely, a decrease in low density lipoprotein receptor-mediated lipid influx, which was mediated through the downregulation of sterol regulatory element-binding protein-2 (SREBP-2) and SREBP cleavage-activating protein (SCAP), and an increase in adenosine triphosphate-binding cassette transporter A1-mediated lipid efflux, which was mediated through the upregulation of the liver X receptor α and peroxisome proliferator-activated receptor α. We conclude that rapamycin shows a clear protective effect on high-glucose PDS-induced PF by improving the disruption of intracellular lipid homeostasis.

Keywords: Rapamycin, high-glucose peritoneal dialysis solution, peritoneal fibrosis, lipid homeostasis

Introduction

Peritoneal dialysis (PD) is an alternative treatment for end-stage renal disease that uses the peritoneum as a permeable barrier for the exchange of noxious substances and water [1]. During PD, long-term exposure to high-glucose concentration PD solutions (PDS) causes damage to the peritoneum, which subsequently can lead to increased synthesis of extracellular matrix (ECM) components by peritoneal mesothelial cells (PMCs), leading to submesothelial fibrosis, ultrafiltration failure, and eventual discontinuation of PD [2, 3].

Recent reports suggest that abnormal lipid homeostasis may contribute to the pathogenesis of multiorgan fibrosis, such as renal fibrosis [4], vascular sclerosis [5], non-alcoholic fatty liver disease [6], and cardiac fibrosis [7]. Interestingly, Chang et al. found that lipid-lowering agents (e.g., statins) inhibit ECM accumulation in high-glucose-treated PMCs and PDS-stimulated rats via the mevalonate pathway. In fact, statins exert these effects by preventing the synthesis of other important isoprenoids of the cholesterol biosynthetic pathway, which suggests that disruption of lipid homeostasis may contribute to the pathogenesis of PF caused by high-glucose concentration PDS [8]. In most cells, intracellular lipids are governed by tight regulation of cholesterol influx and efflux pathways [9]. The low-density lipoprotein receptor (LDLr) in the liver is of primary importance for the binding and internalization of plasma-derived LDL cholesterol and in regulating
intracellular LDL influx. \( \text{LDLr} \) gene expression in mammalian cells is predominantly regulated via a negative-feedback mechanism that depends on mediation of intracellular cholesterol concentrations by sterol regulatory element-binding proteins (SREBPs) and SREBP cleavage-activating protein (SCAP). When cells have sufficient cholesterol, SCAP and SREBP-2 form a complex that is retained in the endoplasmic reticulum (ER), where they remain inactive as transcription factors, resulting in limited intracellular cholesterol by downregulation of \( \text{LDLr} \) [10]. Cholesterol and phospholipid efflux pathways are mediated by cell membrane transporter proteins, such as adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1). Liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) are important nuclear receptors that control the transcription of various specific genes. Studies have suggested that LXRs and PPARs upregulate ABCA1 expression, thereby providing an amplification loop for ABCA1 expression and further decreasing intracellular cholesterol and phospholipids [11].

Rapamycin is a commonly used immunosuppressive agent that inhibits the mammalian target of rapamycin (mTOR) and has been demonstrated to have anti-fibrotic activity in the kidneys [12], liver [13], and cardiovascular system [14], as well as in systemic sclerosis [15]. One of the key actions of rapamycin against fibrosis is inhibition of intracellular lipid accumulation. Our previous work suggests that rapamycin blocks LDLr-mediated cholesterol uptake in HepG2 cells and vascular smooth muscle cells (VSMCs) via inhibition of SREBP-2 and decreased SCAP/SREBP-2 complex translocation from the ER to the Golgi apparatus [13, 14]. Conversely, rapamycin increases cholesterol efflux from VSMCs [16], macrophages [17], and glomerular mesangial cells [18] via increased expression of ABCA1. Therefore, rapamycin may contribute to the maintenance of intracellular lipid homeostasis by both reducing cholesterol uptake and increasing cholesterol efflux, ultimately preventing lipid disorder-mediated organ injury. Recent studies report that rapamycin is also effective in suppressing ECM deposition of PMCs and preventing PF under high-glucose conditions in the peritoneum [19, 20]. However, the underlying relationship involved between rapamycin and PF induced by exposure to high-glucose PDS has not been thoroughly elucidated to date.

The present study was undertaken to explore whether rapamycin inhibits lipid accumulation and ECM deposition of PMCs under high-glucose PDS stimulation, in turn improving lipid disorder-mediated PF.

**Materials and methods**

**Ethics statement**

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Nanjing Drum Tower Hospital (Nanjing City, China). All animals were anesthetized with sodium pentobarbital during surgery, and all efforts were made to minimize discomfort.

**Animal model**

Male C57BL/6 mice purchased from the Model Animal Research Center of Nanjing University were used in this study. Thirty-eight-week-old mice were randomly divided into three groups of 10 mice each. Control mice received a daily intraperitoneal injection of 3 ml phosphate buffered saline (PBS) for 8 weeks, PDS mice received a daily intraperitoneal injection of 3 ml 4.25% glucose PDS (Baxter, 6AB9896) for 8 weeks, and PDS-plus-Rapa mice received daily intragastric administration of 1 mg/kg rapamycin (Selleck Chemicals, s1039) at later time points (weeks 5-8), based on the PD-model group, for a total of 4 weeks. The volume and concentration of PDS and rapamycin used were based on results of previous studies [19, 21]. At termination, peritoneal permeability was tested, and peritoneal tissues (including the parietal peritoneum and visceral peritoneum) were used for histological assessments and western blot analyses.

**Peritoneal permeability**

Modified peritoneal equilibration tests (PETs) were performed to determine the peritoneal permeability before the mice were sacrificed.
After intraperitoneal injection of 3 ml 4.25% glucose PDS, dialysate and blood samples were collected at 0 and 2 h of dwell time. Concentrations of glucose and blood urea nitrogen (BUN) in dialysate and plasma were measured in the laboratory of the Clinical Biochemistry Department in Nanjing Drum Tower Hospital. The peritoneal permeability was determined by glucose absorption from dialysate (D/D0) and the dialysate-to-plasma (D/P) ratio of BUN [21].

Histology and immunohistochemistry staining

The parietal peritoneum was fixed with 4% paraformaldehyde and embedded in paraffin. The thickness of the peritoneum was stained with hematoxylin and eosin (H&E), and imaged under light microscopy (×200). Peritoneum sections were deparaffinized and stained with Masson’s trichrome stain for quantification of fibrosis. The results were observed under light microscopy (×200). For immunohistochemistry staining, deparaffinized sections were placed in citrate-buffered solution (pH 6.0) and heated for antigen retrieval. Endogenous peroxidase was blocked with 3% hydrogen peroxide, and nonspecific antibody binding was blocked with 10% goat serum. Subsequently, sections were incubated with α-smooth muscle actin (α-SMA) (Abcam, ab7817) and collagen I (Santa Cruz, sc-25974) primary antibodies overnight at 4°C followed by incubation with biotinylated secondary antibodies. Finally, a diaminobenzidine tetrahydrochloride substrate was used to initiate the reaction. The results were observed under light microscopy (×200). Semiquantitative analysis was performed with Image-Pro Plus version 5.0 software.

Observation of lipid accumulation

The lipid accumulation in the parietal peritoneum was evaluated by Oil Red O (ORO) staining and filipin staining. The results of ORO staining were observed under light microscopy (×200) and the results of filipin staining were examined by laser microscopy (×200). Semiquantitative analysis was performed with Image-Pro Plus version 5.0 software.

Cell culture

Human PMCs (HPMCs; HmrSV5, Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured with Dulbecco’s Modified Eagle’s Medium (DMEM; Gibco, 10567-014) containing 10% fetal bovine serum (Gibco, 16000044). Experiments were stimulated with 4.25% glucose PDS (Baxter, 6AB9896) and rapamycin (Selleck Chemicals, s1039).

Exposure of HPMCs to high-glucose PDS and rapamycin

Sub-confluent HPMCs grown in a culture dish were incubated with serum-free medium for 24 h to arrest and synchronize the cell growth. Next, four groups were defined as follows: group 1, control (HPMCs without any stimulation); group 2, PDS (HPMCs stimulated with 2 ml 4.25% PDS); group 3, Rapa (HPMCs treated only with 10 ng/ml rapamycin); group 4, PDS plus Rapa (HPMCs co-treated with 2 ml 4.25% PDS and 10 ng/ml rapamycin). The observation time points of cultures were every 24 h.

Quantitative measurement of intracellular free cholesterol/cholesterol ester by high-performance liquid chromatography (HPLC)

Briefly, cells were collected and lipids were extracted by adding 1 ml chloroform/methanol (2:1). The lipid phase was collected, dried in a vacuum, and then dissolved in 2-propanol containing 10% Triton X-100. The concentrations of total and free cholesterol in each sample were analyzed using a standard curve normalized against total cell protein. The concentration of cholesterol ester was calculated by subtracting the amount of free cholesterol from the total cholesterol.

Quantification of specific transcripts by real-time polymerase chain reaction (RT-PCR)

Total RNA was isolated from cultured HPMCs using the guanidinium-phenol-chloroform method. Total RNA (500 ng) was used as a template for reverse transcription PCR for cDNA synthesis, performed using a kit from TaKaRa Biotechnology. Resulting cDNA was divided for separate amplification of target genes using specific primers designed by Taqman Primer Express Software V2.0 and shown in Table 1. RT-PCR was performed in an ABI 7300 RT-PCR system using a SYBR Green PCR kit according to the manufacturer’s protocol (TaKaRa Biotechnology). After RT-PCR, a dissociation curve (melting curve) was constructed for the range of 60-95°C. The relative amount of mRNA was calculated using the comparative threshold.
cycle (ΔΔCt) method. The amplification efficiencies of the target and reference genes were shown to be approximately equal to the slope of the log input amount to Ct < 0.1. Controls consisting of nuclease-free H$_2$O or RNA samples that were not reverse transcribed were negative for the target and reference genes.

**Western blot analysis**

Identical amounts of total protein extracts from cultured HPMCs and peritoneums of C57BL/6 mice were denatured and resolved by electrophoresis. The membranes were blocked with blocking buffer for 1 h at room temperature after gel transfer. Membranes were then incubated overnight at 4°C with primary antibodies for LDLr (Santa Cruz, sc-11824), SREBP-2 (Santa Cruz, sc-271615), SCAP (Abcam, ab190103), ABCA1 (Abcam, ab18180), LXRα (Abcam, ab41902), PPARα (Santa Cruz, sc-398394), α-SMA (Abcam, ab7817), and collagen I (Santa Cruz, sc-25974) followed by horseradish peroxidase-labeled secondary antibodies for 1 h. Finally, the bands were detected using an enhanced chemiluminescence advanced system (Amersham Biosciences, UK).

**Statistical analysis**

All data are expressed as the mean ± standard deviation (SD) and were analyzed with SPSS 16.0 software. One-way ANOVA was used to compare continuous variables among groups where appropriate. The correlation analysis was calculated using Spearman’s rank correlation. A difference was considered significant if the p-value was < 0.05.

**Results**

**Rapamycin prevents PF and functional injury in a PD mouse model**

After 8 weeks of PD in mice, H&E and Masson’s trichrome staining revealed that PDS mice receiving no treatment showed typical features of PF, including thickening of the submesothelial compact zone within the anterior abdominal wall and collagen deposition, compared to normal control mice (Figure 1A). These pathological changes were associated with increased peritoneal permeability, determined by peritoneal equilibration tests (Figure 1B and 1C). In contrast, rapamycin prevented peritoneal thickening and collagen deposition as well as peritoneal dysfunction (Figure 1). These protective effects against PF were further demonstrated by treatment of rapamycin to inhibit α-SMA and collagen I protein expression levels (Figure 2A-D).

**Rapamycin prevents lipid accumulation in a PD mouse model**

We examined the preventive effect of rapamycin on lipid accumulation in response to chronic high-glucose PDS infusion. Compared to the control, a significant increase in lipid accumulation was observed in the high-glucose PDS-treated peritoneal tissue, demonstrated by ORO and filipin staining. However, rapamycin decreased the high-glucose PDS-stimulated lipid droplet deposition (Figure 3A). This finding was consistent with the results from semiquantitative analyses of ORO (Figure 3B) and filipin staining (Figure 3C).

**Relationship between PF and lipid accumulation in a PD mouse model**

As shown in Figure 4, we performed correlation analyses of lipid deposition using data from the semiquantitative analyses and the protein expression levels of α-SMA and collagen I from western blot analyses. Positive correlations were observed between the data from ORO staining and the protein expression levels of α-SMA (r = 0.782, P = 0.013) and collagen I (r = 0.683, P = 0.013).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequences</th>
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<tr>
<td>α-SMA</td>
<td>Sense: 5′ACCCACAATGCTCCCATCTA3′ Anti-sense: 5′GAAGGAATAGCCACGCTCAAG3′</td>
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<tr>
<td>Collagen I</td>
<td>Sense: 5′CTGAAGAACAGCATTGCA3′ Anti-sense: 5′GGCGTGATGGCTATTGTGT3′</td>
</tr>
<tr>
<td>LDLr</td>
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<tr>
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<tr>
<td>SCAP</td>
<td>Anti-sense: 5′TGCTCTCTCAGACGTTGTC3′ Sense: 5′AGAAAGAGAGATCGCGCTGA3′</td>
</tr>
<tr>
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<td>PPARα</td>
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</tr>
<tr>
<td>β-actin</td>
<td>Anti-sense: 5′TTCTCCTTAATGTCACGAGATT3′ Sense: 5′TTCTCCTTAATGTCACGAGATT3′</td>
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**Table 1. Human TaqMan Primers for Real-time PCR**
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Figure 1. Rapamycin prevented PF and functional injury in a mouse model of PD. C57BL/6 mice were treated with PBS (Control), 4.25% glucose PDS for 8 weeks (PDS), or 1 mg/kg rapamycin plus 4.25% glucose PDS for later time points (weeks 5-8) for a total of 4 weeks (PDS plus Rapa). A. H&E staining and Masson’s trichrome staining of parietal peritoneum (original magnification ×200). B. The function of peritoneal permeability checked by PET-glucose. C. The function of peritoneal permeability checked by PET-BUN analysis. The results represent the mean ± SD of five mice in each group. Key: (*) P < 0.05 vs. control, (#) P < 0.05 vs. PDS group.

Rapamycin inhibits ECM deposition and lipid accumulation induced by high-glucose PDS in HPMCs

Consistent with the results from in vivo experiments, exposure of HPMCs to high-glucose PDS increased mRNA and protein expression levels of α-SMA (r = 0.871, P = 0.002) and collagen I (r = 0.793, P = 0.011). The data suggest that the preventive effect of rapamycin against PF could be closely associated with the decrease in lipid accumulation.

Rapamycin inhibits ECM deposition and lipid accumulation induced by high-glucose PDS in HPMCs

Because intracellular lipid homeostasis is controlled by lipid influx and efflux mechanisms, we further investigated the effect of rapamycin on LDLr-mediated intracellular cholesterol influx and ABCA1-mediated intracellular cholesterol efflux in HPMCs. We demonstrated that HPMCs...
Figure 2. Rapamycin prevented α-SMA and collagen I deposition in the parietal peritoneum in a mouse model of PD. C57BL/6 mice treated with PBS (Control), 4.25% glucose PDS for 8 weeks (PDS), or 1 mg/kg rapamycin plus 4.25% glucose PDS for later time points (weeks 5-8) for a total of 4 weeks (PDS plus Rapa). A. Immunohistochemistry staining of α-SMA and collagen I in the area of parietal peritoneum. The positive areas were stained brown (original magnification ×200). B. The values of semiquantitative analysis for the positive areas were expressed as the mean ± SD from five mice in each group. Key: (*) P < 0.05 vs. control, (**) P < 0.01 vs. control, (#) P < 0.05 vs. PDS group. C and D. The protein levels of α-SMA and collagen I of visceral peritoneum were further determined by western blot analyses. Identical total protein concentrations extracted from peritoneal tissues were resolved by gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were subjected to western blotting using anti-mouse polyclonal antibodies against α-SMA, collagen I, or β-actin, which was used as an internal control. The histogram shows the mean ± SD of the densitometric scans of the protein bands from five mice following normalization by comparison with β-actin. Key: (*) P < 0.05 vs. control, (#) P < 0.05 vs. PDS group, (##) P < 0.001 vs. PDS group.
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under high-glucose-PDS conditions exhibited increased mRNA and protein levels of LDLr, SREBP-2, and SCAP (Figure 6A-C). In addition, high-glucose-PDS conditions significantly decreased mRNA and protein levels of ABCA1, LXRα, and PPARα (Figure 6D-F). Treatment with rapamycin significantly abrogated the high-glucose PDS-stimulated activities of these proteins in vitro in HPMCs. Taken together, these data suggest that lipid accumulation due to high-glucose PDS conditions in HPMCs was caused by disruption of intracellular lipid influx and efflux mechanisms, and rapamycin restored intracellular lipid homeostasis.

Discussion

PD-induced PF is associated with progressive increased thickness of the peritoneal membrane, predominantly in the submesothelial compact collagenous zone, and membrane hyperpermeability [22]. Long-term exposure of the peritoneal membrane to conventional high-glucose concentration PDS and glucose degradation products (GDP) may play an important role in the underlying mechanisms of PD structural and functional changes in the peritoneum; however this topic remains unclear. Unfortunately, effective clinical therapy for PF is scarce [23]. Recently, several articles reported that the anti-fibrotic effect of rapamycin may be useful in the treatment of PF [19, 20, 24, 25] and encapsulating peritoneal sclerosis, which is the most severe form of PF [26, 27]. However, the underlying mechanisms of the protective role of rapamycin against PF have not been thoroughly elucidated to date. In this study, we demonstrated that rapamycin has an obvious
effect on PF inhibition by restoring homeostasis of intracellular lipid influx and efflux mechanisms in the peritoneum under high-glucose-PDS conditions.

A previous study reported that C57BL/6 mice receiving daily intraperitoneal injections of 3 ml 4.25% glucose dialysis solution for 4 weeks manifested typical features of PD, including loss of the mesothelial cell monolayer, thickening of the submesothelial compact zone within the anterior abdominal wall, and increased peritoneal permeability, thereby confirming the successful establishment of a PF mouse model [21]. We employed the same animal model, and data shown in Figures 1 and 2 provide evidence that the mice that received high-glucose PDS infusions exhibited typical characteristics of PF, including markedly increased peritoneal thickness, excessive ECM deposition, enhanced peritoneal permeability, as well as higher expression levels of ECM components, such as...
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α-SMA and collagen I. However, rapamycin notably relieved the characteristics of PF and improved peritoneal function, resulting in decreased peritoneal thickness, reduced collagen deposits, decreased peritoneal permeability, and inhibition of α-SMA and collagen I expression levels. The underlying molecular basis of the protective role of rapamycin against high-glucose PDS-induced PF is not clear. Xiang et al. showed that rapamycin notably improved peritoneal function and relieved the signs of high-glucose-induced PF, including reversal of epithelial to mesenchymal transition of the peritoneal mesothelium, as a result of inhibition of Rho GTPase activation [19]. Tamer et al. also demonstrated that rapamycin may be beneficial in preventing or delaying the progression of PF and neoangiogenesis. These alterations in the peritoneal membrane may be connected with reduced tumor necrosis factor-α and transforming growth factor-β (TGF-β) levels [28]. In this study, we observed that high-glucose PDS conditions clearly increased lipid deposition both in vivo and in vitro, and rapamycin reversed the phenomenon. Moreover, there was a parallel decrease in intracellular cholesterol deposition and protein expression levels of α-SMA and collagen I with rapamycin treatment. The data suggest that the preventive effect of rapamycin against PF could be closely associated with decreased lipid accumulation in the peritoneum.

Figure 5. Rapamycin inhibited ECM deposition and lipid accumulation activated by high-glucose PDS in HPMCs. HPMCs were untreated (Control) or treated with 4.25% glucose PDS (PDS), or 10 ng/ml of rapamycin (Rapa), or 4.25% glucose PDS+10 ng/ml of rapamycin (PDS plus Rapa) for 24 h. A. The mRNA expression of α-SMA and collagen I was determined by RT-PCR. Total RNA was extracted from HPMCs, and cDNA was acquired by reverse transcription. β-Actin served as the housekeeping gene. The results represent the mean ± SD from three experiments. Key: (*) P < 0.05 vs. control, (**) P < 0.001 vs. control, (#) P < 0.05 vs. PDS group, (##) P < 0.01 vs. PDS group. B and C. The protein levels of α-SMA and collagen I were examined by western blot analyses. Identical total protein concentrations from HPMCs were resolved by gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were subjected to western blot analysis using anti-human polyclonal antibodies against α-SMA, collagen I, or β-actin, which was used as an internal control. The histogram represents the mean ± SD of the densitometric scans for protein bands from three experiments normalized by comparison with β-actin. Key: (*) P < 0.05 vs. control, (**) P < 0.01 vs. control, (#) P < 0.01 vs. PDS group, (##) P < 0.001 vs. PDS group. D. The concentration of cholesterol ester in HPMCs was measured using the HPLC assay described in the Materials and Methods section. Values are the mean ± SD of triplicate wells from three experiments. Key: (*) P < 0.05 vs. control, (**) P < 0.001 vs. control, (#) P < 0.001 vs. PDS group.
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Figure 6. Rapamycin decreased the LDLr-mediated lipid influx, and increased ABCA1-mediated lipid efflux induced by high-glucose PDS in HPMCs. HPMCs were treated without (Control) or with 4.25% glucose PDS (PDS), or 10 ng/ml of rapamycin (Rapa), or 4.25% glucose PDS +10 ng/ml of rapamycin (PDS plus Rapa) for 24 h. A. The mRNA expression levels of LDLr, SREBP-2, and SCAP was determined by RT-PCR. Total RNA was extracted from HPMCs, and cDNA was acquired by reverse transcription. β-Actin served as the housekeeping gene. The results represent the mean ± SD from three experiments. Key: (*) P < 0.05 vs. control, (**) P < 0.001 vs. control, (#) P < 0.05 vs. PDS group. B and C. The protein levels of LDLr, SREBP-2, and SCAP were examined by western blotting. The identical total protein extracted from HPMCs was isolated by gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were subjected to western blot analyses using anti-human polyclonal antibodies against LDLr, SREBP-2, or β-actin, which was used as an internal control. The histogram represents the mean ± SD of the densitometric scans for protein bands from three experiments, normalized to β-actin protein levels. Key: (*) P < 0.05 vs. control, (**) P < 0.01 vs. control, (#) P < 0.05 vs. PDS group, (##) P < 0.001 vs. PDS group. D. The mRNA expression levels of ABCA1, LXRα, and PPARα were determined by RT-PCR. Total RNA was extracted from HPMCs, and cDNA was acquired by reverse transcription. β-Actin served as the housekeeping gene. The results represent the mean ± SD from three experiments. Key: (*) P < 0.01 vs. control, (**) P < 0.001 vs. control, (#) P < 0.01 vs. PDS group. E and F. The protein levels of ABCA1, LXRα, and PPARα were examined by western blot analysis. Identical total protein concentrations extracted from HPMCs were resolved by gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were subjected to western blot analyses using anti-human polyclonal antibodies against ABCA1, LXRα, or β-actin, which was used as an internal control. The histogram represents the mean ± SD of the densitometric scans for protein bands from three experiments, normalized to β-actin levels. Key: (*) P < 0.05 vs. control, (**) P < 0.01 vs. control, (#) P < 0.05 vs. PDS group.
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LDLr-mediated intracellular cholesterol influx and ABCA1-mediated intracellular cholesterol efflux mechanisms in vitro. Our experiments show that rapamycin prevented lipid accumulation in HPMCs by 1) inhibiting gene expression and protein levels of LDLr, SREBP-2, and SCAP; and 2) upregulating the expression levels of ABCA1, LXRα, and PPARα, thereby overriding the increased cholesterol influx induced by 4.25% glucose PDS. Recently, Inokuchi-Shimizu et al. found that rapamycin significantly inhibited high-glucose PDS-induced ECM accumulation in the peritoneum, which manifested as decreased expression levels of α-SMA and collagen I. These effects correlated with a decrease in the LDLr-mediated lipid influx mechanism, which was mediated through downregulation of SREBP-2 and SCAP expression levels, as well as an increase in the ABCA1-mediated lipid efflux pathway, which was mediated through the upregulation of LXRα and PPARα expression levels. Overall, rapamycin showed an obvious protective effect against high-glucose PDS-induced PF, which was partly due to restoration of lipid homeostasis in PMCs. Abbreviations: α-SMA, alpha-smooth muscle actin; LDLr, low density lipoprotein receptor; SREBP-2, sterol regulatory element-binding protein-2; SCAP, SREBP cleavage activating protein; ABCA1, adenosine triphosphate-binding cassette transporter A1; LXRα, liver X receptor alpha; PPARα, peroxisome-proliferator activated receptor alpha.

In summary, the present study demonstrates that PD-related PF was closely associated with increased accumulation of lipid droplets in the peritoneum. Inhibition of cellular lipid uptake and elevated cholesterol efflux are important aspects for the anti-fibrotic effects of rapamycin on PMCs (Figure 7). These findings suggest that rapamycin treatment may be a promising therapeutic strategy for the preservation of peritoneal membrane integrity in long-term PD patients.

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Disclosure of conflict of interest

None.

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