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Original Article

Beta-arrestin-1 mediating protective effects of penehyclidine hydrochloride on changes of F-actin in lipopolysaccharide-stimulated human pulmonary microvascular endothelial cells

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Abstract: In acute lung injury (ALI) and sepsis, the integrity of the endothelial barrier, maintained by endothelial cytoskeleton of microvascular endothelial cells, is important to maintain normal microvascular function. In response to lipopolysaccharide (LPS), F-actin (fibrous actin, F-actin), an essential component of the endothelial cytoskeleton, has been shown to be critical to maintaining the endothelial integrity. Herein, penehyclidine hydrochloride (PHC), an anticholinergic agent manufactured in China, was verified to inhibit p38 MAPK activation, reduce Hsp27 expression, and inhibit LPS-stimulated reorganization of F-actin and formation of stress fiber in this study. Besides, these roles of PHC in human pulmonary microvascular endothelial cells (HPMVEC) were found to be accompanied with its upregulation of beta-arrestin-1. Furthermore, the silencing of beta-arrestin-1 with a shRNA-containing plasmid resulted in an antagonistic effect vs. PHC on the endothelial F-actin function of HPMVEC. In summary, beta-arrestin-1 plays an important role in mediating protective effect of PHC on changes of F-actin in LPS-stimulated HPMVEC.

Keywords: Beta-arrestin-1 penehyclidine hydrochloride, F-actin, shRNA

Introduction

Lipopolysaccharide (LPS), an essential component of the outer membrane of Gram-negative bacteria, is a potent inflammatory mediator and plays an important role in pathogenesis of sepsis [1]. In sepsis, microvascular endothelial cells play a central role in the control of expression of cytokines and chemokines, which facilitate the trafficking of leukocytes to the infected tissue, and are known to respond to LPS with the loss of vascular barrier function [2]. Microvascular endothelial cells are also responsible for the development of the lung edema that one hallmark of acute lung injury is high pulmonary endothelial permeability [3]. Microvascular endothelial cell together with the associated cytoskeletal networks, ultimately determine the integrity and barrier function of the microvascular endothelium.

Of the major cytoskeletal networks, F-actin cytoskeleton is the most abundant and affects the endothelial barrier function in multiple ways [4]. Beta-arrestins, a known negative feedback regulators of G-protein coupled receptors (GPCRs), might play multiple functions collectively contributing to endothelial barrier properties through regulating the redistribution of F-actin [5]. Acting as scaffolding proteins, beta-arrestins can also lead to the assembly of intracellular signals that can activate or inhibit the function of various signaling cascades, such as the mitogen-activated protein kinases (MAPKs) and NF-kB cascades, ultimately affecting gene expression. This pleiotropic activity of beta-arrestins can regulate both physiologic and pathophysiologic responses and play a central role in the course of pulmonary diseases [6]. Recent studies have uncovered that beta-arrestin 1/2 dual-null mice died shortly after birth

due to pulmonary immaturity [7], and it has been demonstrated that beta-arrestins play a critical role in the development of allergic inflammation in the lung [8], which identify beta-arrestins as potentially important therapeutic targets for pulmonary diseases.

Penehyclidine hydrochloride (PHC) is a new anti-cholinergic drug, which could inhibit biomembrane lipid peroxidation, and decrease cytokines and oxyradicals. The preliminary experiment showed that PHC has no obvious effect on beta-arrestin-2 mRNA expression and our previous studies have found that PHC could upregulate expression of beta-arrestin-1 in pulmonary microvascular endothelial cells [9], and PHC could downregulate pulmonary microvascular permeability during CLP-induced sepsis [10]. However, it is unknown whether the effect of PHC on pulmonary microvascular permeability is dependent on its upregulation of beta-arrestin-1 or not. Then in this study human pulmonary microvascular endothelial cells (HPMVEC) were transfected with a shRNA-containing plasmid that specifically targets beta-arrestin-1 mRNA. F-actin contents and cytoskeleton arrangement, Hsp27 protein expression, p38 MAPK activation, as well as beta-arrestin-1 protein expression, were examined in the incubation of LPS and PHC in HPMVEC.

Materials and methods

Materials

PHC was provided by Lisite Corporation (Chengdu, China). LPS (Escherichia coli 0111: B4) and RPMI 1640 were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Anti-Hsp27 antibody was purchased from Epitomics Inc (Epitomics Inc, Burlingame, CA, USA). Antibodies against p38 MAPK and p-p38 MAPK were purchased from Epitomics Inc (Epitomics Inc, Burlingame, CA, USA). Antibody against beta-arrestin-1 was purchased from Abcam incorporation (Abcam Inc, Cambridge, United Kingdom) and anti-beta-actin antibody was provided by Santa Cruz Biotechnology (Santa Cruz Biotechnology Inc, Dallas, Texas, USA).

Cell culture

HPMVEC were purchased from the ScienCell Research Laboratories (ScienCell, CA, USA). Cells were cultured in RPMI1640, 10% standard newborn calf serum, 50 μg/ml streptomycin, and 50 IU/ml penicillin in a 5% humidified CO_2 atmosphere at 37°C (E191TC, SIM CO_2 INCUBATOR, USA). The medium was changed every other day, and cells were characterized by a typical cobblestone appearance under phase contrast microscope. When the cells were cultured to 80% confluency, the medium was changed and washed twice with PBS. The cells were digested with 0.25% trypsin for 2-3 min and mixed into a suspension. Take the 4-6 generation cells for the later experiment.

Depletion of beta-arrestin-1 in HPMVEC

The plasmidpYr1.1 constructs expressing shRNA directed against human beta-arrestin-1 mRNA and the competent e.coliDH 5α were manufactured by Wuhan Guge biological co. The shRNA sequences were as follows: beta-arrestin-1 shRNA: 5'-AGCTCAAAAAACCTTTGAGATCCCTCCAAAcgtcttgaaTTTGGAGGGATCTCAAAGG-3'. The recombinant plasmid was identified with restriction endonuclease analysis. The plasmid expressing beta-arrestin-1 shRNA contained with green fluorescent protein (GFP) gene. The transfection efficiency of beta-arrestin-1 shRNA was observed by fluorescence microscope. The effects of beta-arrestin-1 knockdown were determined using RT-PCR and we were able to achieve more than 65% transduction efficiency using the plasmid transfection system (data not shown).

Cell groups

HPMVEC were treated with specific beta-arrestin-1 gene-shRNA or empty plasmid. Cells were seeded in 6-well plates (2 ml/well) or in culture flasks (4 ml/flask) with the density of 1×10^5/ml, and randomly divided into empty plasmid group (A group), LPS+ empty plasmid group (B group), PHC+LPS+ empty plasmid group (C group), beta-arrestin 1 gene-shRNA group (D group), LPS+ beta-arrestin 1 gene-shRNA group (E group) and PHC+LPS+ beta-arrestin 1 gene-shRNA group (F group). Cells were treated with 0.1 μg/ml LPS in B group and E group for 60 min, and cells were treated with 2 μg/ml PHC for 60 min and then stimulated with a 0.1 μg/ml concentration of LPS in C group and F group for 60 min.

Laser scanning confocal microscope of F-actin arrangement

The coverslips were taken out when cells were seeded. The coverslips were washed and fixed

with 4% paraformaldehyde for 5 min at room temperature. Then, rhodamine-conjugated phalloidin were added to each coverslip after the cells had grown to confluence and incubated for 40 min in the dark at room temperature. The coverslips were washed in PBS and added with DAPI (Beyotime Institute of Biotechnology, China) in dark at room temperature for 10 min. Morphological features were obtained using a Carl Zeiss 7 confocal laser scanning microscope (Carl Zeiss AG, Jena, Germany). Dual-channel signal acquisition was used. The signal of rhodamine was visualized using an excitation wavelength of 561 nm with fluorescence emission being captured between 565 nm and 650 nm. The signal of DAPI was visualized using an excitation wavelength of 780 nm with fluorescence emission being captured between 450 nm and 500 nm. Images analysis was carried out using the standard software zen 2009.

Determination of F-actin contents

After 1 ml 0.25% trypsin digestion, cells were washed in PBS and collected in flow tubes. The 2 μl anti-F-actin (NH3) antibody (Abcam Inc, Cambridge, United Kingdom) was added and samples were incubated for 40 min at 37°C incubator. Thereafter, cells were centrifuged for 5 min at 1500 rpm, followed by discarding the supernatant and resuspending the cells with 3 ml PBS. Cells were centrifuged for 5 min at 1500 rpm again and the supernatant was discarded. The precipitations were supernatant with 100 μl cold PBS. The mixtures were incubated with 2 μl fluorescein isothiocyanate (FITC)-AffiniPure Goat Anti-Mouse IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA) for 40 min at 37°C incubator. Then samples were centrifuged for 5 min at 1500 rpm, followed by discarding the supernatant and washing with 3 ml PBS. The samples were resuspended in 100 μl PBS before being finally analyzed by flow cytometry (FACsort, BD Biosciences, San Jose, CA, USA).

Immunofluorescence chemistry

The cells were seeded on coverslip-bottom dishes and were treated as indicated, followed by fixation with 4% paraformaldehyde. The cells were then rinsed with PBS and incubated overnight at 4°C with primary anti-Hsp27 antibody. After being washed three times with PBS, cells were incubated with the fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG (KPL, Inc. USA) at room temperature for 50 min. Cells were washed again with PBS and exposed to DAPI (Beyotime Institute of Biotechnology, China) in dark at room temperature for 10 min. Then cells were washed with PBS and dried. The slides were sealed to microscope slides with anti-fluorescence quenching sealed tablets. Several sections were randomly taken and PBS was used instead of antibodies as negative control. The positively-staining sections were analyzed with NIS-elements F3.2 automatic image analysis system.

Western blot analysis

Equal amounts of proteins (40 μg) were loaded onto SDS-polyacrylamide gel, transferred to nylon membranes, and incubated with primary antibody overnight at 4°C. Excess antibody was then removed by washing the membranes in PBS-0.05% Tween-20, and the membranes were incubated in secondary antibodies for 30 min. After being washed in PBS-0.05% Tween 20, the bands were detected by enhanced chemiluminescence (ECL) and the density of the individual bands was quantified by densitometry using Alpha Ease FC software (Genetic Technologies, Inc. Miami, FL, USA).

Statistical analysis

All data were presented as mean ± standard deviation ($\overline{X} \pm S$). Statistical analysis was performed using Student's t-test (SPSS 19.0), and *P* values of less than 0.05 were considered statistically significant.

Results

Role of beta-arrestin-1 in effect of PHC on LPS-induced F-actin arrangement

When exposed to LPS for 60 min, the change in the cell skeleton detected by the confocal laser scanning fluorescence microscope suggests that LPS produced profound effects on the endothelium. Stress fibers were disorder and F-actin (red) was depolymerization and fractured. Cell filopodia was unexpectedly detected, which was quite typical around cell nucleus (blue). PHC partially inhibited LPS-stimulated reorganization of F-actin and formation of stress fiber. And the F-actin became continuous and regular. But in beta-arrestin-1 gene-shRNA group, after PHC incubation, the formation of stress fiber bundles in the central portion of

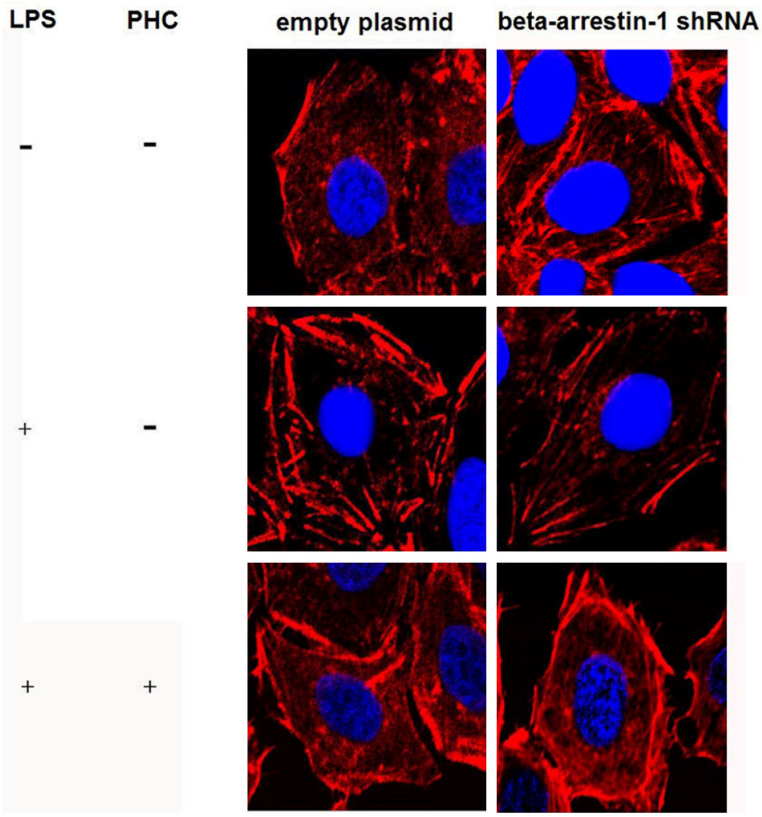

Figure 1. F-actin changes in all groups of HPMVEC. F-actin (red) arrangement were detected using confocal laser scanning fluorescence microscope, and cell nuclei were visualized with DAPI (blue). Human pulmonary microvascular endothelial cells appeared spindle. In control cell, a large number of F-actin was distributed around the cell nucleus, and stress fibers arranged regularlyin the cytoplasm. After exposed to LPS, stress fibers were disorder and F-actin was depolymerization and fractured. Cell filopodia was unexpectedly detected, which was quite typical around cell nucleus. When pretreated with PHC, the F-actin became continuous and neat. But in beta-arrestin-1 gene-shRNA group, after PHC incubation, the formation of stress fiber bundles in the central portion of cells was still obvious, and became discontinuous, shorter, or appeared fuzzy.

cells was still obvious, and became discontinuous, shorter, or appeared fuzzy (**Figure 1**).

Role of beta-arrestin-1 in effect of PHC on F-actin contents

We sought to determine the changes of F-actin contents of human pulmonary microvascular endothelial cells cultured monolayers in response to LPS (0.1 μg/ml) for 60 min. The result showed that the levels of F-actin in HPMVEC were significantly reduced by LPS ($P<0.01$). When pretreated with PHC, the F-actin contents were increased than that in LPS group ($P<0.01$). To test the hypothesis that beta-arrestin-1 plays an important role in the effect ofPHC preventing a decrease in F-actin contents, we pretreated HPMVEC with PHC (2 μg/ml) and then exposed them to LPS in beta-arrestin-1 gene-shRNA transfected cell. However, PHC didn't elicit an elevation in F-actin content during LPS treatment under this condition. Moreover, when exposed to LPS or PHC treatment, there exist significant differences between beta-arrestin-1 gene-shRNA group and empty plasmid group ($P<0.01$) (**Figure 2**).

Role of beta-arrestin-1 in effect of PHC on LPS-induced activation of p38 MAPK and expression of Hsp27

Activation of p38 MAPK and subsequent Hsp27 expression are known to alter F-actin (stress fiber formation) and increase endothelial permeability. Therefore, we sought to determine whether LPS increases activation of p38 MAPK and expression of Hsp27 in HPMVEC and whether PHC inhibits that activation. In our study, LPS induced p38 MAPK activation in HPMVEC (**Figure 4**) and also caused an increase in Hsp27 expression (**Figure 3**). In empty plasmid group, PHC decreased activation of p38 MAPK and expression of Hsp27.

Then, to determine a possible mechanism by which PHC action inhibits activation of p38 MAPK and expression of Hsp27, HPMVECs were transfected with a shRNA-containing plasmid that specifically targets beta-arrestin-1 mRNA. LPS stimulation increased activation of p38 MAPK and expression of Hsp27 but PHC had no effect on activation of p38 MAPK and expression of Hsp27 in LPS-stimulated HPMVEC under this condition (**Figures 3**, **4**).

Expression of beta-arrestin-1 protein in different groups

Western blot from LPS-exposed HPMVEC showed a decrease in beta-arrestin-1 protein expression at different time points of LPS exposure. Pretreatment with PHC 2 μg/ml greatly

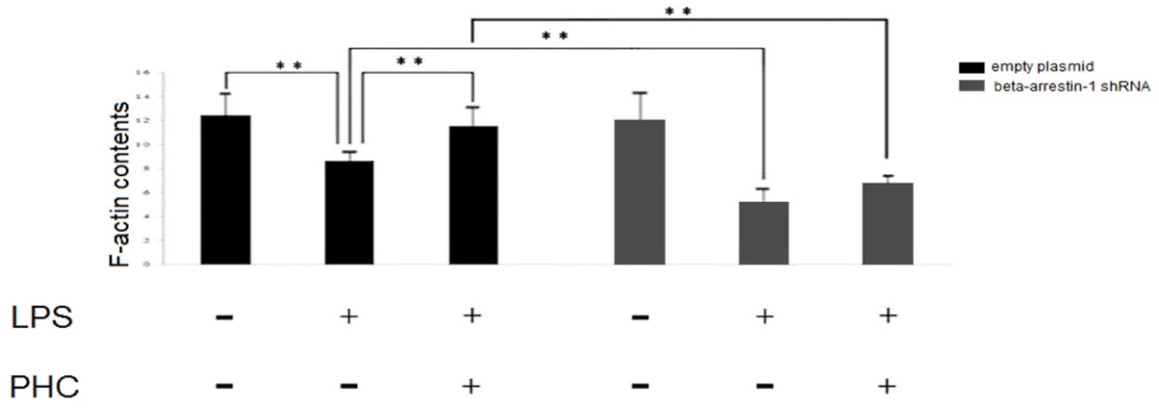

Figure 2. F-actin contents in all groups of HPMVEC. The F-actin contents were detected by flow cytometry. LPS treatment significantly decreased F-actin contents, which was elevated by PHC pretreatment (2 μg/ml). Compared with empty plasmid group, F-actin contents were decreased in beta-arrestin-1 gene-shRNA group. In addition, PHC had no effect on F-actin contents in beta-arrestin-1 gene-shRNA group during LPS treatment. Data are expressed as (mean ± SD) (n=5). $^{*}P<0.05$, $^{**}P<0.01$.

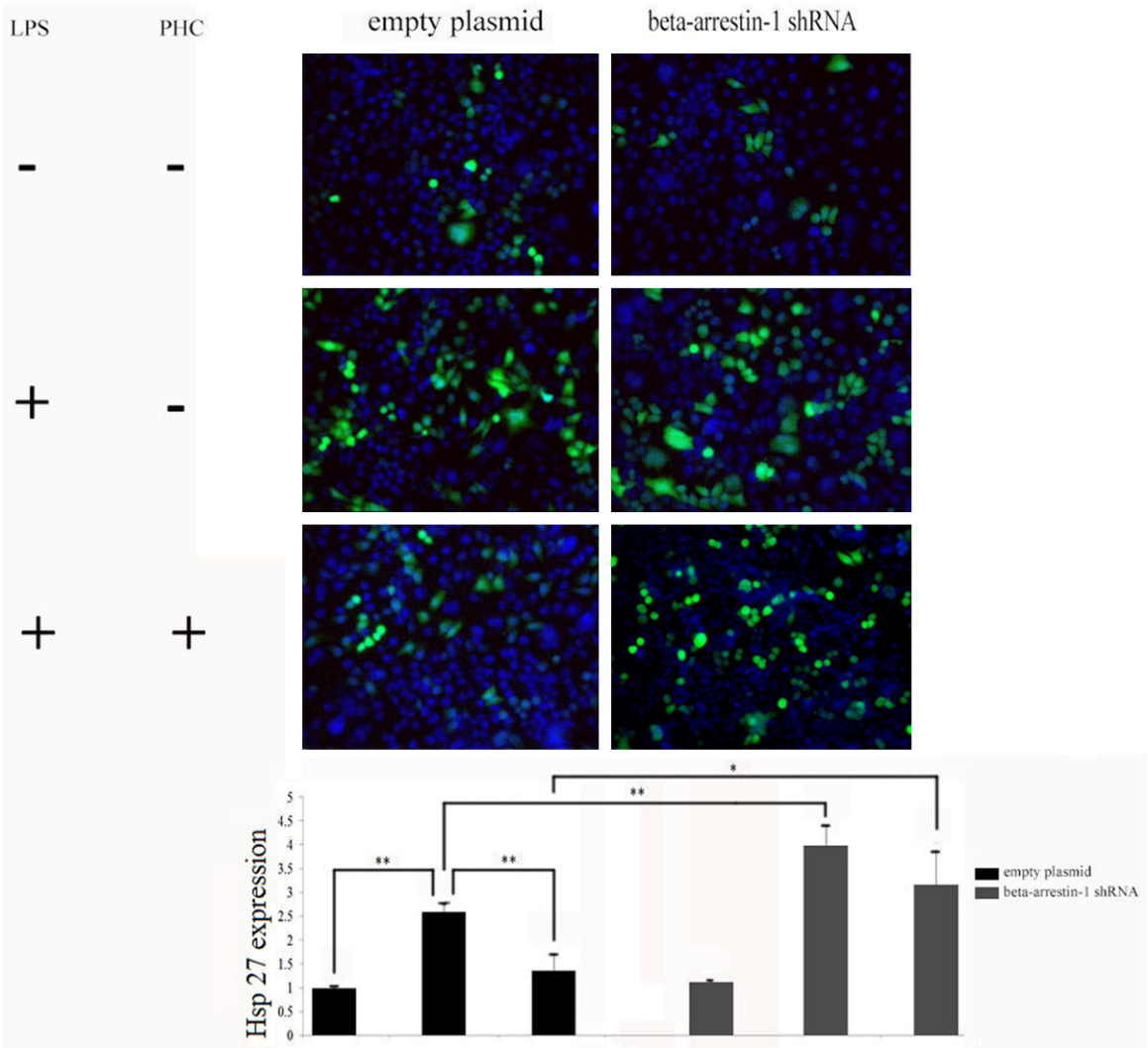

Figure 3. Immunofluorescence analysis of Hsp27 expression in all groups of HPMVEC. Hsp27 (green) expressed in HPMVEC cytoplasm, and cell nuclei were visualized with DAPI (blue). The image is a representative of experi-

ment. In empty plasmid group, PHC attenuated LPS-induced increases in Hsp27 expression. Significant changes of fluorescence signals were observed for cells transfected with beta-arrestin-1 gene-shRNA compared with empty plasmid group. However, PHC didn't elicit a change in expression of Hsp27 proteinsin beta-arrestin-1 gene-shRNA group during LPS treatment. The relative immunofluorescence signal analyzed with NIS-elements F3.2 automatic image analysis system were shown in the bar graph. Data are expressed as (mean ± SD) (n=5). $^{*}P<0.05$, $^{**}P<0.01$.

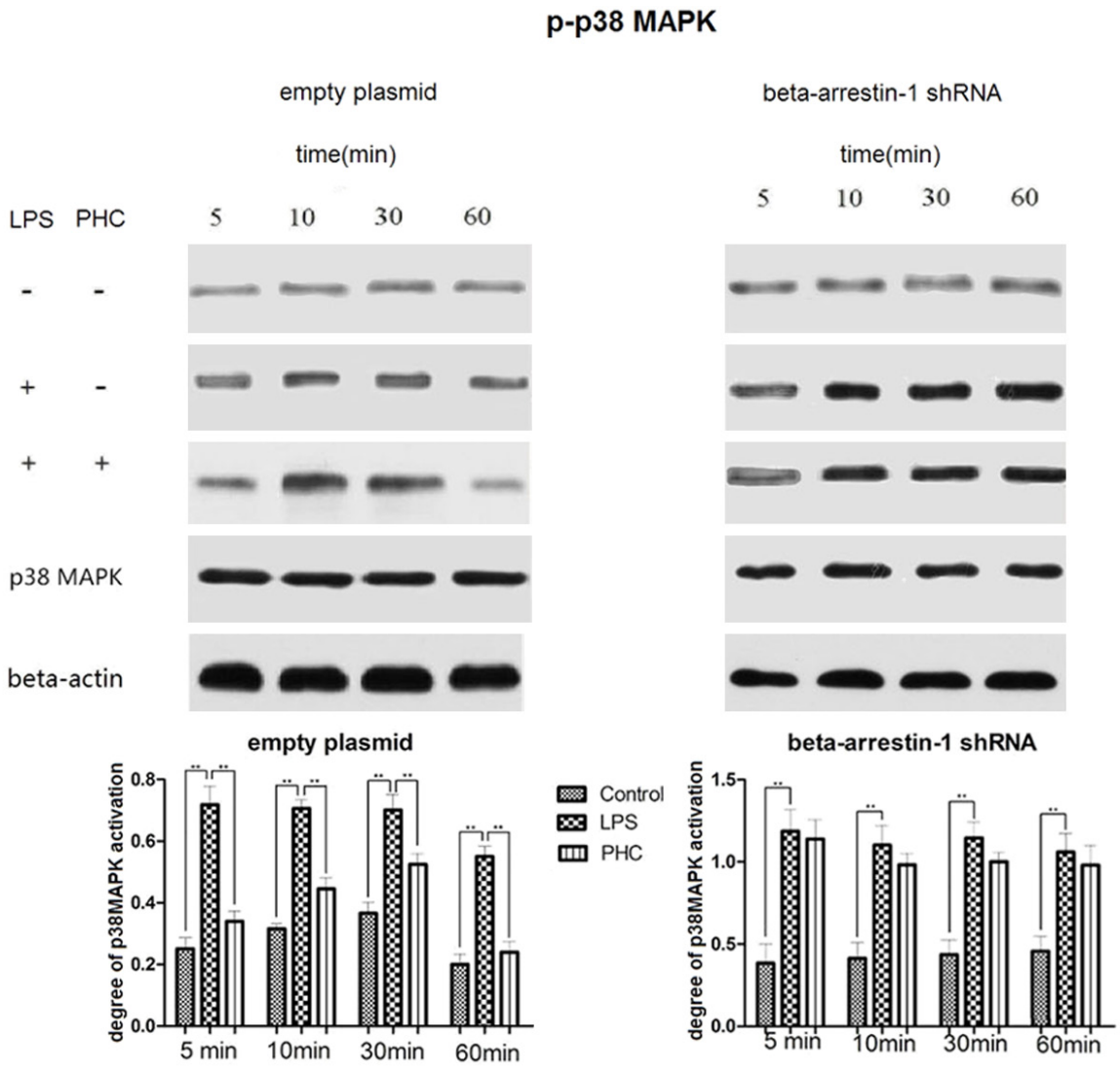

Figure 4. Western blot analysis of p38 activation in all groups of HPMVEC. Equal amounts of protein were electrophoresed and the levels of protein were determined using specific antibodies. LPS induced p38 activation (p38 MAPK phosphorylation) in HPMVEC and PHC decreased activation of p38 in empty plasmid group. Then, to determine a possible mechanism by which PHC action inhibits activations of p38, HPMVECs were transfected with a shRNA-containing plasmid that specifically targets beta-arrestin-1 mRNA. LPS stimulation increased activation of p38 but PHC had no effect on activation of p38 in LPS-stimulated HPMVEC under this condition. Activation of p38 was greater in beta-arrestin-1 gene-shRNA group than that in empty plasmid group. The relative density was shown in the bar graph. Data are expressed as (mean ± SD) (n=5). $^{*}P<0.05$, $^{**}P<0.01$.

increased beta-arrestin-1 expression compared with LPS-treated cells.

To extend the *in vitro* analysis of beta-arrestin-1, HPMVECs were transfected with a shRNA-containing plasmid that specifically targets beta-arrestin-1 mRNA. However, PHC had no effect on beta-arrestin-1 expression under this condition (**Figure 5**).

Discussion

Sepsis is a leading cause of critically ill patients with high mortality and morbidity, and is mainly

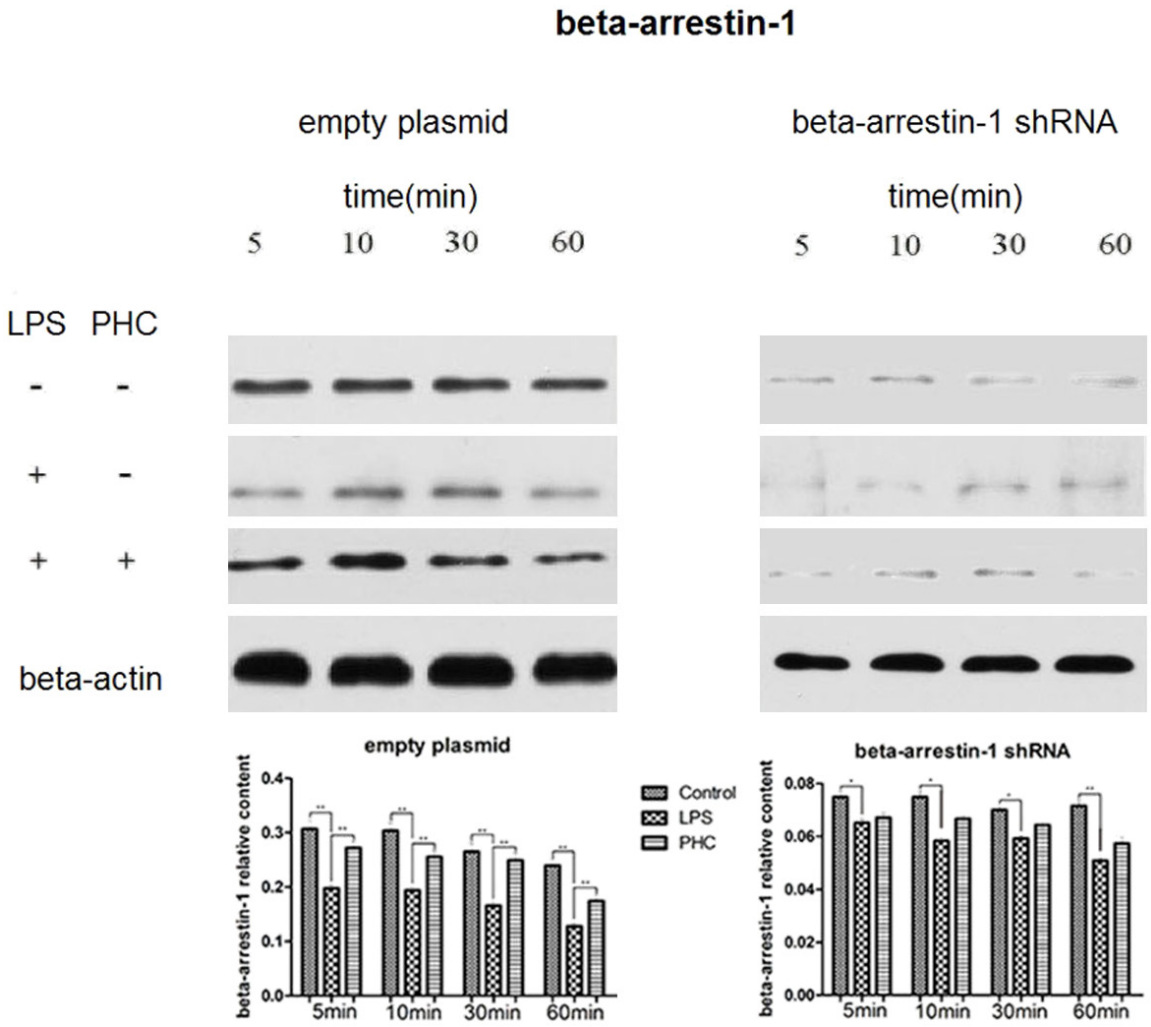

Figure 5. Western blot analysis of beta-arrestin-1 expression in all groups of HPMVEC. Equal amounts of protein were electrophoresed and the levels of protein were determined using specific antibodies. LPS inhibit beta-arrestin-1 expression and PHC increased beta-arrestin-1 expression in empty plasmid group. When HPMVECs were transfected with a shRNA-containing plasmid that specifically targets beta-arrestin 1 mRNA, beta-arrestin-1 expression was inhibited. The relative density was shown in the bar graph. Data are expressed as (mean ± SD) (n=5). $^{*}P<0.05$, $^{**}P<0.01$.

initiated by lipopolysaccharides (LPS)-a component of the cell walls of gram-negative bacteria [11]. Increased lung vascular permeability, the consequence of endothelial cell barrier dysfunction, is a cardinal feature of inflammatory conditions such as acute lung injury (ALI) and sepsis and leads to lethal physiological dysfunction characterized by alveolar flooding, hypoxemia, and pulmonary edema [12]. Microvascular endothelial cells are both key mediators and targets of LPS-induced inflammatory responses, and are central to normal microvascular function, including maintenance of the microvascular permeability barrier [13]. In response to LPS, F-actin (fibrous actin, F-actin), an essential component of the endothelial cytoskeleton, is important to maintain the endothelial integrity. The decrease of F-actin content may result in the increase of microvascular endothelial permeability. Cell cytoskeleton has been shown to be critical to maintaining the endothelial cells integrity. In our study, LPS also induced a progressive rearrangement of the F-actin cytoskeleton. These support the hypothesis that LPS alters endothelial cell integrity by affecting F-actin rearrangement, which is important in maintaining the adhesion of endothelial cells to other kinds of cells. In this study, HPMVEC was used to incubation with LPS, in order to mimic the sep-

tic status in vitro. The results demonstrated that LPS induced a decrease in the contents of F-actin and PHC could increase F-actin contents. Our confocal images also provided the direct evidence that stabilization of F-actin microfilaments by PHC prevented the effect of LPS on cell morphology.

Recent reports by Birukova et al [14] showed that actin reorganization in endothelial membrane was mediated by activation of mitogen-activated protein kinase (MAPK) pathway, which have been shown to play an important role in controlling cell growth, differentiation, and apoptosis. One member of the MAPK family, p38 MAPK, is activated by a variety of intracellular and extracellular stimuli including LPS, TNF-α and et al [15]. After stimulation, p38 MAPK activates several downstream factors such as Hsp27 which are involved in regulation of cell cytoskeleton [16]. Heat shock protein 27 (Hsp27), a central target downstream of p38 MAPK, is known to be a member of the heat shock protein family, which is closely associated with the regulation of actin cytoskeleton rearrangement in response to LPS and various types of stress [17]. Hsp27 could change the content and distribution of F-actin through modulating actin filament dynamics or structure, in a manner dependent on its phosphorylation status, thereby increase the microvascular endothelial permeability [18, 19]. Similar to the previously published data [16], our data here showed that LPS markedly induced the activation of p38 and expression of Hsp27, leading to endothelial F-actin reorganization. Furthermore, we observed that PHC treatment markedly inhibited LPS-induced phosphorylation of p38 MAPK, leading to a decrease in Hsp27 expression. These results demonstrated that PHC improved LPS-induced F-actin reorganization in HPMVECs by blocking the p38 MAPK/Hsp27 pathway.

Beta-arrestins are adaptor proteins that function to regulate G protein-coupled receptor (GPCR) signaling. Beta-arrestins also function as scaffold proteins, interacting with several cytoplasmic proteins. It has been demonstrated that beta-arrestins have been linked to intracellular signaling pathways such as MAPK activation suggesting a direct involvement in signaling cascades [20, 21]. It is well-established that beta-arrestins could prevent the translocation of MAPK into the nucleus, thereby reducing phosphorylation of nuclear substrates and consequently MAPK-dependent gene expression [22]. Furthermore, beta-arrestin 1/2 double knockout cells showed greatly enhanced phosphorylation of MAPK [23] and beta-arrestins-deficient cells also showed a markedly increased activation of the MAPK in LPS-induced mouse embryonic fibroblasts [24]. In the present study, we have shown that LPS significantly decreased beta-arrestin-1 expression in the HPMVEC, thereby leading to p38 MAPK activation and a decrease in F-actin contents. Interestingly, specific inhibition of beta-arrestin-1 with shRNA increased LPS-induced activation of p38 MAPK. These data suggest that beta-arrestin-1 is required for endothelial integrity against LPS-induced p38 MAPK activation.

Penehyclidine hydrochloride (PHC) is an anticholinergic agent manufactured in China, with both antimuscarinic and antinicotinic activity. In this study, our results indicated that treatment of HPMVECs with PHC resulted in a significant increase in expression of beta-arrestin-1, decrease in phosphorylation of p38 MAPK, attenuated LPS-induced actin rearrangement and increased F-actin contents. With the hypothesis that the biological effects of PHC may be likely mediated by beta-arrestin-1, HPMVECs were transfected with a shRNA-containing plasmid that specifically targets beta-arrestin-1 mRNA and beta-arrestin-1 expression was silenced by more than 65%. In this study, compared with LPS alone group, a significant increase in F-actin contents and decreases in p38 MAPK activation as well as Hsp27 expression were not found after PHC treatment in beta-arrestin-1 gene-shRNA group. Furthermore, the gene-repressive effect of PHC on LPS-induced p38 MAPK activation was negatively affected when beta-arrestin-1 was knocked down. These results indicated that PHC interferes with p38 MAPK activation in a beta-arrestin 1-dependent manner. In line with this reasoning, we found that the presence of beta-arrestin-1 was needed for the effect of PHC on pulmonary microvascular F-actin.

In summary, beta-arrestin-1 is required for the physiological and pharmacological functions of PHC. PHC could attenuate p38 MAPK activation and Hsp27 expression by upregulation of beta-arrestin-1 expression, thereby implicating a mechanism by which PHC may exert its pro-

tective effects against LPS-induced endothelial cell injury. Our study also point that the presence of beta-arrestin-1 can allow PHC to exert a very effective control of these beneficial responses by targeting a crucial step leading to microvascular endothelial protection. As PHC is a muscarinic receptor antagonist, the present study needs to be followed by further studies on extending the observations, to investigate whether the upregulation effects of PHC on beta-arrestin-1 is dependent on existence of muscarinic receptor.

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Disclosure of conflict of interest

None.

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References

[1] Zhang Y, Xu X, Ceylan-Isik AF, Dong M, Pei Z, Li Y, Ren J. Ablation of Akt2 protects against lipopolysaccharide-induced cardiac dysfunction: role of Akt ubiquitination E3 ligase TRAF6. J Mol Cell Cardiol 2014; 74: 76-87.

[2] Wilhelmsen K, Khakpour S, Tran A, Sheehan A, Schumacher M, Xu F, Hellman J. The endocannabinoid/endovanilloid N-arachidonoyl dopamine (NADA) and synthetic cannabinoid WIN55, 212-2 abate the inflammatory activation of human endothelial cells. J Biol Chem 2014; 289: 13079-13100.

[3] Grinnell KL, Chichger H, Braza J, Duong H, Harrington EO. Protection against LPS-induced pulmonary edema through the attenuation of protein tyrosine phosphatase-1B oxidation. Am J Respir Cell Mol Biol 2012; 46: 623-632.

[4] Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. Science 2009; 326: 1208-1212.

[5] Jacob C, Yang PC, Darmoul D, Amadesi S, Saito T, Cottrell GS, Coelho AM, Singh P, Grady EF, Perdue M, Bunnett NW. Mast cell tryptase controls paracellular permeability of the intestine, Role of protease-activated receptor 2 and beta-arrestins. J Biol Chem 2005; 280: 31936-31948.

[6] Kaparianos A, Argyropoulou E, Spiropoulos K. The role of Beta-arrestins in respiratory pathophysiology and tumorigenesis: going a step beyond the cell surface. Eur Rev Med Pharmacol Sci 2012; 16: 1781-1794.

[7] Zhang M, Teng H, Shi J, Zhang Y. Disruption of beta-arrestins blocks glucocorticoid receptor and severely retards lung and liver development in mice. Mech Dev 2011; 128: 368-375.

[8] Deshpande DA, Theriot BS, Penn RB, Walker JK. Beta-arrestins specifically constrain beta2-adrenergic receptor signaling and function in airway smooth muscle. FASEB J 2008; 22: 2134-2141.

[9] Zhan J, Xiao F, Zhang ZZ, Wang YP, Chen K, Wang YL. Effect of penehyclidine hydrochloride on beta-arrestin-1 expression in lipopolysaccharide-induced human pulmonary microvascular endothelial cells. Braz J Med Biol Res 2013; 46: 1040-1046.

[10] Zhan J, Liu Y, Zhang Z, Chen C, Chen K, Wang YL. Effect of penehyclidine hydrochloride on expressions of MAPK in mice with CLP-induced acute lung injury. Mol Biol Rep 2011; 38: 1909-1914.

[11] Zhang Y, Zhao C, He W, Wang Z, Fang Q, Xiao B, Liu Z, Liang G, Yang S. Discovery and evaluation of asymmetrical monocarbonyl analogs of curcumin as anti-inflammatory agents. Drug Des Devel Ther 2014; 8: 373-382.

[12] Adyshev DM, Moldobaeva N, Mapes B, Elangovan V, Garcia JG. MicroRNA regulation of nonmuscle myosin light chain kinase expression in human lung endothelium. Am J Respir Cell Mol Biol 2013; 49: 58-66.

[13] Singleton PA, Mirzapoiazova T, Guo Y, Sammani S, Mambetsariev N, Lennon FE, Moreno-Vinasco L, Garcia JG. High-molecular-weight hyaluronan is a novel inhibitor of pulmonary vascular leakiness. Am J Physiol Lung Cell Mol Physiol 2010; 299: L639-651.

[14] Birukova AA, Birukov KG, Gorshkov B, Liu F, Garcia JG, Verin AD. MAP kinases in lung endothelial permeability induced by microtubule disassembly. Am J Physiol Lung Cell Mol Physiol 2005; 289: L75-84.

[15] Kiemer AK, Weber NC, Furst R, Bildner N, Kulhanek-Heinze S, Vollmar AM. Inhibition of p38 MAPK activation via induction of MKP-1: atrial natriuretic peptide reduces TNF-alpha-induced actin polymerization and endothelial permeability. Circ Res 2002; 90: 874-881.

[16] Singh D, McCann KL, Imani F. MAPK and heat shock protein 27 activation are associated with respiratory syncytial virus induction of human bronchial epithelial monolayer disruption. Am J Physiol Lung Cell Mol Physiol 2007; 293: L436-445.
[17] Yang D, Xie P, Guo S, Li H. Induction of MAPK phosphatase-1 by hypothermia inhibits TNF-alpha-induced endothelial barrier dysfunction and apoptosis. Cardiovasc Res 2010; 85: 520-529.
[18] Liu T, Guevara OE, Warburton RR, Hill NS, Gaestel M, Kayyali US. Regulation of vimentin intermediate filaments in endothelial cells by hypoxia. Am J Physiol Cell Physiol 2010; 299: C363-C373.
[19] Hirano S, Rees RS, Yancy SL, Welsh MJ, Remick DG, Yamada T, Hata J, Gilmont RR. Endothelial barrier dysfunction caused by LPS correlates with phosphorylation of HSP27 in vivo. Cell Biol Toxicol 2004; 20: 1-14.
[20] Schreiber G, Golan M, Avissar S. Beta-arrestin signaling complex as a target for antidepressants and as a depression marker. Drug News Perspect 2009; 22: 467-480.
[21] Piu F, Gauthier NK, Wang F. Beta-arrestin 2 modulates the activity of nuclear receptor RAR beta2 through activation of ERK2 kinase. Oncogene 2006; 25: 218-229.
[22] Brown D, Sacks DB. Protein scaffolds in MAP kinase signaling. Cell Signal 2009; 21: 462-469.
[23] Zhao M, Wimmer A, Trieu K, Discipio RG, Schraufstatter IU. Arrestin regulates MAPK activation and prevents NADPH oxidase-dependent death of cells expressing CXCR2. J Biol Chem 2004; 279: 49259-49267.
[24] Wang Y, Tang Y, Teng L, Wu Y, Zhao X, Pei G. Association of beta-arrestin and TRAF6 negatively regulates Toll-like receptor-interleukin 1 receptor signaling. Nat Immunol 2006; 7: 139-147.