RESEARCH ARTICLE

[View Article Online](#)
[View Journal](#) | [View Issue](#)

Protective effects of astragaloside IV against hypoxic pulmonary hypertension

Xiaozheng Zhang,^{†a} Jun Chen,^{†b} Pan Xu^c and Xin Tian^{ib} ^{*a}Cite this: *Med. Chem. Commun.*,
2018, 9, 1715Received 8th July 2018,
Accepted 29th August 2018

DOI: 10.1039/c8md00341f

rsc.li/medchemcomm

The present study aimed to evaluate the protective effects of astragaloside IV (As-IV) against hypoxic pulmonary hypertension (HPH) and its mechanisms of action. Sprague-Dawley rats were used in a model of HPH induced by chronic hypoxia. After hypoxia, the mean pulmonary arterial pressure (mPAP), right ventricular pressure (RVP), and right ventricular hypertrophy index (RVHI) were monitored. Relaxation of the pulmonary artery in response to As-IV was measured. The levels of endothelin-1 (ET-1), angiotensin II (Ang II), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) in serum were assessed. Cell proliferation was detected by the cell counting kit-8 (CCK-8) assay. Treatment with As-IV significantly decreased mPAP, RVP and RV/(LV + S) and attenuated the development of HPH. Moreover, As-IV time-dependently relaxed the pulmonary arteries from HPH rats. In addition, As-IV decreased the levels of ET-1, Ang II, TNF- α , and IL-6 in serum of HPH rats. *In vitro* experiments demonstrated that As-IV also significantly inhibited the proliferation of pulmonary artery smooth muscle cells (PASMCs) subjected to hypoxia. Our findings suggested the therapeutic potential of As-IV in the treatment of pulmonary hypertension.

Introduction

Long-term hypoxia triggers several chronic lung diseases such as hypoxic pulmonary hypertension (HPH). HPH is a common pathophysiological process in the development of various cardiopulmonary diseases, and is characterized by elevated pulmonary artery pressure, pulmonary vascular remodeling and right ventricular hypertrophy.^{1,2} It will lead to right heart failure and death without prompt treatment.^{3,4} However, the pathogenesis of HPH remains unknown and there is no treatment available that reverses it. Therefore, it is urgent to find effective strategies for the treatment of HPH.

Acute hypoxia causes pulmonary vasoconstriction, and chronic hypoxia leads to pulmonary vascular remodeling. Hypoxic pulmonary vasoconstriction and remodeling are two vital factors for the development of HPH.⁵ All hypoxia pathways result in an altered vascular endothelium and smooth muscle function. Hypoxia-induced pulmonary inflammation is the key in the early stage of the occurrence of HPH.⁶ Chronic hypoxia damages vascular endothelial cells and leads to an imbalance between an increase in vasoconstrictors and a decrease in vasodilators, eventually resulting in pulmonary

vascular remodeling as evidenced by proliferation of pulmonary artery smooth muscle cells (PASMCs).^{7,8} Thus, inhibition of vascular remodeling and proliferation of PASMCs is an important approach to the prevention and treatment of HPH.

Astragaloside IV (As-IV) is a high-purity drug extracted from *Astragalus membranaceus* which has been considered to be a widely used herb in China.^{9,10} As-IV elicits a lot of pharmacological effects, such as anti-oxidative,¹¹ anti-inflammatory,¹² cardioprotective and anti-cancer effects.^{13,14} Previous studies have demonstrated that As-IV is protective against bleomycin-induced pulmonary fibrosis and sepsis-induced acute lung injury in rats^{15,16} and these research studies showed that As-IV may have a protective effect against lung diseases. Moreover, As-IV dilates aortic vessels in a dose-dependent manner from normal and spontaneously hypertensive rats.¹⁷ Additionally, it has been documented that As-IV may play a role in resisting short-term hypoxic contraction of the isolated pulmonary artery in rats. However, whether As-IV exerts a protective effect against HPH remains unknown, and the molecular mechanisms underlying As-IV-mediated HPH protective effects require further investigation.

In this study, we aimed to investigate the role of As-IV in HPH and the underlying mechanisms involved.

Methods

2.1. Reagents

As-IV was obtained from Santa Cruz Biotechnology (USA) and dissolved in normal saline (0.85% NaCl solution) prior to

^a Department of Cardiology, Traditional Chinese Medicine Hospital of Shaanxi Province, Xi'an, Shaanxi Province, China. E-mail: tianxin8349@163.com;
Tel: +86 29 87251791

^b Department of Encephalopathy, Traditional Chinese Medicine Hospital of Shaanxi Province, Xi'an, Shaanxi Province, China

^c Clinical Medicine Department, Hubei University of Medicine, Shiyan, Hubei Province, China

[†] These authors contributed equally to this work.

use. Endothelin-1 (ET-1), angiotensin II (Ang II), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) Enzyme-Linked Immunosorbent Assay (ELISA) kits were purchased from Abcam (UK). The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Japan). Methanol and acetonitrile (HPLC grade) were purchased from Thermo Scientific (USA). Sodium pentobarbital was purchased from Guoan Institute of Biotechnology (China).

2.2. Animals

Sprague-Dawley rats (200 ± 20 g) were purchased from the Animal Center of the Air Force Medical University. All experimental procedures involving rats were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Traditional Chinese Medicine Hospital of Shaanxi Province (Xi'an, Shaanxi, China) and approved by the Animal Ethics Committee of Traditional Chinese Medicine Hospital of Shaanxi Province (Xi'an, Shaanxi, China).

2.3. Animal models

The animals were randomly divided into the following groups: (1) the control (normoxic) group; (2) the hypoxia group (rats exposed to hypobaric and hypoxic conditions for 1, 2, and 4 weeks. Normal saline at a dose of 2.0 ml kg^{-1} was intraperitoneally injected every other day, 10 min prior to hypoxia); (3) the hypoxia + As-IV(10) group (the same as the hypoxia group, As-IV at a dose of 10 mg kg^{-1} was intraperitoneally injected every other day, 10 min prior to hypoxia); (4) the hypoxia + As-IV(50) group (the same as the hypoxia group, As-IV at a dose of 50 mg kg^{-1} was intraperitoneally injected every other day, 10 min prior to hypoxia).

An automatic hypoxia instrument was used to induce hypobaric and hypoxic pulmonary hypertension.^{18,19} The hypoxia groups were placed under hypoxic conditions (air pressure is 50 kPa, oxygen concentration is 10%) for 8 hours every day. The rats in the control group were exposed to room air.

2.4. Hemodynamics measurements

After hypoxia, the rats were injected with pentobarbital sodium (60 mg kg^{-1}). A polyethylene microcatheter was inserted into the right ventricle and pulmonary artery and the mean pulmonary arterial pressure (mPAP) and right ventricular pressure (RVP) were recorded.²⁰ Then the right ventricle (RV), left ventricle (LV), and septum (S) were isolated, and RV and LV + S was weighed. The $\text{RV}/(\text{LV} + \text{S})$ ratio was calculated and used as an index for right ventricular hypertrophy.

2.5. Isolated pulmonary arterial rings

The rats were injected with pentobarbital sodium (60 mg kg^{-1}). The pulmonary artery was gently separated and blood was washed out in Krebs solution ($0-4^\circ\text{C}$). A second-grade

branch of the pulmonary artery was picked. Then the pulmonary arteries were isolated and cut into 3 millimeter segments, and the segments were connected to a tension sensor and the pulmonary artery tension was recorded using a multi-channel physiological recording system (RM6280). After equilibration, phenylephrine (PE) at $1 \mu\text{M}$ was added and when the contraction was stable, As-IV ($10 \mu\text{M}$) was added. The effect of As-IV ($10 \mu\text{M}$) on the artery rings of HPH rats was determined. After stabilization, the rings were washed and equilibrated to baseline. Treatment with PE ($1 \mu\text{M}$) induced a vasoconstriction effect. Upon stabilization, As-IV ($50 \mu\text{M}$) was added. The effect of As-IV ($50 \mu\text{M}$) on the artery rings of HPH rats was determined.

2.6. Measurement of serum ET-1, Ang II, TNF- α , and IL-6 levels

After hypoxia, blood was collected and centrifuged at $3000g$ for 10 min. Then the serum was collected. After the above protocol, the levels of ET-1, Ang II, TNF- α , and IL-6 were measured using ELISA kits, respectively. The measurement was carried out strictly according to the manufacturer's instructions.

2.7. Pharmacokinetic analysis

After hypoxia, the rats were fasted for 12 h before receiving intraperitoneal (i.p., $10, 50 \text{ mg kg}^{-1}$) administration of As-IV. $500 \mu\text{L}$ of blood samples were collected from the venous plexus in the eyeground. The plasma samples were withdrawn at 30, 60, 90, 120, 240, 360, 480, and 600 min after i.p. administration. The plasma was obtained by centrifugation at $12000g$ for 10 min and stored at -20°C until HPLC analysis.

2.8. Cell culture

After intraperitoneal injection of pentobarbital sodium (60 mg kg^{-1}), the rats were anaesthetized and the pulmonary artery was carefully separated. The pulmonary artery was cut longitudinally and gently and the endothelium was removed with tweezers. Next, the tunica media was prepared into scraps (1 mm^3) in Dulbecco's modified Eagle's medium (DMEM). The cells were cultured in DMEM containing 20% fetal bovine serum (5% CO_2 , 37°C). The purity of PSMCs was determined by monoclonal antibodies directed against $\alpha\text{-SMA}$. Passages between 3 and 6 were used for the experiments.

2.9. Cell proliferation assay

PASMCs were divided into the following groups: (1) the control (normoxic) group (5% CO_2 , 37°C); (2) the hypoxia group (2% O_2 , 93% N_2 , 5% CO_2 , 37°C); (3) the hypoxia + As-IV ($10 \mu\text{M}$) group, 10 min after As-IV ($10 \mu\text{M}$) was added, the cells were exposed to hypoxia; (4) the hypoxia + As-IV ($50 \mu\text{M}$) group, 10 min after As-IV ($50 \mu\text{M}$) was added, the cells were

exposed to hypoxia. The cells were exposed to hypoxia for 24 hours.

Cell proliferation was detected by counting the number of cells with CCK-8 according to the manufacturer's protocol. The cells were seeded in 96-well plates before exposure to hypoxia. After hypoxia, 10 μ l of CCK-8 solution was added to the wells and sustained for 2 hours. The absorbance at 450 nm of each well was measured, and the diversity rate was calculated.

2.10. Statistical analysis

All data were analyzed using GraphPad Prism 5.0 and presented as the mean $\pm$ SEM. One-way analysis of variance (ANOVA) was used to compare differences among multiple groups. The value, $P < 0.05$, was considered statistically significant.

Results

3.1. Chronic hypoxia induced pulmonary hypertension

After chronic hypoxia for 1, 2, and 4 weeks, the mPAP and RVP of rats were significantly higher compared to those of normoxic rats and increased progressively with prolongation of the hypoxic time (Fig. 1A and B). Meanwhile, long-term hypoxia also led to significant RV hypertrophy as evidenced by increased RV/(LV + S) as the time for hypoxia extended (Fig. 1C). These data suggested that the rats developed severe pulmonary hypertension after chronic hypoxia.

3.2. As-IV attenuated hypoxic pulmonary hypertension

As shown in Fig. 2, treatment with As-IV at 10 mg kg⁻¹ did not alter the mPAP, RVP, and RV/(LV + S) of rats after being exposed to hypobaric hypoxia for two weeks. But compared with the hypoxia group, the mPAP, RVP, and RV/(LV + S) of rats were significantly decreased after administration of As-IV

at 50 mg kg⁻¹. Our data indicated that As-IV exerted depressive effects on HPH and RV hypertrophy.

3.3. As-IV relaxed the pulmonary arteries of HPH rats in a time-dependent manner

To clarify whether As-IV could relax the pulmonary artery, isolated pulmonary artery rings were obtained. As shown in Fig. 3, the relaxation appeared at 1 min, and peaked at 10 min after As-IV (10 μ M) administration. The maximal relaxation was 9.42% in HPH rats. Moreover, this relaxation appeared at 1 min, and peaked at 10 min after As-IV (50 μ M) administration. The maximal dilating effect was 21.194% in HPH rats. The ability of As-IV to relax the pulmonary artery is elevated with the increase of concentration. These results indicated that As-IV elicited a significant relaxation effect on pulmonary artery rings from HPH rats.

3.4. As-IV decreased the levels of ET-1 and Ang II in HPH rats

To investigate the effect of As-IV on vasoactive substances, we measured the levels of ET and Ang II in serum under hypoxic conditions. Fig. 4 illustrates that, after two weeks of hypobaric hypoxia, the serum ET and Ang II levels increased significantly compared with those in the control group. Treatment with As-IV (10 mg kg⁻¹) did not change the serum ET and Ang II levels in HPH rats. But administration of As-IV (50 mg kg⁻¹) significantly reduced the serum ET and Ang II levels in hypoxic rats, suggesting that As-IV could decrease the levels of ET-1 and Ang II in serum.

3.5. As-IV decreased the levels of TNF- α and IL-6 in HPH rats

Next, we sought to demonstrate the effect of As-IV on hypoxic pulmonary inflammation responses in rats, and thus the serum TNF- α and IL-6 levels were assayed after hypoxia for two weeks. As shown in Fig. 5, it was proved that the levels of TNF- α and IL-6 in serum in the hypoxic group significantly decreased compared with those in the control group. As-IV

Fig. 1 Hemodynamic index under chronic hypoxia. (A) Changes of mPAP in rats exposed to chronic hypoxia. (B) Changes of RVP in rats exposed to chronic hypoxia. (C) Changes of RV/(LV + S) in rats exposed to chronic hypoxia. Data are represented as the mean $\pm$ SEM, $n = 4$ per group. * $P < 0.05$ and ** $P < 0.01$.

Fig. 2 Effect of As-IV on the hemodynamic index in HPH rats. (A) Effects of As-IV on mPAP of rats under chronic hypoxia. (B) Effects of As-IV on RVP of rats under chronic hypoxia. (C) Effects of As-IV on RV/(LV + S) of rats under chronic hypoxia. Data are represented as the mean $\pm$ SEM, $n = 4$ per group. * $P < 0.05$ and ** $P < 0.01$.

(10 and 50 mg kg⁻¹) treatment remarkably reversed the hypoxia-induced elevation of the TNF- α and IL-6 levels. Our results indicated that As-IV decreased the serum TNF- α and IL-6 levels in a dose-dependent manner in HPH rats.

3.6. Pharmacokinetics of As-IV in rats

Concentration–time profiles after i.p. administration (10 and 50 mg kg⁻¹) of As-IV are shown in Fig. 6. The plasma concentration–time data of i.p. administration were analyzed, assuming a bi-exponential disposition curve. After As-IV administration at 10 mg kg⁻¹, the time to reach the peak concentration and the maximal plasma concentration were found to be 60 min and 7.27 μ g ml⁻¹. In contrast, the time to reach the peak concentration and the maximal plasma concentration were found to be 60 min and 25.21 μ g ml⁻¹ after As-IV (50 μ M) administration.

Fig. 3 Time-dependent relaxation effect of As-IV on pulmonary arteries from HPH rats. Data are represented as the mean $\pm$ SEM, $n = 4$ per group. * $P < 0.05$ and ** $P < 0.01$.

3.7. As-IV decreased PASMC proliferation under hypoxic conditions

To demonstrate the effect of As-IV on PASMC proliferation, we further detected cell viability by the CCK-8 assay. As shown in Fig. 7, compared with the cells from the control group, the OD optimum in the CCK-8 assay increased by 54.6% after hypoxia for 24 hours. The CCK-8 assay showed that As-IV (50 μ M) treatment significantly inhibited PASMC proliferation under hypoxic conditions for 24 hours, while there was no significant difference for PASMC proliferation between the hypoxic group and the hypoxic + As-IV (10 μ M) group. These findings suggested that As-IV significantly inhibited PASMC proliferation subjected to hypoxia.

Discussion

In the present study, we established animal and cell models to study the protective effects of As-IV against hypoxic pulmonary hypertension. Our data suggested that As-IV reduced the mPAP, RVP, and RV/(LV + S) of HPH rats, exerting its protective effects in pulmonary circulation. We further demonstrated that As-IV relaxed the pulmonary arteries of HPH rats. Moreover, at the cellular level, As-IV inhibited the proliferation of PASMCs induced by hypoxia. These combined effects could suppress the occurrence and development of HPH. Our results also indicated that As-IV decreased the levels of ET-1 and Ang II in HPH rats and exerted an anti-inflammatory effect by reducing the TNF- α and IL-6 levels in serum. The plasma concentration–time data were analyzed, assuming a bi-exponential disposition curve. Given these results, we demonstrated that As-IV significantly attenuated HPH and had potential for treatments of HPH.

HPH is caused by vascular injury caused by hypoxia, resulting in early pulmonary vasoconstriction, later pulmonary vascular remodeling, and increased pulmonary vascular resistance characterized by pulmonary arterioles, which can

Fig. 4 Effect of As-IV on ET-1 and Ang II levels in serum of HPH rats exposed to chronic hypoxia. (A) Effects of As-IV on the ET-1 level in serum of HPH rats under hypoxia. (B) Effects of As-IV on the Ang II level in serum of HPH rats under hypoxia. Data are represented as the mean $\pm$ SEM, $n = 4$ per group. * $P < 0.05$ and ** $P < 0.01$.

Fig. 5 Effect of As-IV on TNF- α and IL-6 levels in serum of HPH rats subjected to hypoxia. (A) Effects of As-IV on the TNF- α level in serum of HPH rats under hypoxia. (B) Effects of As-IV on the IL-6 level in serum of HPH rats under hypoxia. Data are represented as the mean $\pm$ SEM, $n = 4$ per group. * $P < 0.05$ and ** $P < 0.01$.

cause serious disorders of the cardiopulmonary function and ultimately lead to death of the patients due to right heart fail-

Fig. 6 The concentration-time profiles for As-IV in rats after intraperitoneal administration. Data are represented as the mean $\pm$ SEM, $n = 4$ per group.

ure.^{21,22} At present, the drug treatment of HPH is mainly a pulmonary vasodilator, with high selectivity, quick onset and short action time. As-IV is the main component of saponins extracted from *Astragalus membranaceus*.²³ For decades, its pharmacokinetics, pharmacological effects and mechanisms have been extensively studied, and great progress has been made. As-IV has the effects of resisting the injury of organ ischemia, oxidation, and inflammation,^{24–26} and has a certain relaxation effect on the normal isolated aorta and mesenteric artery in rats. In the present study, we found that As-IV significantly suppressed the increase of mPAP, RVP and RV/(LV + S) in HPH rats. Moreover, our data indicated that As-IV could relax the pulmonary artery rings in HPH rats and remarkably inhibit PASM proliferation after 24 hours of hypoxia exposure. Therefore, our results demonstrated that As-IV elicited an anti-HPH effect.

Vasoactive substances play a crucial role in the occurrence and progression of HPH. ET-1 is an important vasoconstrictor factor. Previous studies indicated that ET-1 has a strong

Fig. 7 Effect of As-IV on PASM cell proliferation subjected to hypoxia. Proliferation was measured in rat PASM cells treated with As-IV (10 and 50 μ M) and subjected to hypoxia for 24 hours. Cell viability was measured using the CCK-8 assay. Data are represented as the mean $\pm$ SEM, $n = 4$ per group. * $P < 0.05$ and ** $P < 0.01$.

and lasting contraction effect on PASM cells, and significantly increases the PASM cell proliferation.²⁷ Ang II is another important vasoactive material besides ET-1. Recent studies on pulmonary circulation indicated that Ang II could directly stimulate vasoconstriction and increase pulmonary vascular resistance.²⁸ The elevation of ET-1 and Ang II levels is closely related to the development of HPH.²⁹ Our results demonstrated that the levels of ET-1 and Ang II in serum significantly increased after being exposed to chronic hypoxia for 2 weeks, thereby HPH may be established. We also found that As-IV prevented the elevated levels of ET-1 and Ang II induced by hypoxia. These data suggested that As-IV decreased the levels of ET-1 and Ang II in HPH rats.

Inflammatory cytokines appear in the early stage of the inflammatory response and contribute to the severity of HPH.³⁰ IL-6 plays a role in the inflammatory response of HPH.³¹ TNF- α , TNF, a pro-inflammatory cytokine mainly produced by macrophages and monocytes, is responsible for the pathogenesis of inflammation.³² We demonstrated that the levels of TNF- α and IL-6 in serum were elevated under hypoxic conditions. Moreover, our study suggested that As-IV significantly prevented the hypoxia-induced increase of TNF- α and IL-6 levels in a dose-dependent manner. These results showed that As-IV significantly decreased hypoxia-induced inflammation.

In conclusion, the present study revealed that As-IV effectively attenuated HPH as evidenced by the inhibition of pulmonary artery remodeling and PASM cell proliferation. The treatment mechanism of As-IV might be involved in the reduction of the levels of inflammatory cytokines and vasoconstrictor factors. These results provide evidence that As-IV may be a potential drug against HPH in clinical practice.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

Acknowledgements

This study was supported by grants from the Youth Science and Technology Innovation Personnel Training Project of Shaanxi Province, China (No. 2017KJXX-84), the National Natural Science Foundation of China (No. 81402055), and the Research Foundation of Shaanxi Administration of Traditional Chinese Medicine, China (No. 2017JCMS009).

References

- H. J. Bogaard, K. Abe, N. A. Vonk and N. F. Voelkel, *Chest*, 2009, 135(3), 794–804.
- M. Humbert, *Eur. Respir. Rev.*, 2010, 19(115), 59–63.
- J. A. Cruz, E. M. Bauer, A. I. Rodriguez, A. Gangopadhyay, N. S. Zeineh, Y. Wang, S. Shiva, H. C. Champion and P. M. Bauer, *Am. J. Physiol.*, 2012, 302(12), H2518–H2527.
- C. M. Schannwell, S. Steiner and B. E. Strauer, *J. Physiol. Pharmacol.*, 2007, 58 Suppl 5(Pt 2), 591–602.
- S. Duong-Quy, S. Riviere, Y. Bei, C. Duong-Ngo, N. N. Le-Dong, T. Hua-Huy and A. T. Dinh-Xuan, *Rev. Mal. Respir.*, 2012, 29(8), 956–970.
- T. Minamino, H. Christou, C. M. Hsieh, Y. Liu, V. Dhawan, N. G. Abraham, M. A. Perrella, S. A. Mitsialis and S. Kourembanas, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, 98(15), 8798–8803.
- T. Tajsic and N. W. Morrell, *Compr. Physiol.*, 2011, 1(1), 295–317.
- L. Yu, Y. Tu, X. Jia, K. Fang, L. Liu, L. Wan, C. Xiang, Y. Wang, X. Sun, T. Liu, D. Yu, W. Cao, Y. Song and Y. Fan, *Cell. Physiol. Biochem.*, 2017, 42(1), 55–67.
- S. Ren, H. Zhang, Y. Mu, M. Sun and P. Liu, *J. Tradit. Chin. Med.*, 2013, 33(3), 413–416.
- L. Li, X. Hou, R. Xu, C. Liu and M. Tu, *Fundam. Clin. Pharmacol.*, 2017, 31(1), 17–36.
- D. Gui, Y. Guo, F. Wang, W. Liu, J. Chen, Y. Chen, J. Huang and N. Wang, *PLoS One*, 2012, 7(6), e39824.
- W. J. Zhang, P. Hufnagel, B. R. Binder and J. Wojta, *Thromb. Haemostasis*, 2003, 90(5), 904–914.
- W. D. Zhang, H. Chen, C. Zhang, R. H. Liu, H. L. Li and H. Z. Chen, *Planta Med.*, 2006, 72(1), 4–8.
- X. Cheng, J. Gu, M. Zhang, J. Yuan, B. Zhao, J. Jiang and X. Jia, *Int. Immunopharmacol.*, 2014, 23(1), 304–313.
- W. N. Yu, L. F. Sun and H. Yang, *Inflammation*, 2016, 39(5), 1835–1841.
- R. Huang and M. Li, *Saudi Pharm. J.*, 2016, 24(3), 341–347.
- W. D. Zhang, C. Zhang, X. H. Wang, P. J. Gao, D. L. Zhu, H. Chen, R. H. Liu and H. L. Li, *Planta Med.*, 2006, 72(7), 621–626.
- Y. Zhou, Y. Wang, X. Wang, X. Tian, S. Zhang, F. Yang, H. Guo, R. Fan, N. Feng, M. Jia, X. Gu, Y. Wang, J. Li and J. Pei, *Cell. Physiol. Biochem.*, 2017, 44(5), 1965–1979.
- Q. Wu, H. Y. Wang, J. Li, P. Zhou, Q. L. Wang, L. Zhao, R. Fan, Y. M. Wang, X. Z. Xu, D. H. Yi, S. Q. Yu and J. M. Pei, *PLoS One*, 2013, 8(5), e60850.

- 20 E. D. Michelakis, M. S. McMurtry, X. C. Wu, J. R. Dyck, R. Moudgil, T. A. Hopkins, G. D. Lopaschuk, L. Puttagunta, R. Waite and S. L. Archer, *Circulation*, 2002, **105**(2), 244–250.
- 21 H. Nouredine, G. Gary-Bobo, M. Alifano, E. Marcos, M. Saker, N. Vienney, V. Amsellem, B. Maitre, A. Chaouat, C. Chouaid, J. L. Dubois-Rande, D. Damotte and S. Adnot, *Circ. Res.*, 2011, **109**(5), 543–553.
- 22 N. M. Fine, L. Chen, P. M. Bastiansen, R. P. Frantz, P. A. Pellikka, J. K. Oh and G. C. Kane, *Circ. Cardiovasc. Imaging*, 2013, **6**(5), 711–721.
- 23 H. J. Kwon and Y. D. Park, *J. Chromatogr. A*, 2012, **1232**, 212–217.
- 24 T. Chen, R. Wang, W. Jiang, H. Wang, A. Xu, G. Lu, Y. Ren, Y. Xu, Y. Song, S. Yong, H. Ji and Z. Ma, *Inflammation*, 2016, **39**(1), 483–492.
- 25 L. H. Qiu, X. J. Xie and B. Q. Zhang, *Biol. Pharm. Bull.*, 2010, **33**(4), 641–646.
- 26 D. Gui, J. Huang, Y. Guo, J. Chen, Y. Chen, W. Xiao, X. Liu and N. Wang, *Cytokine*, 2013, **61**(3), 970–977.
- 27 I. Hubloue, B. Rondelet, F. Kerbaul, D. Biarent, G. M. Milani, M. Staroukine, P. Bergmann, R. Naeije and M. Leeman, *Crit. Care*, 2004, **8**(4), R163–R171.
- 28 T. K. Jeffery and J. C. Wanstall, *Pharmacol. Ther.*, 2001, **92**(1), 1–20.
- 29 C. Orte, J. M. Polak, S. G. Haworth, M. H. Yacoub and N. W. Morrell, *J. Pathol.*, 2000, **192**(3), 379–384.
- 30 J. Qian, Y. Ye, L. Lv, C. Zhu and S. Ye, *Int. Immunopharmacol.*, 2014, **21**(2), 426–431.
- 31 A. Chaouat, L. Savale, C. Chouaid, L. Tu, B. Sztrymf, M. Canuet, B. Maitre, B. Housset, C. Brandt, P. Le Corvoisier, E. Weitzenblum, S. Eddahibi and S. Adnot, *Chest*, 2009, **136**(3), 678–687.
- 32 Z. Tianzhu, Y. Shihai and D. Juan, *Inflammation*, 2014, **37**(6), 1976–1983.