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Original article

Puerarin protects pulmonary arteries from hypoxic injury through the BMPRII and PPARγ signaling pathways in endothelial cells

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ABSTRACT

Background: Recent evidence indicates that Puerarin has a protective effect on pulmonary arteries. In the present study, we aimed to investigate whether Puerarin could protect pulmonary arterial endothelial cells from hypoxic injury and determine its potential targets.
Methods: In our study, human pulmonary arterial endothelial cells (HPAECs) were injured by hypoxic (1% O_2) incubation. Cell viability was detected by a cell counting kit (CCK8). The production of nitric oxide (NO) was detected by Griess reagent and endothelin-1 (ET-1) was detected by the ELISA method. Oxidative stress was measured by a fluorescence microscope *via* the fluorescent probe DCFH-DA. Western blotting was employed for studying the mechanism.
Results: The results show that Puerarin protects HPAECs from hypoxia-induced apoptosis and slightly improves cell viability. Puerarin increases NO and decreases ET-1 to prevent the imbalance between vasoactive substances induced by hypoxia in HPAECs. Puerarin also inhibits the oxidative stress induced by hypoxia. The results from the Western blot show that Puerarin activates the BMPRII/Smad and PPARγ/PI3K/Akt signaling pathways.
Conclusion: In conclusion, Puerarin protects HPAECs from hypoxic injury through the inhibition of oxidative stress and the activation of the BMPRII and PPARγ signaling pathways. This work provides insight into the development of Puerarin as a treatment for hypoxic pulmonary hypertension.

Introduction

Pulmonary artery hypertension (PAH) is a severe disease characterized by elevated pulmonary vascular resistance. If PAH is left untreated, the disease could cause right ventricular failure and result in a high fatality rate. The proliferative vascular remodeling process is one of the main causes of this disease [1]. Hypoxia contributes to the development of pulmonary artery remodeling. Lung diseases and/or hypoxia could induce sustained contraction of pulmonary arteries, damage the function of endothelial cells and contribute to one of the five groups of pulmonary hypertension [2,3]. PAH induced by hypoxia is a disease that has been widely studied but has not been well-managed. To discover novel compounds having therapeutic effect on vascular remodeling induced by hypoxia might contribute to the treatment of pulmonary arterial hypertension.

The root of *Pueraria lobate* (Gegen) is one of the most popular Chinese herbal medicines, and it has been used in the treatment of several diseases including cardiovascular diseases [4,5]. Puerarin (Fig. 1) is one of the main isoflavone glycosides found in the root of *Pueraria lobata*. Puerarin has been found to have various biological activities, such as anti-oxidative activity and protective effects against diabetes, cancer and cardiovascular and nervous systems diseases [6–9]. In our preliminary study, puerarin was found to protect the function of pulmonary arteries in rats with PAH induced by monocrotaline (MCT). Moreover, there has been a report about puerarin promoting apoptosis in pulmonary arterial smooth muscle cells under a hypoxic environment [10]. It is known that hypoxia is also one of the key causes of PAH. Hypoxia may induce abnormal proliferation and dysfunction of pulmonary arterial cells and is often used as a model in *in vitro* studies. Therefore, in the present study, we aimed to study the effect of

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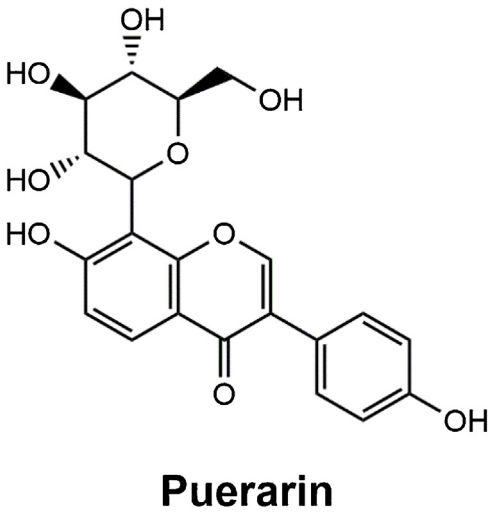

Fig. 1. Chemical structure of puerarin (7-hydroxy-3-(4-hydroxyphenyl)-1-benzopyran-4-one 8-(β-D-glucopyranoside). $C_{21}H_{20}O_9$, MW: 416.38.

puerarin on pulmonary arterial endothelial cells under a hypoxic environment to determine a possible targets of puerarin. This work might contribute to the further development of puerarin in the treatment of PAH.

Materials and methods

Reagents and cell culture

Puerarin was provided by the Beijing Key Laboratory of Polymorphic Drugs in our institute. Puerarin was extracted, separated and purified from the root of Gegen. The purity of puerarin was higher than 99%, and the structure was confirmed by their physicochemical property analysis and spectral or NMR evidence. The impurities have no influence on the following experiments.

DCFH-DA (2′,7′-Dichlorofluorescin diacetate) and Hoechst 33342 were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Cell Counting Kit-8 (CCK8) was purchased from Dojindo (Japan). All the primary and secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

Primary cultured human pulmonary arterial endothelium cells (HPAEC) (Catalog#3100) were purchased from ScienCell (Carlsbad, CA, USA). HPAECs were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Aukland, New Zealand) supplemented with 10% fetal bovine serum (FBS) (Gibco, Aukland, New Zealand). All experiments were carried out with cells at passages of 3–9.

Cell viability under hypoxia environment

HPAECs were cultured in 96-well plates with a density of 5×10^4/mL. After 24 h, the cells were serum-starved for 24 h and then treated with or without puerarin (3, 10, or 30 μM) for 3 h in the normal incubator. The control group remained in the normal incubator and the experimental group was moved into the tri-gas incubator of a hypoxic environment (1% O_2/5% CO_2). Forty-eight hours later, the cell viability was detected by the CCK8 kit. The schematic for the experimental design is shown in Fig. 2.

Western blotting assay

The cells were seeded into 60 mm culture dishes at a density of 5×10^4/mL and treated in the same way as mentioned above. All proteins in HPAECs were lysed and extracted by a RIPA lysis buffer and quantified by the BCA method. Protein samples were separated by 10–12% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in 5% BSA. After 2 h of blocking, membranes were incubated with primary antibodies at 4 °C overnight, including β-actin, Bax, Bcl2, BMPRII, phosphor-Smad1/5, PPARγ, PI3K, AKT, phosphor-AKT and phosphor-eNOS (Ser1177) (1:1000 dilution). After incubation with HRP-conjugated secondary antibodies (1:2000 dilution) for 2 h at room temperature, protein bands were subsequently detected with an enhanced ECL system. β-Actin was used as an internal control.

NO detection

Nitric oxide (NO) produced by HPAECs were detected by the Griess reaction as previous described [11]. Briefly, after hypoxic treatment, the supernatant was collected from each well. One hundred microliters of cellular supernatant was incubated with the same volume of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride, and 2.5% phosphoric acid) at room temperature for 10 min. The level of nitrite was determined by absorbance at 540 nm.

ET-1 detection

Endothelin-1 (ET-1) produced by HPAECs were detected by the ELISA method according to the instructions provided by the manufacturer (Cusbio, Wuhan, China). In brief, at the end of the experiment, cell supernatant was added to the microplate precoated with antibody specific for ET-1. After removing any unbound substances, a biotin-conjugated antibody specific for ET-1 was added to the wells. Avidin conjugated HRP was added to the wells after washing. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and the color developed. Color development was stopped, and the intensity of the color was measured at a wavelength of 450 nm, which was in proportion to the amount of ET-1.

ROS detection

HPAECs were cultured in black 96 well plates with a clear bottom at a density of 5×10^4/mL. The cells were treated in the

Fig. 2. The schematics for the experimental design.

same way as above. After 48 h incubation under a hypoxic environment, the intracellular ROS level was measured by DCFH-DA. The cells were washed with prewarmed PBS and then covered with a serum-free DMEM containing 10 μM DCFH-DA and 10 μM Hoechst. The cells were placed back into the incubator and incubated for 30 min. The cells were washed with PBS to remove the extra dye and then covered with 100 μL PBS. Images were taken using the HCS system (ArrayScan, Thermo, USA).

Statistical analysis

All data are expressed as the mean ± SEM. The analysis of statistical data was performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). All data were submitted to one-way ANOVA followed by Dunnett's *post hoc* test. A *p*-value less than 0.05 was considered statistically significant.

Results

The protective effect of puerarin on the cell viability under a hypoxic environment

To investigate the effect of puerarin on HPAECs, the cells were pretreated with 3, 10 or 30 μM puerarin before being cultured under a hypoxic environment (1% O_2) for 48 h. At the end of the experiment, cell viability was detected by the CCK8 assay. The results (Fig. 3) show that puerarin could slightly increase the cell viability of HPAECs after 48 h of hypoxia without significant differences. Western Blot assay shows that puerarin increased the expression of anti-apoptosis protein Bcl2 and decreased the ratio of Bax/Bcl2, indicating that puerarin could attenuate the apoptosis of HPAECs induced by hypoxia.

Puerarin improves NO and suppresses ET-1 production under a hypoxic environment

NO is an important vasodilator in the circulatory system. The abnormal decrease in NO production could cause sustained vasoconstriction. After 48 h of hypoxia treatment, the dysfunction of HPAECs resulted in a significant decrease in NO production (Fig. 4A). Pre-incubation with 30 μM puerarin significantly increased the level of NO, indicating that puerarin could protect HPAECs from the dysfunction induced by hypoxia.

On the other hand, ET-1 is the most potent endogenous vasoconstrictor. When hypoxia occurred, production of ET-1 was increased (Fig. 4B). However, treatment with different concentrations of puerarin suppressed the level of ET-1. In other words, puerarin could relieve the imbalance between NO and ET-1 induced by hypoxia and further attenuate the abnormal contraction in pulmonary arteries.

Puerarin inhibited the production of ROS induced by hypoxia

Hypoxia could cause oxidative stress in endothelial cells and result in over production of ROS. In the present study, a specific fluorescent probe for ROS was used to detect the oxidative stress under a hypoxic environment. The results (Fig. 5) show that the formation of ROS in HPAECs after 48 h of hypoxia was significantly increased. Treatment with puerarin could dose-dependently decrease the formation of ROS. Therefore, puerarin may inhibit the oxidative stress in HPAECs induced by hypoxia and further protect HPAECs from hypoxia injury.

Puerarin inhibited the downregulation of BMPRII/Smad signaling pathway induced by hypoxia

The BMPRII/Smads pathway is an important signaling pathway involved in the progress of PAH. This pathway is found to be downregulated in PAH patients. In our study, the results from the Western blotting assay (Fig. 6) show that the expression of BMPRII and the phosphorylation level of the substrate of BMPRII named Smad1/5 were decreased after 48 h of hypoxic treatment. Puerarin (30 μM) could significantly inhibit the downregulation of BMPRII, and 10 and 30 μM Puerarin could increase the phosphorylation level of Smad1/5 to some extent. The intervention effect of puerarin on the BMPRII/Smad signaling pathway might explain its protective effect on HPAECs under hypoxia environments.

The influences of puerarin on the PPARγ/PI3K/Akt signaling pathways

Furthermore, it was found that hypoxia could downregulate the expression of PPARγ and PI3K and could further inhibit the phosphorylation levels of Akt and eNOS. On the other hand, the results (Fig. 7) show that pretreatment with puerarin could increase the expression of PPARγ especially at the concentration of 30 μM. Puerarin also slightly increased the expression of PI3K.

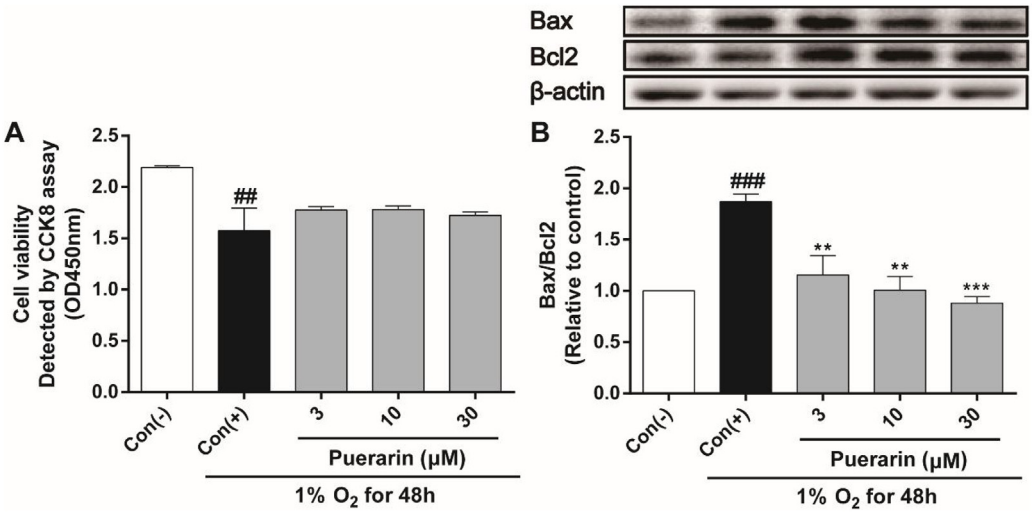

Fig. 3. Puerarin inhibited the apoptosis of HPAECs induced by hypoxia. (A) Puerarin slightly increased the cell viability of HPAECs after 48 h hypoxia injury without significant difference. (B) WB shows that different concentration of puerarin could enhance the expression of Bcl2 and downregulated the ratio of Bax/Bcl2. The results are expressed as the means ± SEM of three experiments. ## $p<0.01$ *vs.* Con(-), ###$p<0.001$ *vs.* Con(-), *** $p<0.001$, **$p<0.05$ *vs.* Con(+).

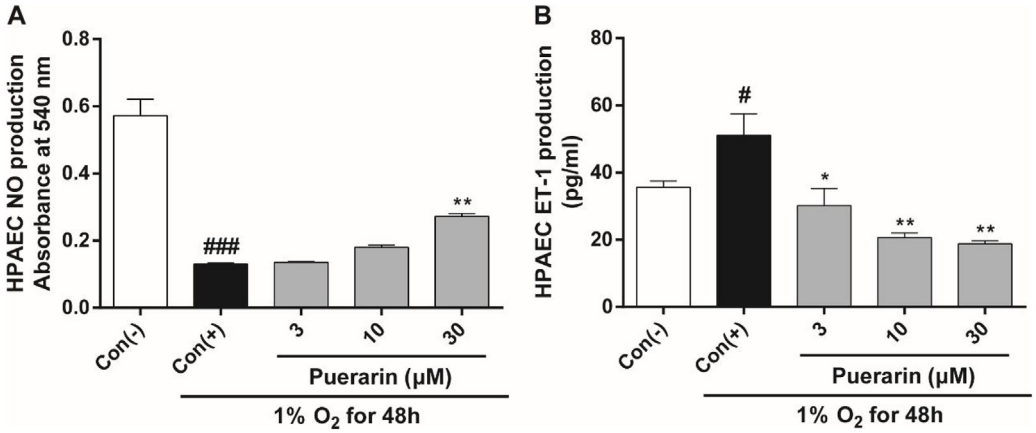

Fig. 4. The effect of puerarin on (A) NO production and (B) ET-1 production in HPAEC cultured in hypoxia environment. The results are expressed as the means ± SEM of three experiments. ###$p < 0.001$ *vs.* Con(-), #$p < 0.05$ *vs.* Con(-), *$p < 0.05$ *vs.* Con(+), **$p < 0.01$ *vs.* Con(+).

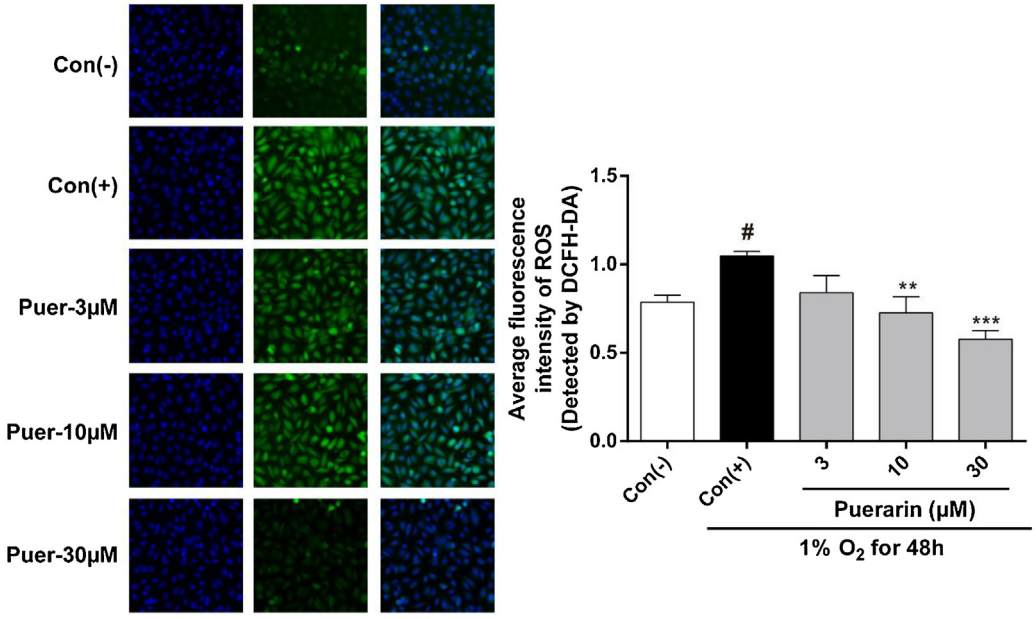

Fig. 5. The effect of puerarin on ROS production in HPAEC induced by hypoxia. Intracellular ROS was indicated by DCFH-DA probe and the representative images are taken by high-content cytometer. Average fluorescence intensity of ROS was analyzed. The results are expressed as the means ± SEM of three experiments. #$p < 0.05$ *vs.* Con(-), **$p < 0.01$ *vs.* Con(+), ***$p < 0.001$ *vs.* Con(+).

Puerarin (30 μM) increased the phosphorylation level of Akt, and 10 and 30 μM puerarin increased the phosphorylation level of eNOS at the site of Ser 1177. In other words, puerarin might activate PI3K/Akt through PPARγ and further stimulate eNOS to elevate the production of NO.

Discussion

Puerarin is a kind of isoflavone glycoside found in traditional Chinese medicine and has multiple biological activities [12]. In our previous study, we found that puerarin exhibited a positive effect on rats with PAH induced by MCT and improved the function of pulmonary arteries, which made us question whether puerarin has a protective effect on pulmonary artery cells. In the present study, we aimed to study the effect of puerarin on pulmonary arterial endothelium cells (HPAECs) and preliminarily discuss the underlying mechanisms.

Pulmonary hypertension due to lung diseases and/or hypoxia is one of the five subgroups of the disease. Hypoxia can cause abnormal constriction of pulmonary arteries and dysfunction of smooth muscle cells and endothelial cells [13]. Therefore, in the present study, hypoxia was used to establish a model of dysfunctional HPAECs *in vitro*. A hypoxic environment can cause apoptosis in endothelial cells. This finding suggests that endothelial cell apoptosis may trigger both degenerative and reactive proliferative events that result in the remodeling of pulmonary arteries [14]. Previous reports show that puerarin could inhibit the proliferation of HPASMCs induced by hypoxia [10]. However, in the present study, puerarin inhibited the apoptosis of HPAECs induced by hypoxia. We think that puerarin has a dual effect on HPASMCs and HPAECs. Puerarin has both anti-apoptosis and pro-apoptosis effects, and it plays different roles at different stages of cell damage. In any case, the protection of HPAECs from apoptosis could contribute to the early treatment of PAH. Bax was the first identified pro-apoptotic member of the Bcl2 protein family. The ratio of Bax to Bcl2 reflects apoptosis in cells. Although puerarin only slightly increased the cell viability of HPAECs, it significantly inhibited the ratio of Bax to Bcl2, which means that puerarin

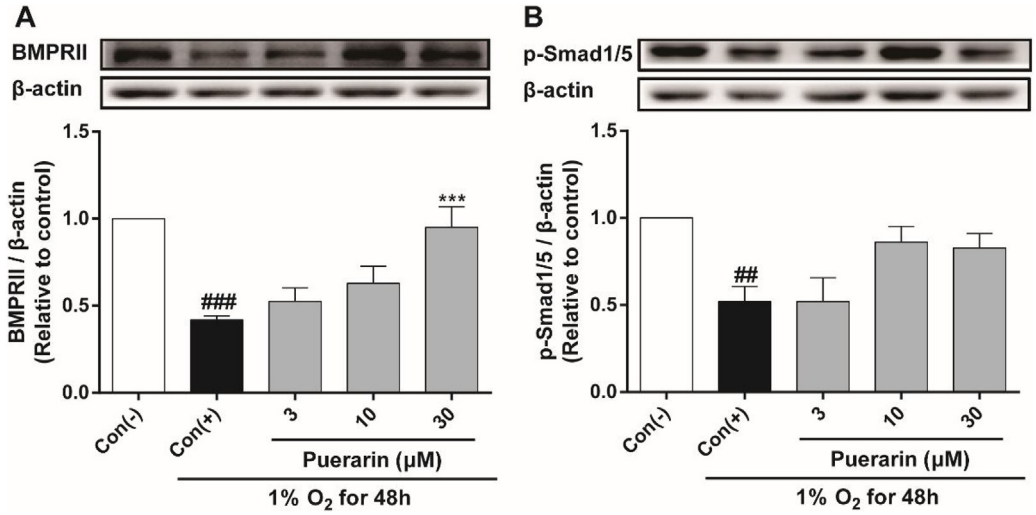

Fig. 6. Effect of puerarin on the BMPRII/Smad pathway in HPAEC. Densitometric analysis of the relative amount of proteins compare to the β-actin was shown in the lower panel and the representative pictures were shown in the upper panel: (A) BMPRII and (B) p-Smad1/5. The results are expressed as the means ± SEM of four experiments. ##$p < 0.01$ *vs.* Con(-), ###$p < 0.001$ *vs.* Con(-), ***$p < 0.001$ *vs.* Con(+).

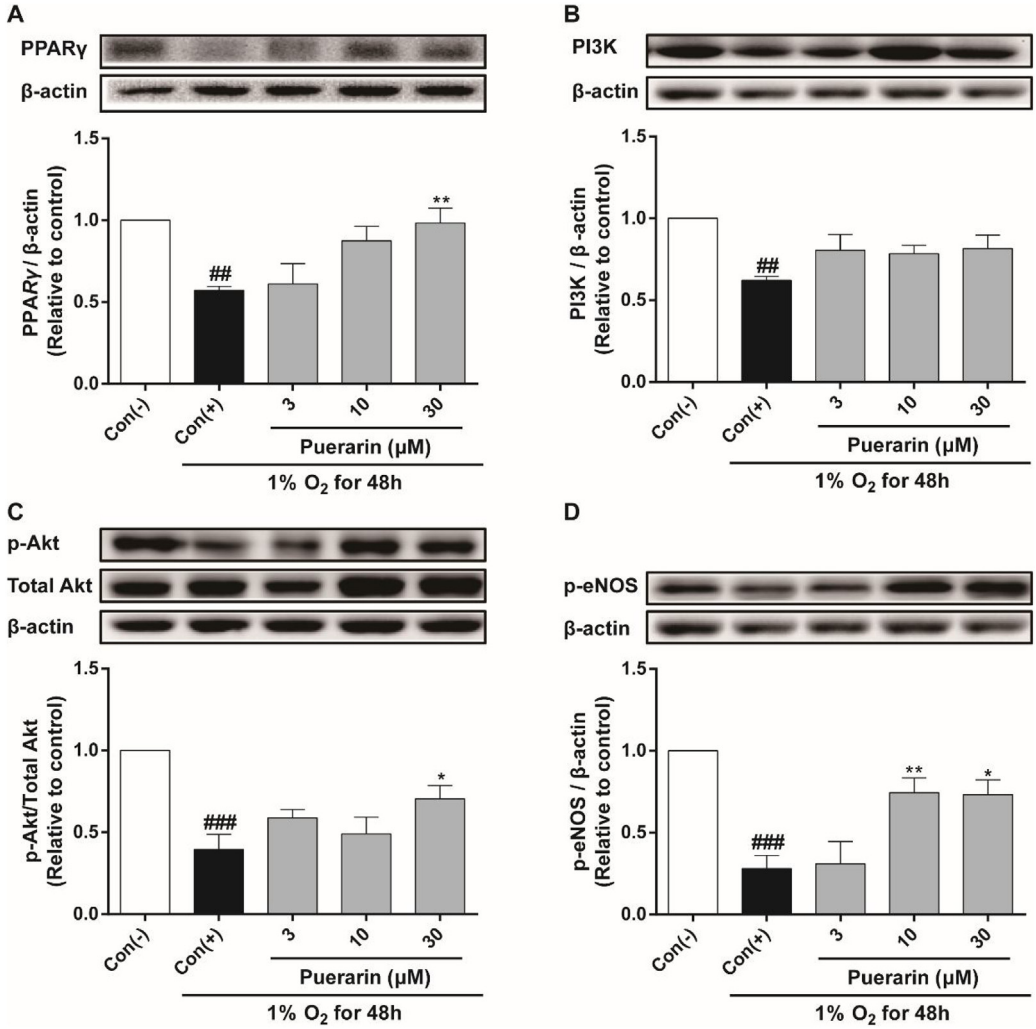

Fig. 7. Effect of puerarin on the PPARγ/PI3K/Akt/eNOS pathway in HPAEC. Densitometric analysis of the relative amount of proteins compare to the β-actin was shown in the lower panel and the representative pictures were shown in the upper panel: (A) PPARγ, (B) PI3K, (C) phosphorylation level of Akt and (D) phosphorylation level of eNOS at Ser 1177. The results are expressed as the means ± SEM of four experiments. ##$p < 0.01$ *vs.* Con(-), ###$p < 0.001$ *vs.* Con(-), *$p < 0.05$ *vs.* Con(+), **$p < 0.01$ *vs.* Con(+).

inhibited the progress of apoptosis induced by hypoxia. We think that puerarin inhibited the initial period of the apoptosis process, and its influence on cell viability is not significant. The influence of puerarin on cell viability might be obvious when the hypoxic incubation is longer.

NO and ET-1 are both produced by endothelium cells, and these molecules relax and contract vessels, respectively. ET-1 is a potent intrinsic vasoconstrictor, which plays an important role in the development of pulmonary hypertension [15]. To date, antagonists of ET-1 receptors are the first-line treatment option for patients with PAH [16]. Normally, the production and release of NO and ET-1 are in a dynamic equilibrium [17]. Under a hypoxic environment, however, the balance is disrupted. The effect of ET-1 overwhelms that of NO, which results in the abnormal constriction of pulmonary arteries. From the *in vitro* experiment, we detected the levels of NO and ET-1 in cell supernatant. Treatment with puerarin significantly inhibited the decrease in NO and the increase in ET-1 in a dose-dependent manner, which might further maintain the normal vascular tension.

In addition to the abnormal release of vascular tone regulators, hypoxia could induce oxidative stress in HPAECs. Reactive oxygen species (ROS) are important physiological messengers in vascular cells. However, an imbalance between ROS production and metabolism can lead to oxidative stress, which will become harmful to cells. In the pulmonary vasculature, the NADPH oxidase is a main source of ROS. Hypoxia causes PAH and is associated with increased NADPH oxidases [18,19]. Hypoxia can aggravate the infiltration of the cell and cause oxidative stress by increasing the level of ROS through NADPH oxidase, mitochondrial electron transport, xanthine oxidase pathway and so on [20]. ROS have been considered as therapeutic targets [21]. Similar to other flavonoid compounds, puerarin has an anti-oxidative effect and protects cells from oxidative stress [6,22]. In our study, it was found that puerarin could decrease the levels of ROS in HPAECs under a hypoxic environment. The inhibition on oxidative stress might be the most important property of puerarin in protection pulmonary arteries.

In the following study, the Western blotting assay was used to explain the underlying mechanisms of the above effects. The BMPRII/Smads signaling pathway has been reported to be important in PAH. BMPRII gene mutations have been recognized to cause heritable PAH [23]. Meanwhile, the downregulation of this pathway was also observed in rats with PAH induced by monocrotaline [24]. In the present study, hypoxia decreased the expression of BMPRII [25]. High doses of puerarin could inhibit the downregulation of BMPRII in HPAECs induced by hypoxia, and further increase the phosphorylation level of Smad1/5.

In the lungs of patients with pulmonary hypertension, the expression of peroxisome proliferator-activated receptor γ (PPARγ) is decreased [26]. The expression of PPARγ in endothelium cells was also downregulated by a hypoxic culture according to our results. PPARγ is a kind of ligand-activated nuclear transcription factor and is associated with hypertension, obesity, diabetes, atherosclerosis and so on [27,28]. PPARγ is also observed to be expressed in endothelial cells, and its activation reduces the level of ET-1 and increases NO [29]. Hypoxia significantly reduces the expression of PPARγ, while treatment with puerarin inhibits such a reduction. Studies show that PPARγ can promote PI3K expression, which further activates Akt and phosphorylates eNOS to produce NO [30,31]. Although puerarin does not significantly influence the expression of PI3K, the phosphorylation levels of Akt and eNOS are increased. These results also explain the enhancement in NO production induced by puerarin under a hypoxic environment.

In conclusion, puerarin treatment protects HPAECs from hypoxic injury. Puerarin probably inhibits the apoptosis of HPAECs induced by hypoxia and slightly increases cell viability. Puerarin increases NO production and reduces ET-1 production. Primary studies of the mechanism show that puerarin protects HPAECs under a hypoxic environment mainly through its inhibition of oxidative stress, and it might also influence the BMPRII/Smad and PPARγ/PI3K/Akt/eNOS signaling pathways. This study shows that puerarin could protect pulmonary arteries through its effects on endothelium cells. And this natural compound might have potential in the treatment of PAH especially PAH induced by hypoxia.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Author contributions

Tianyi Yuan: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Funding acquisition, Visualization. Huifang Zhang: Methodology, Investigation, Visualization. Di Chen: Methodology, Investigation, Visualization. Yucai Chen: Validation, Investigation. Yang Lyu: Resources. Lianhua Fang: Data curation, Supervision, Funding acquisition. Guanhua Du: Supervision, Funding acquisition, Project administration.

References

[1] Velayati A, Valerio MG, Shen M, Tariq S, Lanier GM, Aronow WS. Update on pulmonary arterial hypertension pharmacotherapy. Postgrad Med 2016;128:460–73.
[2] Kim HJ, Yoo HY. Hypoxic pulmonary vasoconstriction and vascular contractility in monocrotaline-induced pulmonary arterial hypertensive rats. Korean J Physiol Pharmacol 2016;20:641–7.
[3] Wang LN, Yu WC, Du CH, Tong L, Cheng ZZ. Hypoxia is involved in hypoxic pulmonary hypertension through inhibiting the activation of FGF2 by miR-203. Eur Rev Med Pharmacol Sci 2018;22:8866–76.
[4] Han P, Gao D, Zhang W, Liu S, Yang S, Li X. Puerarin suppresses high glucose-induced MCP-1 expression via modulating histone methylation in cultured endothelial cells. Life Sci 2015;130:103–7.
[5] Hwang YP, Kim HG, Hien TT, Jeong MH, Jeong TC, Jeong HG. Puerarin activates endothelial nitric oxide synthase through estrogen receptor-dependent PI3-kinase and calcium-dependent AMP-activated protein kinase. Toxicol Appl Pharmacol 2011;257:48–58.
[6] Cheng Y, Leng W, Zhang J. Protective effect of puerarin against oxidative stress injury of neural cells and related mechanisms. Med Sci Monit 2016;22:1244–9.
[7] Chen T, Chen H, Wang Y, Zhang J. In vitro and in vivo antitumour activities of puerarin 6''-O-xyloside on human lung carcinoma A549 cell line via the induction of the mitochondria-mediated apoptosis pathway. Pharm Biol 2016;54:1793–9.
[8] Zhang X, Liu Y, Han Q. Puerarin attenuates cardiac hypertrophy partly through increasing Mir-15b/195 expression and suppressing non-canonical transforming growth factor beta (tgfbeta) signal pathway. Med Sci Monit 2016;22:1516–23.
[9] Zhang Y, Wang H, Yu L, Chen J. The Puerarin improves renal function in STZ-induced diabetic rats by attenuating eNOS expression. Ren Fail 2015;37:699–703.
[10] Chen C, Chen C, Wang Z, Wang L, Yang L, Ding M, et al. Puerarin induces mitochondria-dependent apoptosis in hypoxic human pulmonary arterial smooth muscle cells. PLoS One 2012;7: e34181.
[11] Du L, Miao X, Jia H, Gao Y, Liu K, Zhang X, et al. Detection of nitric oxide in macrophage cells for the assessment of the cytotoxicity of gold nanoparticles. Talanta 2012;101:11–6.
[12] Zhou YX, Zhang H, Peng C. Puerarin: a review of pharmacological effects. Phytother Res 2014;28:961–75.
[13] Rosenkranz S. Pulmonary hypertension 2015: current definitions, terminology, and novel treatment options. Clin Res Cardiol 2015;104:197–207.

[14] Yamaji-Kegan K, Takimoto E, Zhang A, Weiner NC, Meuchel LW, Berger AE, et al. Hypoxia-induced mitogenic factor (FIZZ1/RELMalpha) induces endothelial cell apoptosis and subsequent interleukin-4-dependent pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2014;306:L1090–103.
[15] Sun XZ, Li SY, Tian XY, Hong Z, Li JX. Effect of Rho kinase inhibitor fasudil on the expression ET-1 and NO in rats with hypoxic pulmonary hypertension. Clin Hemorheol Microcirc 2018.
[16] Nadeau V, Potus F, Boucherat O, Paradis R, Tremblay E, Iglarz M, et al. Dual ETA/ETB blockade with macitentan improves both vascular remodeling and angiogenesis in pulmonary arterial hypertension. Pulm Circ 2018;8:2045893217741429.
[17] Feng EZ, Yang SY, Huang NX, Yin H, Zhang Y, Tian ZX. Plasma endothelin-1 and nitric oxide correlate with ligustrazine alleviation of pulmonary artery hypertension in patients of chronic cor pulmonale from high altitude plateau during acute exacerbation. Zhongguo Ying Yong Sheng Li Xue Za Zhi 2014;30:532–7.
[18] Cheng F, Lan J, Xia W, Tu C, Chen B, Li S, et al. Folic acid attenuates vascular endothelial cell injury caused by hypoxia via the inhibition of ERK1/2/NOX4/ROS pathway. Cell Biochem Biophys 2016;74:205–11.
[19] Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. Diabetes Care 2008;31(Suppl 2):S170–180.
[20] Yuan T, Zhang H, Chen Y, Jiao X, Xie P, Fang L, et al. The protective effect of DL0805 derivatives on pulmonary artery cells and the underlying mechanisms study. Curr Vasc Pharmacol 2017;15:469–76.
[21] Freund-Michel V, Guibert C, Dubois M, Courtois A, Marthan R, Savineau JP, et al. Reactive oxygen species as therapeutic targets in pulmonary hypertension. Ther Adv Respir Dis 2013;7:175–200.
[22] Li X, Cai W, Lee K, Liu B, Deng Y, Chen Y, et al. Puerarin attenuates diabetic kidney injury through the suppression of NOX4 expression in podocytes. Sci Rep 2017;7:14603.
[23] Liu D, Morrell NW. Genetics and the molecular pathogenesis of pulmonary arterial hypertension. Curr Hypertens Rep 2013;15:632–7.
[24] Chen YC, Yuan TY, Zhang HF, Wang DS, Yan Y, Niu ZR, et al. Salvianolic acid a attenuates vascular remodeling in a pulmonary arterial hypertension rat model. Acta Pharmacol Sin 2016;37:772–82.
[25] Takahashi K, Kogaki S, Matsushita T, Nasuno S, Kurotobi S, Ozono K. Hypoxia induces alteration of bone morphogenetic protein receptor signaling in pulmonary artery endothelial cell. Pediatr Res 2007;61:392–7.
[26] Rabinovitch M. PPARgamma and the pathobiology of pulmonary arterial hypertension. Adv Exp Med Biol 2010;661:447–58.
[27] Stump M, Mukohda M, Hu C, Sigmund CD. PPARgamma regulation in hypertension and metabolic syndrome. Curr Hypertens Rep 2015;17:89.
[28] Soskic SS, Dobutovic BD, Sudar EM, Obradovic MM, Nikolic DM, Zaric BL, et al. Peroxisome proliferator-activated receptors and atherosclerosis. Angiology 2011;62:523–34.
[29] Sutliff RL, Kang BY, Hart CM. PPARgamma as a potential therapeutic target in pulmonary hypertension. Ther Adv Respir Dis 2010;4:143–60.
[30] Peng X, Chen R, Wu Y, Huang B, Tang C, Chen J, et al. PPARgamma-PI3K/AKT-NO signal pathway is involved in cardiomyocyte hypertrophy induced by high glucose and insulin. J Diabetes Complications 2015;29:755–60.
[31] Yasuda S, Kobayashi H, Iwasa M, Kawamura I, Sumi S, Narentuoya B, et al. Antidiabetic drug pioglitazone protects the heart via activation of PPAR-gamma receptors, PI3-kinase, Akt, and eNOS pathway in a rabbit model of myocardial infarction. Am J Physiol Heart Circ Physiol 2009;296:H1558–65.