Original Research

Valsartan ameliorates high glucose-induced peritoneal fibrosis by blocking mTORC1 signaling

Jing Liu, Yuan Feng, Cheng Sun, Wei Zhu, Qing-Yan Zhang, Bo Jin, Qiu-Yuan Shao, Yang-Yang Xia, Peng-Fei Xu, Miao Zhang and Chun-Ming Jiang

Institute of Nephrology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing City, Jiangsu Province 210008, China

Corresponding author: Chun-Ming Jiang. Email: guloujiang@sina.com

Impact statement

Our study provided new insight into the mechanism underlying the preservation of the peritoneum by valsartan. The results demonstrated that the mice receiving chronic high glucose (HG) peritoneal dialysis solution infusion showed a typical feature of peritoneal fibrosis (PF), as well as higher expression of α-smooth muscle actin (α-SMA) and collagen I. *In vitro*, HG increased the protein expression of α-SMA and collagen I in a dose-dependent manner, while valsartan significantly ameliorated these pathological changes. Interestingly, there was a parallel decrease in the activity of mammalian target of rapamycin complex 1 (mTORC1) and the protein expression levels of α-SMA and collagen I upon treatment with valsartan *in vivo* and *in vitro*. Moreover, the mTOR agonist MHY1485 reversed the downregulation of α-SMA and collagen I *in vitro*, even in the presence of valsartan. Altogether, our findings reported for the first time that valsartan exerts a protective effect against HG-induced PF by inhibiting the activity of the mTORC1 pathway.

Abstract

Our previous study demonstrated that the mammalian target of rapamycin complex 1 (mTORC1) pathway is activated in peritoneal fibrosis under high glucose condition. This study aimed to investigate whether valsartan inhibits high glucose-induced peritoneal fibrosis via decreasing the activity of the mTORC1 pathway. We used high glucose peritoneal dialysis solution in a mouse peritoneal dialysis model to induce peritoneal fibrosis *in vivo* and high glucose in human peritoneal mesothelial cells (HPMCs) to stimulate extracellular matrix accumulation *in vitro*. After injections of peritoneal dialysis solution containing 4.25% glucose for four weeks, mice showed typical features of peritoneal fibrosis, including markedly increased peritoneal thickness, excessive matrix deposition, increased peritoneal permeability, and higher expression of extracellular matrix proteins, such as α-smooth muscle actin (α-SMA) and collagen I. Oral gavage of valsartan significantly ameliorated these pathological changes at both week 6 and week 8. These effects of valsartan were closely correlated with a decrease in the activation of the mTORC1 signal, which was mediated by the downregulation of the protein expression of phosphorylated (p)-mTOR, p-eukaryotic initiation factor 4E-binding protein 1, and p-p70 S6 kinase 1. Further research showed that the protein expression of mTORC1 signal was positively correlated with the expression of both α-SMA and collagen I in the peritoneum. *In vitro*, high glucose increased the protein expression of α-SMA and collagen I in a dose-dependent manner, while valsartan significantly inhibited high glucose-induced extracellular matrix accumulation in HPMCs. The effect was also accompanied by a decrease in the activation of the mTORC1 signal. Furthermore, the mTOR agonist MHY1485 reversed the downregulation of extracellular matrix components in HPMCs, even in the presence of valsartan. We conclude that valsartan exerts a protective effect against high glucose-induced peritoneal fibrosis via suppressing the activity of the mTORC1 pathway.

Keywords: Valsartan, high-glucose peritoneal dialysis solution, human peritoneal mesothelial cell, mammalian target of rapamycin complex 1, extracellular matrix accumulation, peritoneal fibrosis

***Experimental Biology and Medicine* 2020; 245: 983–993. DOI: 10.1177/1535370220919364**

Introduction

Peritoneal dialysis (PD) is a well-accepted renal replacement therapy for end-stage renal disease (ESRD).[1] However, long-term exposure to bioincompatible PD solutions (PDS), especially the high glucose (HG)-PDS, can result in the structural and functional impairment of the peritoneum by inducing the abnormal accumulation of extracellular matrix (ECM) in peritoneal mesothelial cells (PMCs), which is a pathological feature of peritoneal fibrosis (PF), and ultimately, ultrafiltration failure.[2]

Mounting evidence has suggested that the mammalian target of rapamycin complex 1 (mTORC1) represents an important signaling node during fibrogenesis.[3,4] Woodcock *et al.*[5] demonstrated that mTORC1 signaling is a critical mechanism for collagen synthesis in human lung fibroblasts through the application of transforming growth factor-β1 (TGF-β1), and thereby leads to idiopathic pulmonary fibrosis. Andrikopoulos *et al.*[6] suggested that trametinib ameliorates kidney fibrosis by suppressing TGF-β1-induced extracellular-regulated kinase 1/2 in cultured primary human renal fibroblasts, thereby attenuating downstream mTORC1 activation and reducing the expression of the ECM marker α-smooth muscle actin (α-SMA), and decreasing the collagen deposition in renal fibroblasts. More recently, our results showed that the mTORC1 pathway can be activated by HG-PDS and that it subsequently phosphorylated the downstream effectors p70 S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1), which exacerbated ECM formation in human PMCs (HPMCs). The data suggest that the activation of mTORC1 is involved in HG-mediated PF.[7] Therefore, targeting mTORC1 may be a promising therapeutic strategy for the preservation of peritoneum integrity in long-term PD patients.

Recent studies have deduced that the renin-angiotensin system (RAS) is involved in PF under HG stress.[8] Elements of the RAS, including angiotensin II (AngII), and AngII type 1 receptor (AT1R), have been detected in cultured HPMCs.[9] As noted, data from both animal model and limited human trials have indicated that AT1R plays a very important role in the pathogenesis of PD-related PF. Moreover, it has been proven that systemically administered AT1R blockers (ARBs) alleviate the progression of PF, seemingly independent of their effects on systemic blood pressure.[10,11] However, the mechanism by which ARBs protect against PF remains to be explored. A study by Mavroeidi suggested that there is a close connection between the RAS and the mTOR signaling pathway.[12] Diniz *et al.*[13] suggested that AT1R mediates the induction of cardiomyocyte hypertrophy via triiodothyronine through the activation of protein kinase B (AKT)/glycogen synthase kinase-3β (GSK-3β)/mTOR pathway. ARBs and AT1R silencing block cardiomyocyte hypertrophy or ameliorate or completely block AKT/GSK-3β/mTOR activation. Research indicates that both tissue damage and mTOR activation are simultaneously mitigated by RAS inhibitors. Whaley-Connell *et al.*[14] demonstrated that telmisartan attenuates proteinuria as well as tubulointerstitial fibrosis observed in Ren2 rats, in part by reducing mTORC1 signaling. However, whether ARBs function downstream of mTORC1 to prevent PD-related peritoneal injury has not been clarified.

The purpose of this research was to explore whether valsartan protects against PF under HG stimulation by inhibiting the mTORC1 pathway.

Materials and methods

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Animal model

Male C57BL/6 mice (22–26 g) purchased from the Model Animal Research Center of Nanjing University were used in this study. After one week of acclimatization, the mice were randomly divided into four groups: (1) control (daily intraperitoneal injections of 3 mL of phosphate-buffered saline (PBS), $n = 8$); (2) control+valsartan (control mice administered orally by gavage at a daily dose of 30 mg/kg valsartan, $n = 8$); (3) PDS (daily intraperitoneal injections of 3 mL of 4.25% glucose PDS (Baxter, 6AB9896), $n = 8$ for each time point); and (4) PDS+valsartan (PDS mice treated with valsartan, $n = 8$ for each time point). Valsartan was purchased from the Beijing Novartis Company (Beijing, China). The mice in the control and PDS groups received the same volume of distilled water daily by gavage. The mice were sacrificed at three time points (four, six, and eight weeks) after treatment.

Peritoneal permeability

Modified peritoneal equilibration tests (PETs) were performed to assess peritoneal permeability. The method was carried out as Yu *et al.*[15] described.

Histology and immunohistochemical staining of the peritoneum

The thickness and fibrosis of the parietal peritoneum were measured in tissue sections stained with hematoxylin and eosin (HE), and Masson's trichrome under light microscopy (100×). Semiquantitative analysis of peritoneal thickness and fibrosis was performed with Image-Pro Plus version 6.0 software and was expressed as the mean of five independent measurements for each section. For immunohistochemical staining, the sections were incubated with primary antibodies against α-SMA (Abcam, ab7817) and collagen I (Santa Cruz, sc-25974). The results were observed under light microscopy (200×).

Semiquantitative analysis of the positive immune staining was performed with Image-Pro Plus version 6.0 software.

Cell culture

HPMCs (HmrSV5, Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in serum-free Dulbecco's modified Eagle's medium (DMEM) containing 0.2% bovine serum albumin for 24 h to arrest and synchronize cell growth. First, to mimic the environment of HG-PDS, DMEM was supplemented with 84 mM, 138 mM, and 236 mM glucose for 24 h. In addition, the control was treated with a normal glucose (NG) concentration of 5.6 mM. Second, to examine the effect of valsartan on ECM accumulation, the cells were preincubated with 236 mM glucose (HG group) with or without different dosages of valsartan (Sigma, SML0142) for 24 h. Third, the positive control experiment was designed with buffer

containing NG, with or without AngII (MCE, 4474–91-3), and with or without valsartan. Finally, MHY1485 (Selleck Chemicals, s7811) was chosen to activate the mTOR pathway. The cells were pretreated with 2 μM MHY1485 for 30 min before treatment with valsartan.

Western blot analysis

Identical amounts of total protein from the peritoneum and cultured HPMCs were denatured and later subjected to electrophoresis. The membranes were incubated with primary antibodies against α-SMA (Abcam, ab7817), collagen I (Santa Cruz, sc-25974), mTOR (Abcam, ab51089), p-mTOR (Abcam, ab51044), 4EBP1 (Abcam, ab32024), p-4EBP1 (Abcam, ab47365), S6K1 (Abcam, ab32359), and p-S6K1 (Abcam, ab131459) overnight at 4°C, then the membranes were incubated for 1 h in horseradish peroxidase-labeled secondary antibodies. Finally, the immunoreactive protein was detected using an enhanced chemiluminescence advanced system (Amersham Biosciences, UK).

Statistical analysis

All results were expressed as the mean ± standard deviation (SD) and were analyzed with SPSS 16.0. Comparisons among multiple groups were made by two-way analysis of variance (ANOVA). Correlation analysis was performed using the Spearman rank-order correlation. Differences were defined significant at $P < 0.05$.

Results

Valsartan ameliorates peritoneal histopathologic changes and functional damage in a PD mouse model

In contrast to normal control mice, mice that received injections of 4.25% PDS for four weeks developed overt typical features of PF, including thickening of the submesothelial compact zone within the anterior abdominal wall and collagen deposition (Figure 1(a) to (d)). Similarly, PF mice exhibited increased peritoneal permeability from week 4 to week 8, as determined by PETs (Figure 1(e) and (f)). In contrast, valsartan significantly attenuated peritoneal thickness and collagen accumulation as well as peritoneal impairment at both week 6 and week 8 (Figure 1).

Valsartan alleviates ECM accumulation in a PD mouse model

ECM accumulation is a key process of PF. We detected the expression of critical ECM markers, including α-SMA and collagen I, by immunohistochemical staining (Figure 2(a) to (d)) and Western blot (Figure 2(e) to (g)). Consistent with the histological and functional injury, the protein expression levels of both α-SMA and collagen I were increased in PD mice at week 4 and further aggravated until week 8 compared with the control. Valsartan treatment obviously suppressed the ECM accumulation in peritoneum from weeks 6 to 8 (Figure 2).

Valsartan restrains the activation of the mTORC1 pathway in a PD mouse model

We examined the preventive role of valsartan against the expression of the mTORC1 pathway in response to chronic HG-PDS infusion. Compared to that in the control, the protein expressions of p-mTOR, p-4EBP1, and p-S6K1 were increased in the HG-PDS-treated peritoneal tissue from weeks 4 to 8, as demonstrated by Western blot. However, valsartan substantially inhibited the HG-PDS-induced activation of the mTORC1 pathway at each time point (Figure 3).

Correlation between PF and the activation of the mTORC1 pathway in a PD mouse model

As demonstrated in Figure 4, there was an obvious positive correlation between the protein expression of p-mTOR and both α-SMA ($r = 0.822$, $P < 0.001$) and collagen I ($r = 0.697$, $P < 0.05$), between the protein expression of p-4EBP1 and both α-SMA ($r = 0.838$, $P < 0.001$) and collagen I ($r = 0.896$, $P < 0.01$), and between the protein expression of p-S6K1 and both α-SMA ($r = 0.897$, $P < 0.001$) and collagen I ($r = 0.606$, $P < 0.01$). The data suggest that the preventive effect of valsartan against ECM accumulation is closely associated with the inhibition of mTORC1 pathway.

Valsartan prevents HG-induced ECM accumulation in vitro

To explore the effects of different glucose concentrations and exclude the effect of osmolality, HPMCs were stimulated with various concentrations of glucose (84 mM, 138 mM, 236 mM) that are equal to PDS containing 1.5%, 2.5%, and 4.25% glucose, and 236 mM mannitol for 24 h. Mannitol was used as a high osmotic pressure control. The results showed that, compared with NG treatment, 236 mM glucose significantly increased the expression of α-SMA and collagen I, and the osmotic pressure control had no such effect (Figure 5(a) and (b)). Based on the data, we used 236 mM glucose for 24 h as the HG group. We next tested the effect of valsartan on the expression of ECM markers in HPMCs after exposure to HG. HPMCs were treated with 1 μM and 10 μM valsartan, and the content of ECM components such as α-SMA and collagen I decreased in a dose-dependent manner, even in the presence of HG (Figure 5(c) and (d)). Hence, we treated HPMCs with 10 μM valsartan for 24 h in subsequent experiments. Lastly, the positive control experiment was designed with buffer containing NG, with or without 10^{-7}M AngII, and with or without valsartan. Compared with the NG, HG and AngII had the same effect on the upregulation of α-SMA and collagen I, while cotreatment with valsartan decreased the HG-induced or AngII-induced upregulation of α-SMA and collagen I (Figure 5(e) and (f)).

Valsartan inhibits HG-induced ECM accumulation through the inactivation of the mTORC1 pathway in vitro

Exposure of HPMCs to AngII increased the protein expression levels of p-mTOR, p-4EBP1, and p-S6K1, as assessed

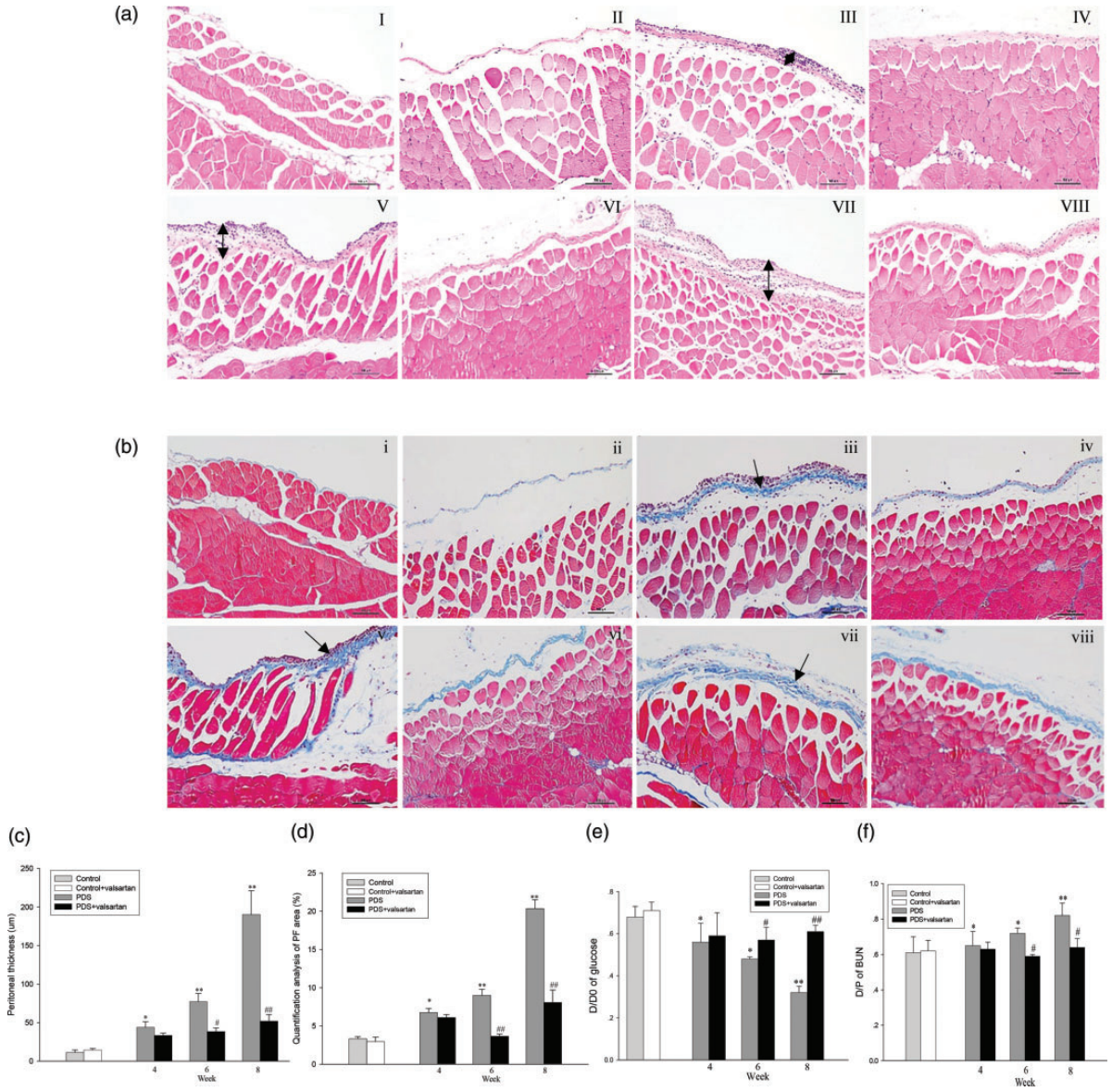

Figure 1. Effect of valsartan on peritoneal histopathological and functional injury in a mouse model of PD. C57BL/6 mice were treated with PBS (control, I and i), 30 mg/kg/d valsartan plus PBS for eight weeks (control+valsartan, II and ii), 4.25% glucose PDS (PDS) for four weeks (III, iii), six weeks (V, v), or eight weeks (VII, vii), or 30 mg/kg/d valsartan plus 4.25% glucose PDS (PDS+valsartan) for four weeks (IV, iv), six weeks (VI, vi), or eight weeks (VIII, viii). (a) H&E staining and (b) Masson's trichrome staining of the parietal peritoneum (Scale Bar = 100 μm, original magnification, 100×). (c) Thickness of the peritoneum. (d) The area of PF. (e) Peritoneal permeability, as determined by PET-glucose. (f) Peritoneal permeability, as determined by PET-BUN analysis. The results represent the mean ± SD of six mice from each group. $^{*}P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. PDS group at the same time point, respectively; $^{\#\#}P < 0.001$ vs. PDS group at the same time point. (A color version of this figure is available in the online journal.)

by Western blot, and HG had the similar role as the AngII. Cotreatment with valsartan ameliorated the HG-induced or AngII-induced upregulation of components of the mTORC1 pathway (Figure 6(a) and (b)). To further determine the mechanisms associated with the regulation of ECM accumulation by valsartan, we subsequently analyzed the effect of the specific mTOR agonist MHY1485 on valsartan-mediated α-SMA and collagen I expression. The data showed that, compared with valsartan, MHY1485 dramatically increased the expression of α-SMA and collagen I, even in the presence of valsartan, as determined by Western blot (Figure 6(c)and (d)). Taken together, these results suggest that the protective effect of valsartan against PF is related to the downregulation of the activity of the mTORC1 pathway.

Discussion

PF is a severe complication associated with the treatment of ESRD with PD.[16] The pathogenesis of PF is characterized by the excessive deposition of ECM in PMCs, macrophage infiltration, and progressive peritoneal thickening with a raising presence of myofibroblasts. PMCs are the main composition of peritoneum, and convincing evidence has highlighted the deposition of ECM in PMCs plays a critical role in the PF.[17,18] Recently, some studies have reported that

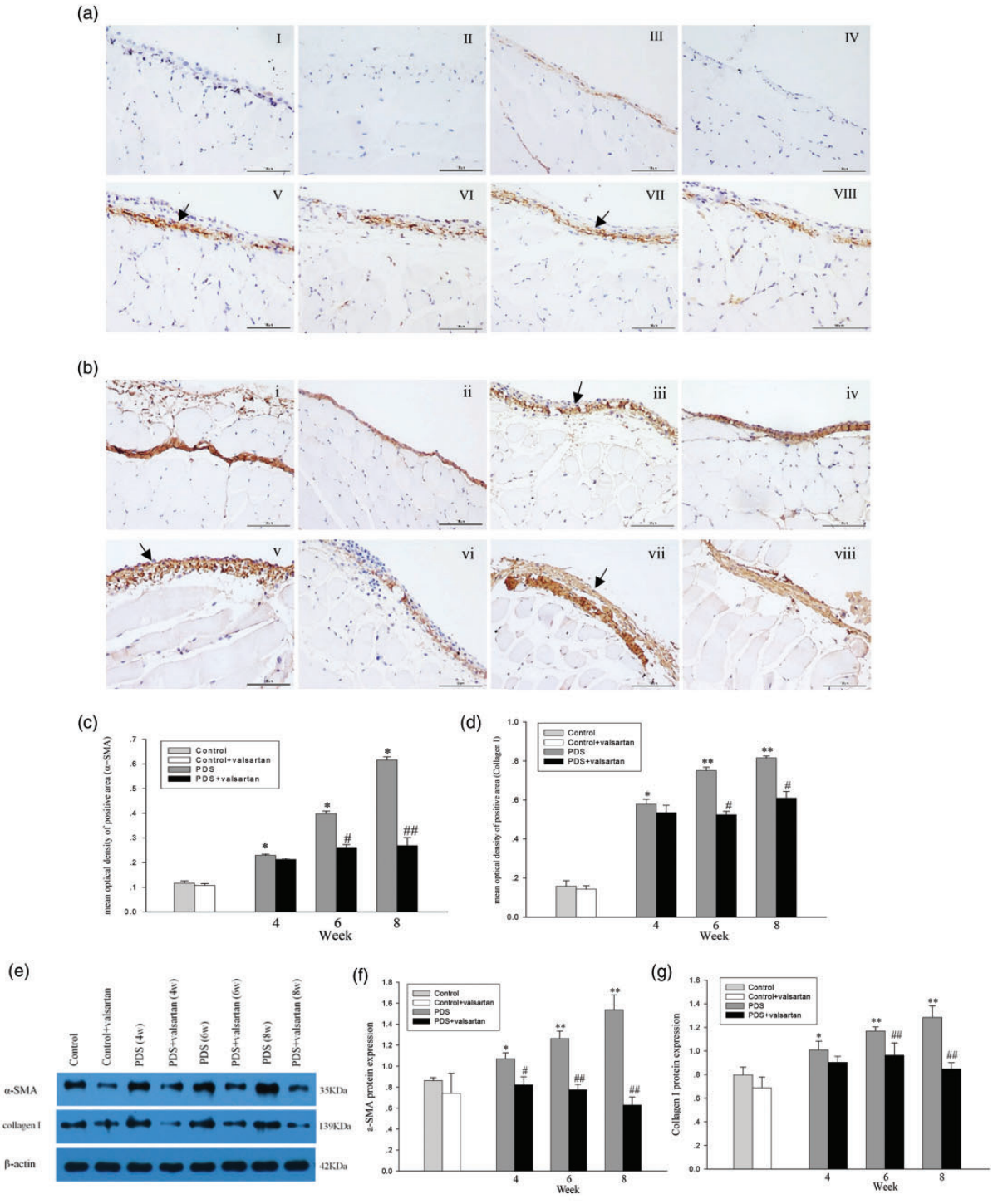

Figure 2. Effect of valsartan on ECM deposition in the peritoneum of a mouse model of PD. C57BL/6 mice were treated with PBS (control, I and i), 30 mg/kg/d valsartan plus PBS for eight weeks (control+valsartan, II and ii), 4.25% glucose PDS (PDS) for four weeks (III, iii), six weeks (V, v), or eight weeks (VII, vii), or 30 mg/kg/d valsartan plus 4.25% glucose PDS (PDS+valsartan) for four weeks (IV, iv), six weeks (VI, vi), or eight weeks (VIII, viii). Immunohistochemical staining for (a) α-SMA and (b) collagen I in the area of the parietal peritoneum. The positive areas were stained brown (scale bar = 200 μm, original magnification, 200×). (c and d) The values of semiquantitative analysis for the positive areas are expressed as the mean ± SD of six mice from each group. $^{*}P < 0.05$ vs. control; $^{**}P < 0.001$ vs. control; $^{\#}P < 0.05$ vs. PDS group at the same time point; $^{\#\#}P < 0.01$ vs. PDS group at the same time point. (e–g) The protein levels of α-SMA and collagen I in the visceral peritoneum were further determined by Western blot analysis. The histogram shows the mean ± SD of the densitometric scans of the protein bands from six mice following normalization b to β-actin. $^{*}P < 0.05$ vs. control; $^{**}P < 0.001$ vs. control; $^{\#}P < 0.01$ vs. PDS group at the same time point; $^{\#\#}P < 0.001$ vs. PDS group at the same time point. (A color version of this figure is available in the online journal.)

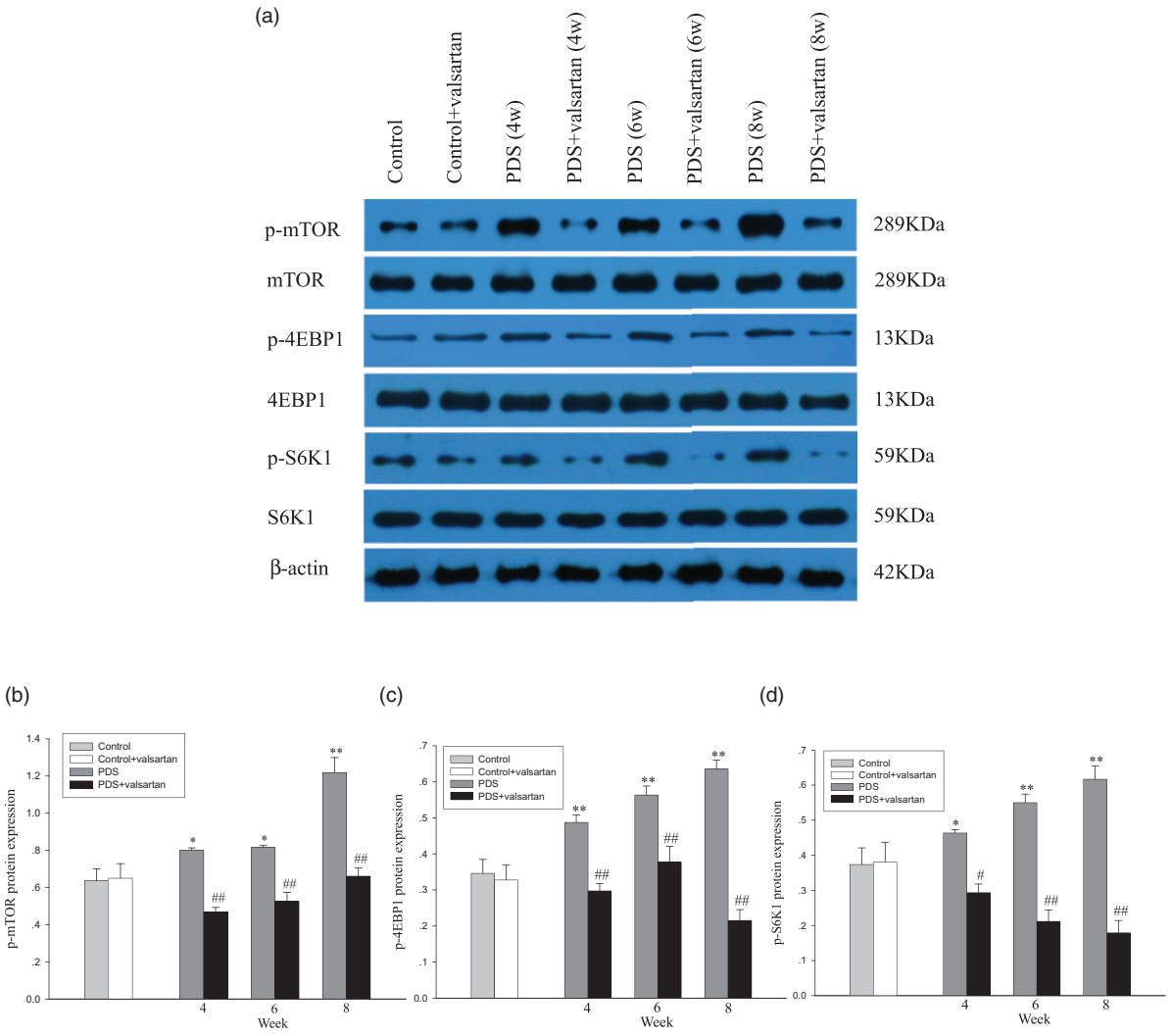

Figure 3. Effect of valsartan on the expression of the mTORC1 pathway in the peritoneum of a mouse model of PD. C57BL/6 mice were treated with PBS (control), 30 mg/kg/d valsartan plus PBS for eight weeks (control+valsartan), or 4.25% glucose PDS (PDS) or 30 mg/kg/d valsartan plus 4.25% glucose PDS (PDS+valsartan) for three time points (four, six, or eight weeks). (a–d) The protein expression levels of p-mTOR, mTOR, p-4EBP1, 4EBP1, p-S6K1, and S6K1 were determined by Western blot analysis. The histogram shows the mean ± SD of the densitometric scans of the protein bands from six mice following normalization to β-actin. $^{*}P < 0.05$ vs. control; $^{**}P < 0.001$ vs. control; $^{\#}P < 0.05$ vs. PDS group at the same time point; $^{\#\#}P < 0.001$ vs. PDS group at the same time point.

AngII is constitutively expressed by PMCs and initiates the production of TGF-β1, thus contributing to ECM deposition and the induction of PF.[19] ARBs may therefore be a powerful tool for preserving peritoneal function beyond blood pressure reduction.[20,21] However, the mechanism of the protective role of ARBs against PF has not been thoroughly investigated. In this study, we demonstrated that valsartan obviously inhibits PF by decreasing the activity of the mTORC1 pathway in the peritoneum under HG stimulation.

Our previous study suggested that the C57BL/6 mice that receive daily intraperitoneal injections of HG-PDS for four weeks manifest typical features of PF.[22] Although the idea animal model should combine both uremia and daily PD treatment, only few studies report on PD in uremic animals, which is most likely caused by the difficult procedure needed to induce uremia, and low rate of success especially when combined with long-term exposure to PDS. In this study, we used the daily PD treatment animal model without uremia, and the data showed that the mice that received chronic HG-PDS infusion also manifested typical characteristics of PF from four weeks; moreover, these pathological features were even more robust at week 6 and week 8. However, treatment with valsartan reversed the phenomenon after week 6. *In vitro*, HG increased the protein expression of α-SMA and collagen I in a dose-dependent manner, while valsartan significantly inhibited HG-induced ECM accumulation in HPMCs. Ersoy *et al.*[23] demonstrated that when irbesartan was added to the drinking water of Wistar rats, the formation of adhesions and the increase in peritoneal thickness caused by the bacterial/dextran mixture were obviously decreased by more than 80% and 90%, respectively. Nakamoto *et al.*[24] showed that subsets of rats that received a 1.35% glucose solution of pH 3.5 were treated with oral olmesartan or oral amlodipine for six weeks. However, the 25-mm Hg increase in blood pressure was completely prevented by

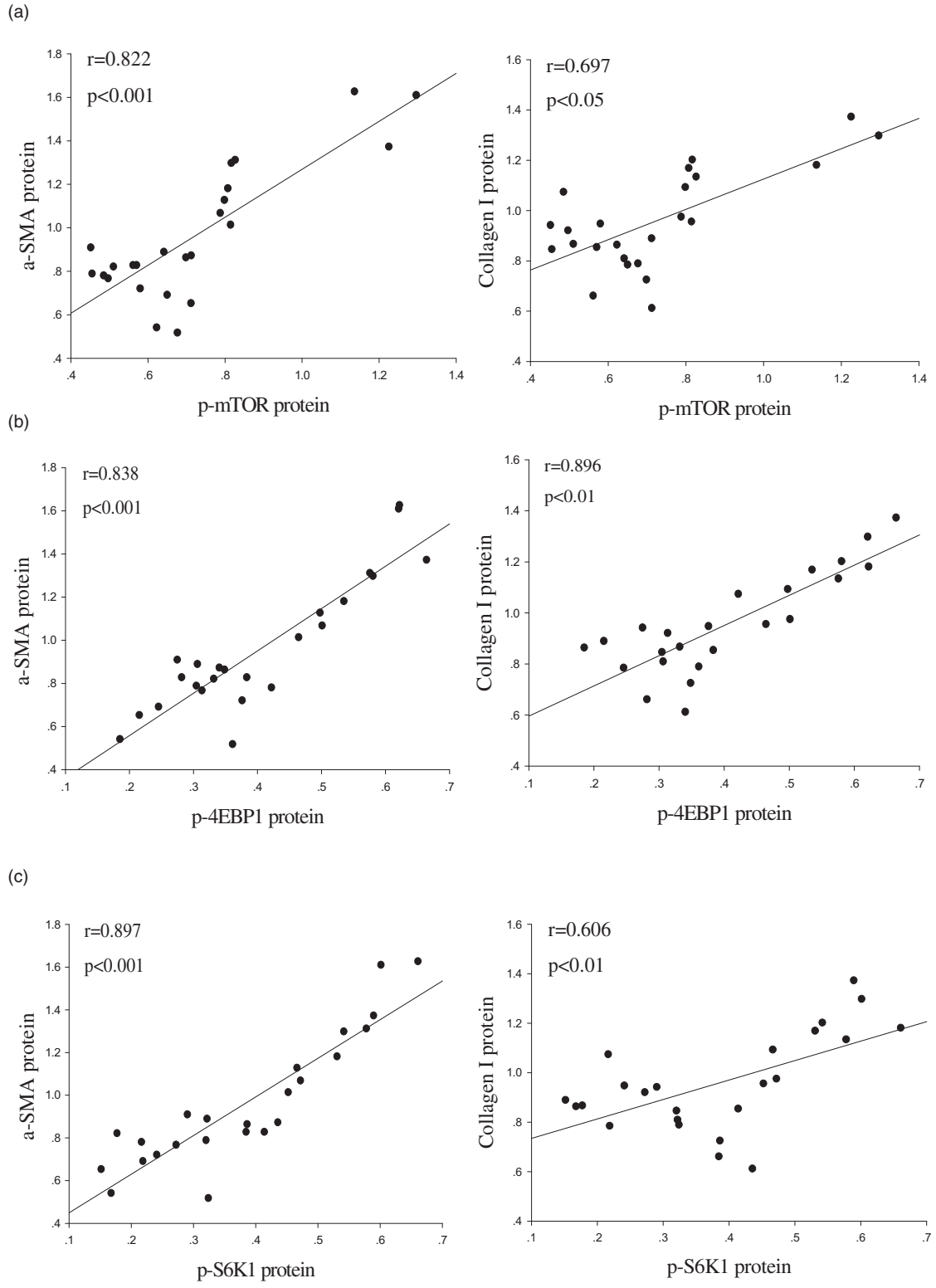

Figure 4. Correlation analysis between ECM accumulation and the expression of the mTORC1 pathway in the peritoneum of a PD mouse model. C57BL/6 mice were treated with PBS (control), 30 mg/kg/d valsartan plus PBS for eight weeks (control+valsartan), or 4.25% glucose PDS (PDS) or 30 mg/kg/d valsartan plus 4.25% glucose PDS (PDS+valsartan) for three time points (four, six, or eight weeks). According to Western blot data of α-SMA and collagen I expression, ECM accumulation showed positive correlation with (a) p-mTOR, (b) p-4EBP1, and (c) p-S6K1 protein levels. The *P*-values were two-tailed, and $P < 0.05$ was considered significant.

both antihypertensive drugs and olmesartan, but not amlodipine prevented the development of adhesions and thickened peritoneum. The protective effect of ARBs has also been shown in chronic PD patients. Jing *et al.*[25] found that chronic treatment with ARBs mitigates the increase in small molecule transport that results over time from PD therapy, and further analysis showed that the concentrations of fibronectin, TGF-β1, and vascular

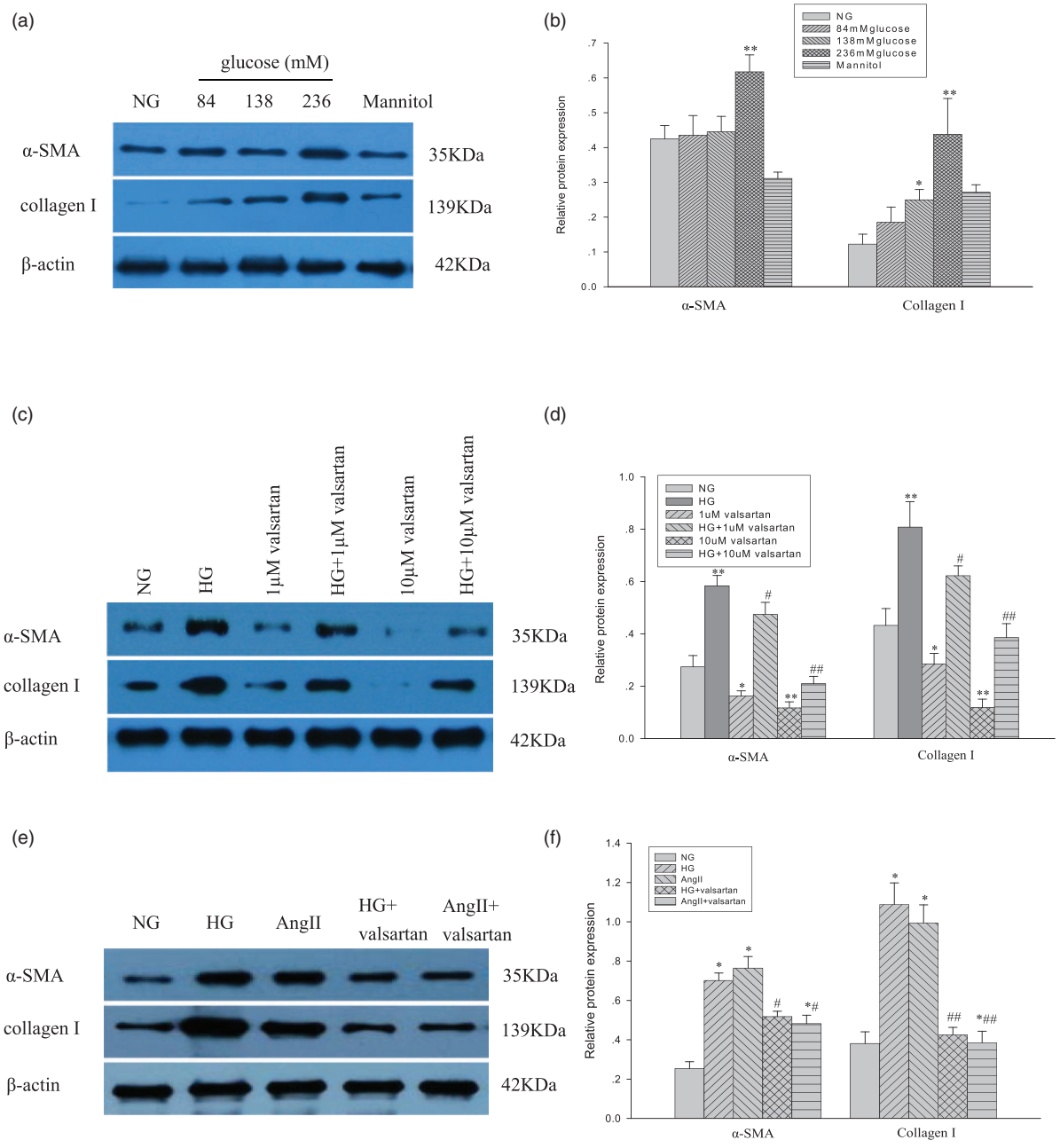

Figure 5. Effect of valsartan on ECM deposition activated by HG in HPMCs. (a and b) HPMCs were stimulated with serum-free medium containing 5.6 mM glucose (NG) or high concentrations of glucose (84, 138, and 236 mM) or 236 mM mannitol (mannitol) for 24 h. The protein levels of α-SMA and collagen I were examined by Western blot analysis. The histogram represents the mean±SD of the densitometric scans of the protein bands from three experiments normalized to β-actin. $^{*}P < 0.05$ vs. NG group; $^{**}P < 0.001$ vs. NG group. (c and d) HPMCs were exposed to serum-free medium containing 5.6 mM glucose (NG) or serum-free medium containing 236 mM glucose (HG), 1 μM valsartan, HG plus 1 μM valsartan, 10 μM valsartan, or HG plus 10 μM valsartan. The protein levels of α-SMA and collagen I were examined by Western blot analysis. The histogram represents the mean ± SD of the densitometric scans for protein bands from three experiments normalized to β-actin. $^{*}P < 0.05$ vs. NG group; $^{**}P < 0.001$ vs. NG group; $^{\#}P < 0.01$ vs. HG group; $^{\#\#}P < 0.001$ vs. HG group. (e and f) HPMCs were treated with 5.6 mM glucose (NG) or with 236 mM glucose (HG), or 10^{-7} M AngII (AngII), or 236 mM glucose plus 10 μM valsartan (HG+valsartan), or 10^{-7} M AngII plus 10 μM valsartan (AngII+valsartan) for 24 h. The protein levels of α-SMA and collagen I were examined by western blot analysis. The histogram represents the mean ± SD of the densitometric scans for protein bands from three experiments normalized to β-actin. $^{*}P < 0.001$ vs. NG group; $^{\#}P < 0.01$ vs. HG group; $^{\#\#}P < 0.001$ vs. HG group; $^{*\#}P < 0.01$ vs. AngII group; $^{*\#\#}P < 0.001$ vs. AngII group.

endothelial growth factor in the intraperitoneal fluid are significantly lower over a one-year period in patients treated with ARBs. These studies suggest that ARBs can improve peritoneal injury induced by either HG or other infectious factors. However, the underlying molecular basis of the protective role of ARBs against HG-induced PF is not clear.

Next, we observed that HG induced the activation of mTORC1 pathway *in vivo* and *in vitro*. These data were consistent with those of our previous *in vitro* study.[7] Conversely, valsartan treatment significantly suppressed the activity of mTORC1 signaling from weeks 4 to 8. Interestingly, the expression of p-4EBP1 and p-S6K1 in the PDS+valsartan group was too much decreased compared

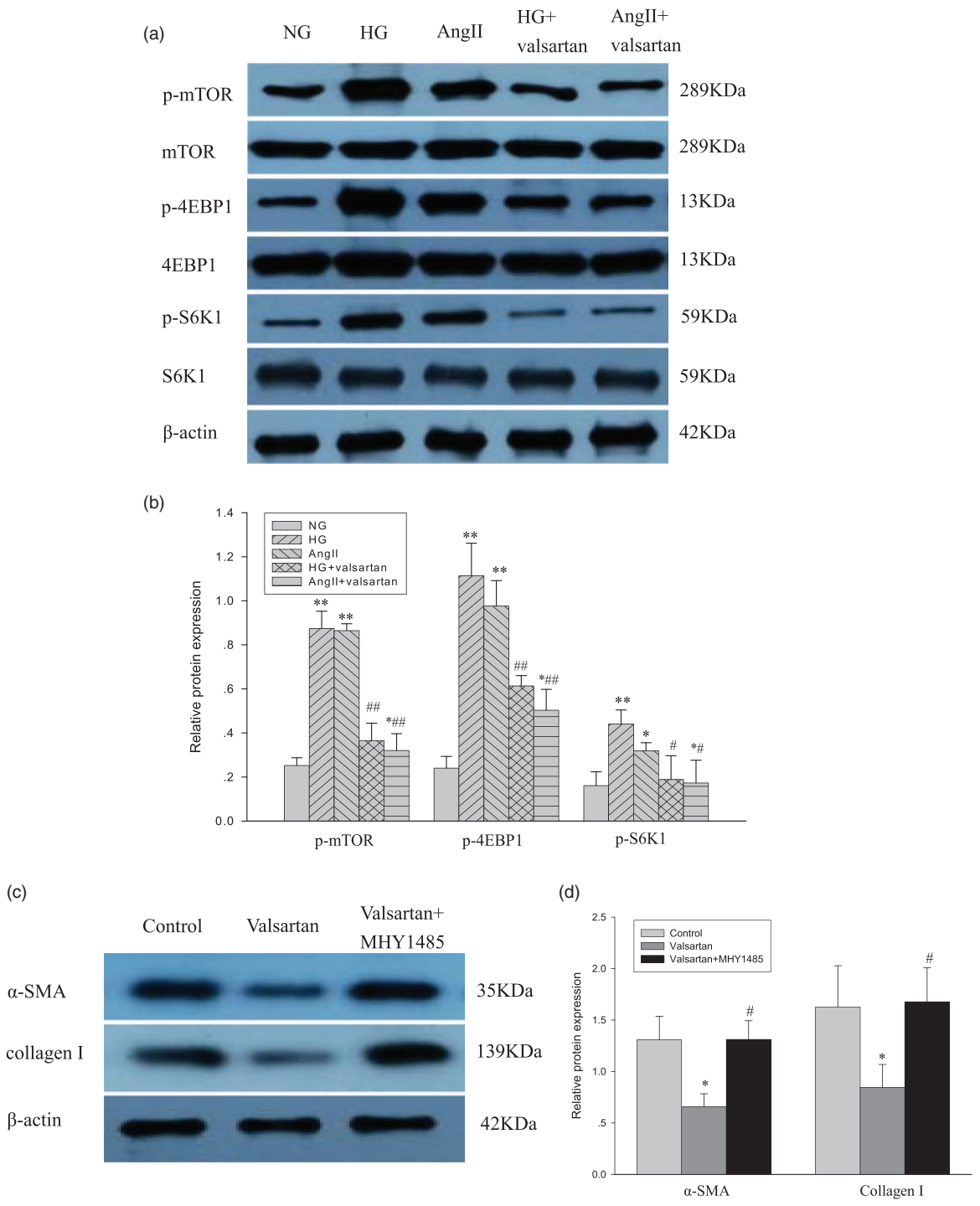

Figure 6. Effect of valsartan on the expression of the mTORC1 pathway activated by HG in HPMCs. (a and b) HPMCs were treated with 5.6 mM glucose (NG) or with 236 mM glucose (HG), or 10^{-7} M AngII (AngII), or 236 mM glucose plus 10 μM valsartan (HG+valsartan), or 10^{-7} M AngII plus 10 μM valsartan (AngII+valsartan) for 24 h. The protein levels of p-mTOR, mTOR, p-4EBP1, 4EBP1, p-S6K1, and S6K1 were examined by Western blot analysis. The histogram represents the mean ± SD of the densitometric scans of the protein bands from three experiments normalized to β-actin. $^{*}P < 0.05$ vs. NG group; $^{**}P < 0.01$ vs. NG group; $^{\#}P < 0.01$ vs. HG group; $^{\#\#}P < 0.001$ vs. HG group; $^{*\#}P < 0.05$ vs. AngII group; $^{*\#\#}P < 0.001$ vs. AngII group. (c and d) HPMCs were treated without (control) or with 10 μM valsartan (valsartan) or 10 μM valsartan plus 2 μM MHY1485 (valsartan+MHY1485) for 24 h. The protein levels of α-SMA and collagen I were examined by Western blot analysis. The histogram represents the mean ± SD of the densitometric scans for protein bands from three experiments normalized to β-actin. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. valsartan group.

with the control+valsartan group. Nessim *et al.* demonstrated that components of the RAS including AT1R are constitutively expressed within peritoneum, and are increased in the presence of HG-PDS. And valsartan exerts its effect mainly through antagonizing the AT1R,[26] so we speculate that valsartan might play a better role on blocking mTORC1 signal when cotreated with PDS. Further correlation analysis found that there was a parallel decrease in the activity of mTORC1 and the protein expression of ECM markers upon treatment with valsartan.

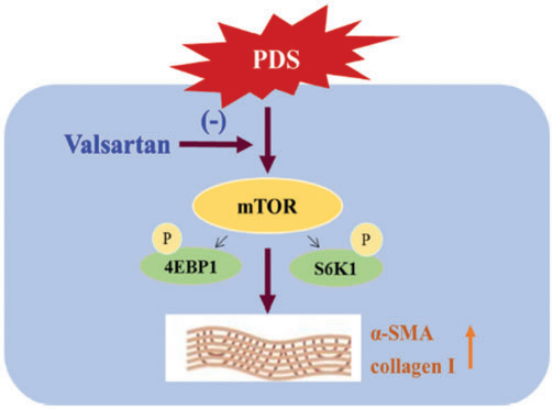

Figure 7. Schematic model of the protective mechanism of valsartan against HG-induced PF in PMCs. Valsartan significantly inhibits HG-induced ECM accumulation in the peritoneum, which manifests as decreased expression levels of α-SMA and collagen I. These effects are correlated with a decrease in the expression of the mTORC1 pathway, which is mediated by the downregulation of p-mTOR, p-4EBP1, and p-S6K1 levels. Overall, valsartan exerts an obvious protective effect against HG-induced PF, which is partly due to the inhibition of the mTORC1 pathway in PMCs. (A color version of this figure is available in the online journal.)

Moreover, the positive control experiment was designed with AngII and then cotreated with valsartan *in vitro*, results showed that AngII had the similar role with HG on the ECM accumulation and the mTORC1 activation, while cotreatment with valsartan inhibited the AngII-induced these effects. Therefore, the protective effect of valsartan on the peritoneum seems to be closely related to the decrease of mTORC1 activity.

To further explain the potential regulation of mTOR and ECM accumulation in HPMCs, we detected the effect of the specific mTOR agonist MHY1485 on valsartan-mediated ECM expression. The results showed that MHY1485 reversed the downregulation of α-SMA and collagen I in HPMCs, even in the presence of valsartan. Recently, Gong *et al.* found that mTOR/p70S6K signaling is highly activated in HK-2 cells cultured under HG but is inhibited by transfection with AT1R siRNA. The results imply that preventing AT1R inhibits renal tubulointerstitial fibrosis induced by HG through the inactivation of the mTORC1 signaling.[27] Takenaka *et al.* suggested that exogenous klotho supplementation represses mTORC1 signaling to reduce renal hypertrophy and restore the autoregulatory ability of the glomerular filtration rate in stroke-prone spontaneously hypertensive rats, and the potential mechanism is that klotho binds AT1R to suppress AngII signal transduction; therefore, mTORC1 is an important downstream effector of AT1R that participates in organ injury.[28] Collectively, our results are partially consistent with the conclusion of these studies, which showed that blocking AT1R with valsartan prevents PF by inactivating mTORC1 signaling.

In summary, the present study demonstrated that HG-related PF is closely associated with the activation of mTORC1. Valsartan can control PF and is associated with the inhibition of mTORC1 activity (Figure 7). Altogether, our data provide new insight into the mechanism underlying the preservation of the peritoneum by valsartan and supply a foundation for therapeutic strategies for long-term PD patients.

Authors' contributions: All authors contributed to this research article.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This study was funded by the Nanjing Medical Science and Technique Development Foundation (QRX17120, YKK18068), Project of Jiangsu Province Department of Health (H201645), Six talent peaks project in Jiangsu Province (2016-SWYY-089), and Jiangsu Province's Key Provincial Talents Program (QNRC2016028).

ORCID iD

Jing Liu https://orcid.org/0000-0001-6424-9719

REFERENCES

1. Zhang Z, Jiang N, Ni Z. Strategies for preventing peritoneal fibrosis in peritoneal dialysis patients: new insights based on peritoneal inflammation and angiogenesis. *Front Med* 2017;**11**:349–58
2. Zhou Q, Bajo MA, Del Peso G, Yu X, Selgas R. Preventing peritoneal membrane fibrosis in peritoneal dialysis patients. *Kidney Int* 2016;**90**:515–24
3. Lu Q, Chen YB, Yang H, Wang WW, Li CC, Wang L, Wang J, Du L, Yin XX. Inactivation of TSC1 promotes epithelial-mesenchymal transition of renal tubular epithelial cells in mouse diabetic nephropathy. *Acta Pharmacol Sin* 2019;**40**:1555–67
4. Sun X, Wei W, Ren J, Liang Y, Wang M, Gui Y, Xue X, Li J, Dai C. Inhibition of 4E-BP1 phosphorylation promotes tubular cell escaping from G2/M arrest and ameliorates kidney fibrosis. *Cell Signal* 2019;**62**:109331
5. Woodcock HV, Eley JD, Guillotin D, Plate M, Nanthakumar CB, Martufi M, Peace S, Joberty G, Poeckel D, Good RB, Taylor AR, Zinn N, Redding M, Forty EJ, Hynds RE, Swanton C, Karsdal M, Maher TM, Bergamini G, Marshall RP, Blanchard AD, Mercer PF, Chambers RC. The mTORC1/4E-BP1 axis represents a critical signaling node during fibrogenesis. *Nat Commun* 2019;**10**:6
6. Andrikopoulos P, Kieswich J, Pacheco S, Nadarajah L, Harwood SM, O'Riordan CE, Thiemermann C, Yaqoob MM. The MEK inhibitor trametinib ameliorates kidney fibrosis by suppressing ERK1/2 and mTORC1 signaling. *J Am Soc Nephrol* 2019;**30**:33–49
7. Liu J, Zhu W, Jiang CM, Feng Y, Xia YY, Zhang QY, Xu PF, Zhang M. Mammalian target of rapamycin complex 1 activation disrupts the Low-Density lipoprotein receptor pathway: a novel mechanism for extracellular matrix accumulation in human peritoneal mesothelial cells. *Am J Nephrol* 2018;**48**:357–68
8. Morinelli TA, Luttrell LM, Strungs EG, Ullian ME. Angiotensin II receptors and peritoneal dialysis-induced peritoneal fibrosis. *Int J Biochem Cell Biol* 2016;**77**:240–50
9. Nessim SJ, Bargman JM. The peritoneal-renal syndrome. *Nat Rev Nephrol* 2013;**9**:302–6
10. Kocak G, Azak A, Astarci HM, Huddam B, Karaca G, Ceri M, Can M, Sert M, Duranay M. Effects of renin-angiotensin-aldosterone system blockade on chlorhexidine gluconate-induced sclerosing encapsulated peritonitis in rats. *Ther Apher Dial* 2012;**16**:75–8

11. Trost Rupnik A, Pajek J, Gucek A, Osredkar J, Kovac D, Bren A, Klancic D, Saksida S, Rus I, Globokar M, Drozg A, Lesnik M, Plesivcnik Z, Ekart R, Lopert S, Lindic J. Influence of renin-angiotensin-aldosterone system-blocking drugs on peritoneal membrane in peritoneal dialysis patients. *Ther Apher Dial* 2013;**17**:425–30
12. Mavroeidi V, Petrakis I, Stylianou K, Katsarou T, Giannakakis K, Perakis K, Vardaki E, Stratigis S, Ganotakis E, Papavasiliou S, Daphnis E. Losartan affects glomerular AKT and mTOR phosphorylation in an experimental model of type 1 diabetic nephropathy. *J Histochem Cytochem* 2013;**61**:433–43
13. Diniz GP, Carneiro-Ramos MS, Barreto-Chaves ML. Angiotensin type 1 receptor mediates thyroid hormone-induced cardiomyocyte hypertrophy through the akt/GSK-3beta/mTOR signaling pathway. *Basic Res Cardiol* 2009;**104**:653–67
14. Whaley-Connell A, Habibi J, Panfili Z, Hayden MR, Bagree S, Nistala R, Hyder S, Krueger B, Demarco V, Pulakat L, Ferrario CM, Parrish A, Sowers JR. Angiotensin II activation of mTOR results in tubulointerstitial fibrosis through loss of N-cadherin. *Am J Nephrol* 2011;**34**:115–25
15. Yu JW, Duan WJ, Huang XR, Meng XM, Yu XQ, Lan HY. MicroRNA-29b inhibits peritoneal fibrosis in a mouse model of peritoneal dialysis. *Lab Invest* 2014;**94**:978–90
16. Mehrotra R, Devuyst O, Davies SJ, Johnson DW. The current state of peritoneal dialysis. *J Am Soc Nephrol* 2016;**27**:3238–52
17. Devuyst O, Margetts PJ, Topley N. The pathophysiology of the peritoneal membrane. *J Am Soc Nephrol* 2010;**2**:1077–85
18. Helmke A, Nordlohne J, Balzer MS, Dong L, Rong S, Hiss M, Shushakova N, Haller H. Von vietinghoff S. CX3CL1-CX3CR1 interaction mediates macrophage-mesothelial cross talk and promotes peritoneal fibrosis. *Kidney Int* 2019;**95**:1405–17
19. Li YS, Ni SY, Meng Y, Shi XL, Zhao XW, Luo HH, Li X. Angiotensin II facilitates fibrogenic effect of TGF-beta1 through enhancing the down-regulation of BAMBI caused by LPS: a new pro-fibrotic mechanism of angiotensin II. *PLoS One* 2013;**8**:e76289
20. Nongnuch A, Assanatham M, Panorchan K, Davenport A. Strategies for preserving residual renal function in peritoneal dialysis patients. *Clin Kidney J* 2015;**8**:202–11
21. Kyuden Y, Ito T, Masaki T, Yorioka N, Kohno N. Tgf-beta1 induced by high glucose is controlled by angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker on cultured human peritoneal mesothelial cells. *Perit Dial Int* 2005;**25**:483–91
22. Liu J, Jiang CM, Feng Y, Zhu W, Xia JB, Zhang YY, Xu QY, Zhang PF. M. Rapamycin inhibits peritoneal fibrosis by modifying lipid homeostasis in the peritoneum. *Am J Transl Res* 2019;**11**:1473–85
23. Ersoy R, Celik A, Yilmaz O, Sarioglu S, Sis B, Akan P, Yenicerioglu Y, Ormen M, Camsari T. The effects of irbesartan and spironolactone in prevention of peritoneal fibrosis in rats. *Perit Dial Int* 2007;**27**:424–31
24. Nakamoto H, Imai H, Fukushima R, Ishida Y, Yamanouchi Y, Suzuki H. Role of the renin-angiotensin system in the pathogenesis of peritoneal fibrosis. *Perit Dial Int* 2008;**28**:S83–87
25. Jing S, Kezhou Y, Hong Z, Qun W, Rong W. Effect of renin-angiotensin system inhibitors on prevention of peritoneal fibrosis in peritoneal dialysis patients. *Nephrology* 2010;**15**:27–32
26. Nessim SJ, Perl J, Bargman JM. The renin-angiotensin-aldosterone system in peritoneal dialysis: is what is good for the kidney also good for the peritoneum?. *Kidney Int* 2010;**78**:23–8
27. Gong Q, Hou F. Silencing of angiotensin II type-1 receptor inhibits high glucose-induced epithelial-mesenchymal transition in human renal proximal tubular epithelial cells via inactivation of mTOR/p70S6K signaling pathway. *Biochem Biophys Res Commun* 2016;**469**:183–8
28. Takenaka T, Inoue T, Miyazaki T, Kobori H, Nishiyama A, Ishii N, Hayashi M, Suzuki H. Klotho ameliorates medullary fibrosis and pressure natriuresis in hypertensive rat kidneys. *Hypertension* 2018;**72**: 1151–9

(Received November 21, 2019, Accepted March 25, 2020)