Journal of Biosciences and Medicines, 2017, 5, 1-10
http://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Notoginsenoside R_1 Attenuates Hypoxia and Hypercapnia-Induced Vasoconstriction *In Vitro* by Reducing the Expression of p38

Congcong Zhang[1], Meiping Zhao[1], Mengxiao Zheng[1], Longsheng Song[2], Wantie Wang[1*]

[1]Department of Pathophysiology, Wenzhou Medical University, Wenzhou, China
[2]Division of Cardiovascular Medicine University of Iowa Carver College of Medicine, Iowa City, America
Email: *wwt@wmu.edu.cn

How to cite this paper: Zhang, C.C., Zhao, M.P., Zheng, M.X., Song, L.S. and Wang, W. (2017) Notoginsenoside R_1 Attenuates Hypoxia and Hypercapnia-Induced Vasoconstriction *In Vitro* by Reducing the Expression of p38. *Journal of Biosciences and Medicines*, **5**, 1-10.
https://doi.org/10.4236/jbm.2017.58001

Received: June 16, 2017
Accepted: July 30, 2017
Published: August 2, 2017

Abstract

Notoginsenoside R_1, the main active ingredient of Panax notoginseng saponins (PNS), has been proposed to play fatal roles in the development of hypoxic hypercapnia-induced pulmonary vasoconstriction (HHPV). Subsequently, pulmonary arterial smooth muscle cells (PASMCs) lead to pulmonary vascular system remodeling and chronic pulmonary disease in the development of HHPV. Despite considerable studies have contributed to pulmonary disease, the mechanism of how Notoginsenoside R_1 affects HHPV remains unclear. In this view, we will discuss the effect of notoginsenoside R_1 by investigating the expression of p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway in PASMCs under hypoxia and hypercapnia condition. The third order PASMCs of Sprague Dawley (SD) rats were cultured with various concentrations (8, 40, 100 mg/L, respectively) of Notoginsenoside R_1. Our data showed that the protein and mRNA expression levels of p-38 MAPK were higher in hypoxic hypercapnia group compared with hypoxic DMSO and normoxia control groups ($p < 0.01$). In R_1 treatment groups, the level of p-p38 MAPK protein and p38 MAPK mRNA were significantly decreased with different degrees ($p < 0.01$, each). This study provides the evidence that Notoginsenoside R_1 treatment may contribute to attenuate HHPV via decreasing the protein and mRNA expression levels of p-38 MAPK.

Keywords

Hypoxic Hypercapnia, p38 MAPK, Notoginsenoside R_1, Pulmonary Arterial Smooth Muscle Cells

1. Introduction

Hypoxia hypercapnia-induced pulmonary arterial hypertension (HHPH) is a

chronic and progressive disease with poor diagnosis and leads to right heart failure and eventually death due to lack of proper therapy. HHPH is featured with pulmonary vasoconstriction, elevated pulmonary vascular pressures and chronic right heart failure and leads to death [1] [2] [3] [4] [5]. Recently, the fifth World Symposium on PH updated the previous classification of pulmonary hypertension (PH), and indicated that HHPH belongs to Group 3: respiratory diseases lead to pulmonary hypertension like pulmonary fibrosis, COPD, lung emphysema or interstitial lung disease [6]. Of note, the median survival of untreated patients was limited to 3 years [7], but even in those receiving formal treatment, the results are still not optimistic.

Recently, it has been accepted that pulmonary arterial hypertension (PAH) is a result of proliferative remodeling rather than pulmonary vasculature vasoconstriction [8], therefore, the therapies that target vasoconstriction may not be beneficial. Another challenge is that PAH is associated with inflammatory disease like schistosomiasis and factors like tyrosine kinase [9], scleroderma, viral infections with HIV, Loss of Kv1.5,, or loss-of-function mutations in BMPR2. [9] [10] [11] [12]. In this study, we explored the effect of panax notoginseng saponins (PNS) attenuating pulmonary vascular remodeling that precedes PAH.

PNS, a compound of various saponins, is extracted from traditional Chinese medicine Panax notoginseng [13]. Recent studies have shown that PNS has effects on the blood vessels dilation, the removal of free radicals and the specific inhibition of Ca^{2+} channels of vascular smooth muscle cells [14] [15]. The main bioactive components are ginsenoside Rg1, ginsenoside Rb1 and notoginsenoside R_1 [16] [17]. Literature shows that PNS has an effect on attenuating chronic hypoxia hypercapnia-induced experimental pulmonary hypertension in rats, and also inhibits HHPV rats [18]. However, the underlying mechanism of how notoginsenoside R_1 has effect on hypoxic hypercapnia-induced vasoconstriction has not been well understood.

In addition, the p38 mitogen-activated protein kinase (P38 MAPK) signaling pathway has attracted to more investigators as it is crucial to fibroblast proliferation in the hypoxic model, and the inhibition of P38 MAPK can attenuate or block the pulmonary vascular proliferative response induced by hypoxic [19] [20]. In this study, we intended to clarify the effect of notoginsenoside R_1 on attenuating HHPH and explore the underlying mechanism though detecting the expression of p38 at mRNA and protein levels.

2. Materials and Methods

2.1. Animal Preparation

The use of laboratory animals observed the international guidelines and ethnic. 10 adult male Sprague-Dawley rats, weighing 200 - 220 g, were used in all experiments and were provided by the Experimental Animal Center of Wenzhou Medical University, China, Animal license NO: SCXK (Zhejiang 2008-0156). The animals were allowed to drink and eat freely and housed at 21˚C to 23˚C.

2.2. Reagents and Antibodies

Ginsenoside R_1 (>98% pure, Figure 1) was purchased from the Department of Organic chemistry, College of Preclinical Medicine, Jilin University (Jilin, China), High-glucose Dulbecco's modified Eagle's medium, fetal bovin serum (Gibco, US), acetycholine (sigma, USA), RT-PCR kit (Takara Biotechnology, China), monoclonal antibody to SM-alpha-actin, SABC-FITC (POD) kit (Takara Biotechnology, China), DAB staining kit (Takara Biotechnology, China), anti-phospho-p38 (rabbit anti-mouse) and anti-p38 (rabbit anti- mouse) monoclonal antibodies and goat anti-rabbit IgG/HRP (Cell Signal Technology), BCA protein assay kit, enhanced chemiluminescent kit (Pierce, US).

2.3. Primary Cell Culture and Grouping

Briefly, pulmonary vessels were dissected free from the muscular tissue and endothelial cell layers were removed by gentle abrasion after killing rats by cervical dislocation. The third or fourth order branches were separated from pulmonary artery branch under the microscope and washed by Krebs buffer five times. Subsequently, wiped the medial artery was gently wiped with sterile swabs to remove the endothelial cells. The remaining tissue was incubated with collagenase I in 37˚C for 3 hours, then the solution was centrifuged at 1000 r/min for 7 min, then the floccule was resuspended in DMEM containing 20% FBS. Cells were incubated in a humidified atmosphere of 5% CO_2 in air at 37.0˚C. The PASMCs were identified with inverted phase contrast microscope or using fluorescence microscopy after fluorescence staining. Cells (used between passage 2 and 5) were subsequently maintained in DMEM containing 10% FBS with 5% CO2 at 37.0˚C. After reaching the concentration of 80%, the PASMCs were starved at least 24 h before medicine treatment to make the cells stop growth [20]. The cell groups were divided up into four groups: 1) normoxia group (N group, 5% CO_2, 21% O_2); 2) hypoxia and hypercapnia group (H group, 6% CO_2, 1% O_2); 3) DESO control group with hypoxia and hypercapnia (HD group, 0.05% DMSO with 6% CO_2, 1%O_2); 4) notoginsenoside R_1 treatment group

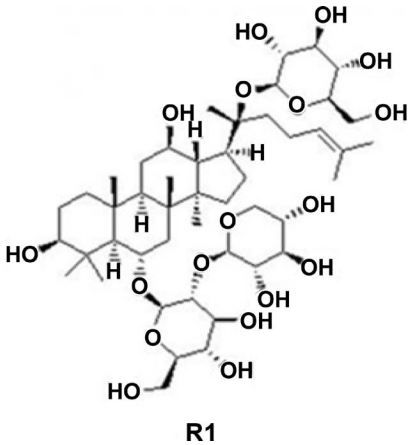

Figure 1. The chemical structure of R_1.

(RgL, RgM, RgH with 6% CO_2, 1% O_2). Cells were grown in serum-free DMEM for 24 h at 37.0˚C.

2.4. Western Blotting

After washed by cold PBS three times, PASMCs were homogenized with Radio Immunoprecipitation Assay (RIPA) lysate (containing 1 mM Phenylmethanesulfonyl fluoride (PMSF) and 10 mM NaF). Then homogenates were centrifuged for 5 min at 4˚C, and the supernatants were collected and conserve at −80˚C if not required. The protein concentration was assessed by using a BCA technique as the standard. Then 40 ug protein samples were separated on the sample buffer for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Proteins were then transferred to polyvinylidene difluoride (PVDF) membranes and the blot was blocked at 25˚C - 27˚C for 1 h in 5% skim milk in Tris-buffered saline plus Tween 0.05%. PVDF membranes were then incubated overnight at 4˚C with an anti-phosphospe-p38 MAPK antibody (1:1000). These were three times washed by Tris Buffered Saline, with Tween (TBST) and then incubated with anti-rabbit IgG antibodies (1:4000) at room temperature for 1 h. The bound antibodies were detected by using enhanced chemiluminscence (ECL). The test used p38 MAPK as an internal control and had been repeated for eight times.

2.5. RT-PCR

Gene expression of p38 MAPK was analyzed using semi-quantitative RT-PCR [21]. RNA was isolated from the cells using TRIzol (Beyotime, China). Then these were reverse transcribed to cDNA with the following gene primers: p38 5'-TCC AAG GGC TAC ACC AAA TC-3' (forward) and 5'-TGT TCC AGG TAA GGG TGA GC-3' (reverse). The primers of β-actin: 5'-GAG ACC TTC AAC ACC CCA GCC-3' (forward) and 5'-TCG GGG GAT CGG AAC CGC TCA-3' (reverse).

2.6. Statistical Analysis

Data are reported as means ± SE and SPSS17.0 was used to analyze the data. Data between more than two groups were analyzed by one-way ANOVA. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Effect of Ginsenoside R_1 on the Level of p-p38 MAPK Protein under Hypoxia and Hypercapnia Condition

According to the analysis of western blot (**Figure 2**), compared with PASMCs in N Group, H group and HD group had a higher the level of p-p38 MAPK protein. However, the level of p-p38 MAPK protein significantly decreased in ginsenoside R_1 treatment group compared with PASMCs in H group and HD group ($p < 0.05$). We confirmed that ginsenoside R_1 could reduce the level of p-p38 MAPK protein, and the concentration of 100 mg/L had the best effect.

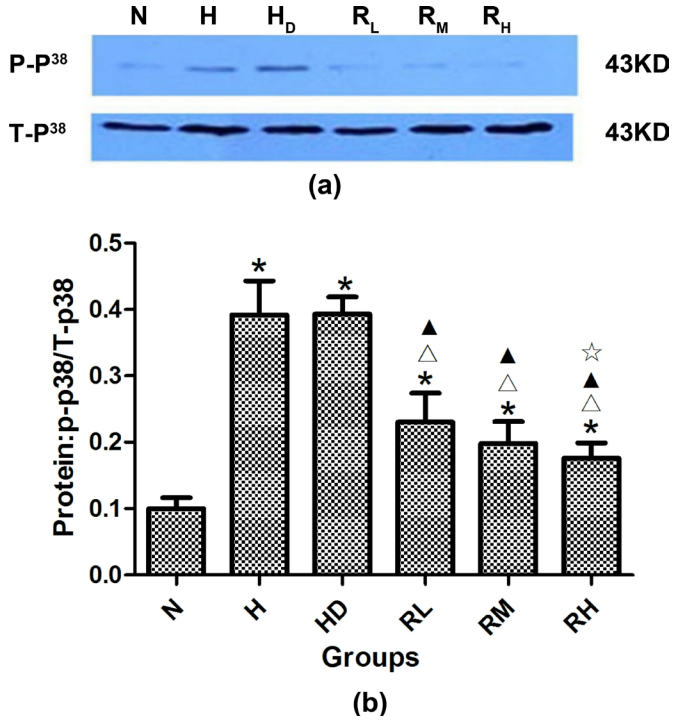

Figure 2. The level of p-p38 MAPK protein in different groups. (a) The level of p-p38 MAPK protein by the analysis of western blot; (b) Comparison of the level of p-p38 MAPK protein in different groups. Data are expressed as means ± standard error of the mean. n = 8 per group. *P < 0.01 vs N group; △P < 0.01 vs H group; ▲P < 0.01 vs HD group; ☆P < 0.05 vs RL group.

3.2. Effect of Ginsenoside R_1 on the Level of p38 MAPK mRNA in PASMCs under Hypoxia and Hypercapnia Condition

According to real-time PCR analysis (**Figure 3**), compared with N group, the expression level of p38 MAPK mRNA exhibited a significant increase in H group and HD group. The expression of p38 MAPK mRNA also significantly decreased in ginsenoside R_1 treatment group compared with H group and HD group. We concluded that there was decreased the level of p38 MAPK mRNA in ginsenoside R_1 treatment group, suggesting down regulation of p38 MAPK mRNA by ginsenoside R_1.

4. Discussion

Currently, there has been the evidence that persistent inflammation and vascular remodeling is a mark of HHPV. Furthermore, Pulmonary artery fibroblasts express all four isoforms of p38 MAPK; however, in HHPV, only p38 *α*- and *β*-isoforms are phosphorylated [22]. The p38 MAPK system is increasingly recognized as a crucial pathway of inflammatory response in pulmonary vascular disease but its specific role in this way is unclear. To facilitate this, previous study had investigated the role of the p38 MAPK pathway in both *in vitro* and *in vivo* models of HHPV, and the expression of phosphorylated p38 MAPK and p38 MAPK*α* had increased [23] [24] [25], indicating the activation of this path-

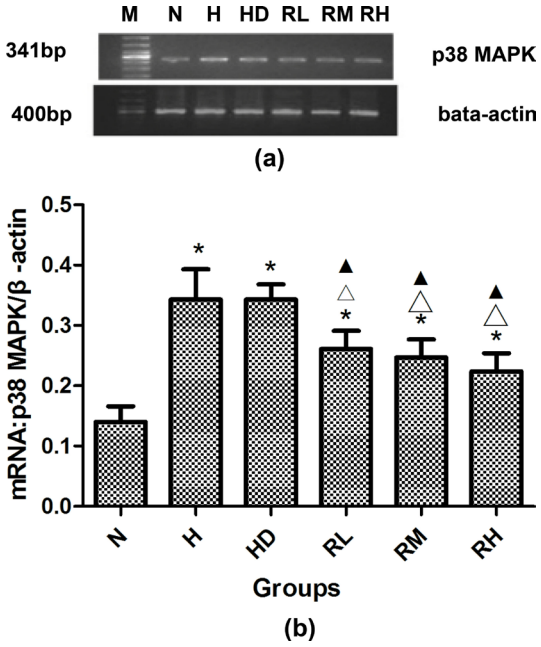

Figure 3. The level of p38 MAPK mRNA in different groups. (a) The level of p38 MAPK mRNA by the analysis of RT-PCR; (b) Comparison of the level of p38 MAPK mRNA in different groups. Data are expressed as means ± standard error of the mean. n = 8 per group.* $P < 0.01$ vs N group; $^{\triangle}P < 0.01$ vs H group; $^{\blacktriangle}P < 0.01$ vs HD group.

way in the PVremod A reduction of IL-6 levels and suggesting that the p38 MAPK and the α-isoform plays a significantly role in pulmonary hypertension potentially mediated through IL-6 [26].

Hypoxic and hypercapnia pulmonary hypertension (HHPH) was a chronic pulmonary disease with poor prognosis and high mortality, its clinical symptoms was apparent, such as dyspnea, right-sided heart failure, and it was characterized by an increase resistance in pulmonary artery [27]. Mechanisms associated with HHPV in PASMCs was calcium, which released from the smooth muscle sarcoplasmic reticulum via ryanodine receptors pivotal [28] [29] [30], and consistent constriction though myofilament calcium sensitisation, which was because of the release of uncertain vasoconstrictor from the endothelium [29] [30]. Hypoxic and hypercapnia also modulated the activity of voltage-gated potassium channels (Kv) in the plasma membrane of PASMCs [31] [32] [33] [34].

It is generally agreed that PNS participates in the process of cell differentiation, proliferation and in Ca^{2+} entry [35], regulates the balance of cell apoptosis and autophagy. Recently, compelling evidence has recently appeared that PNS has effect on pulmonary arterial hypertension and pulmonary heart disease. In our study, we confirmed that the expression level of p38 MAPK mRNA showed a significant higher under hypoxia and hypercapnia condition, and with the treatment of single ginsenoside R_1 the level of p38 MAPK mRNA had decreased ($p < 0.01$). Our present data showed that single ginsenoside R_1 at the concentration of 100 mg/L had the optimum efficiency to prevent the phosphorylation of

p38 MAPK in response to hypoxia and hypercapnia condition.

It is possible that excessive proliferation of PASMCs is the major pathological mechanism of pulmonary arterial vascular remodeling and finally leads to pulmonary hypertension under hypoxia and hypercapnia condition. Recent preclinical study suggested that the antiproliferative activity of notoginseng R_1 extract is most probably linked to cell cycle arrest and the induction of cell apoptosis [36]. MAPK family is considered as the main mediators in cell proliferation and hypertrophy, furthermore, ERK signaling pathway also has effect on smooth muscle cell proliferation [37]. Since the increase of ERK under hypoxia and hypercapnia condition in previous study, further experiments should be developed to verify the contribution of ERK in the pathomechanism of HHPV.

5. Conclusion

In summary, the findings from this study extended evidence that p38 MAPK is a crucial intermediary in the functional consequences of acute hypoxia in pulmonary hypertension. This provided that PNS can relieve the pulmonary vasoconstrictions under hypoxia and hypercapnia condition though inhibiting the activation of p38 MAPK signaling pathway. Although our present research was performed in isolate PASMCs, which does not directly perform an experiment on humans, it provides a novel therapeutic means for patients with pulmonary hypertension for ginsenoside Rg1 treatment.

Acknowledgements

This work was supported by the key project of Traditional Chinese Medicine Development Plan of Zhejiang province (2008ZA017, 2013ZZ011) and the Key Construction Academic Subject (Traditional Chinese Medicine) of Zhejiang Province (2012-XK-A28).

References

[1] Humbert, M. (2009) Update in Pulmonary Hypertension 2008. *American Journal of Respiratory and Critical Care Medicine*, **179**, 650-656. https://doi.org/10.1164/rccm.200901-0136UP

[2] Mooi, W.J. and Grungerg K. (2006) Histopathology of Pulmonary Hypertensive Diseases. *Current Diagnostic Pathology*, **12**, 429-440. https://doi.org/10.1016/j.cdip.2006.07.003

[3] Morrell, N.W., Adnot, S., Archer, S.L., Dupuis, J., Jones, P.L., MacLean, M.R., *et al.* (2009) Cellular and Molecular Basis of Pulmonary Arterial Hypertension. *Journal of the American College of Cardiology*, **54**, 20-31. https://doi.org/10.1016/j.jacc.2009.04.018

[4] Pietra, G.G., Capron, F., Stewart, S., Leone, O., Humbert, M., Robbins, I.M., *et al.* (2004) Pathologic Assessment of Vasculopathies in Pulmonary Hypertension. *Journal of the American College of Cardiology*, **43**, 25S-32S. https://doi.org/10.1016/j.jacc.2004.02.033

[5] Rajagopalan, N., Simon, M.A., Suffoletto, M.S., Shah, H., Edelman, K., Mathier, M.A., *et al.* (2009) Noninvasive Estimation of Pulmonary Vascular Resistance in

Pulmonary Hypertension. *Echocardiography*, **26**, 489-494. https://doi.org/10.1111/j.1540-8175.2008.00837.x

[6] Simonneau, G., Gatzoulis, M.A., Adatia, I., Celermajer, D., Denton, C., Ghofrani, A., Gomez Sanchez, M.A., Krishna Kumar, R., Landzberg, M., Machado, R.F., Olschewski, H., Robbins, I.M. and Souza, R. (2013) Updated Clinical Classification of Pulmonary Hypertension. *Journal of the American College of Cardiology*, **62**, D34-41. https://doi.org/10.1016/j.jacc.2013.10.029

[7] McLaughlin, V.V., Archer, S.L., Badesch, D.B., Barst, R.J., Farber, H.W., Lindner, J.R., Mathier, M.A., McGoon, M.D., Park, M.H., Rosenson, R.S., Rubin, L.J., Tapson, V.F., Varga, J., Harrington, R.A., Anderson, J.L., Bates, E.R., Bridges, C.R., Eisenberg, M.J., Ferrari, V.A., Grines, C.L., Hlatky, M.A., Jacobs, A.K., Kaul, S., Lichtenberg, R.C., Moliterno, D.J., Mukherjee, D., Pohost, G.M., Schofield, R.S., Shubrooks, S.J., Stein, J.H., Tracy, C.M., Weitz, H.H. and Wesley, D.J. (2009) ACCF/AHA 2009 Expert Consensus Document on Pulmonary Hypertension a Report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; And the Pulmonary Hypertension Association. *Journal of the American College of Cardiology*, **119**, 2250-2294.

[8] Michelakis, E.D., Wilkins, M.R. and Rabinovitch, M. (2008) Emerging Concepts and Translational Priorities in Pulmonary Arterial Hypertension. *Circulation*, **118**, 1486-1495. https://doi.org/10.1161/CIRCULATIONAHA.106.673988

[9] Moreno-Vinasco, L., Gomber-Maitland, M., Maitland, M., Desai, A., Singleton, P., Sammani, S., Sam, L., Liu, Y., Husain, A., Lang, R., Ratain, M., Lussier, Y. and Garcia, J. (2008) Genomic Assessment of a Multikinase Inhibitor, Sorafenib, in a Rodent Model of Pulmonary Hypertension. *Physiological Genomics*, **33**, 278-291. https://doi.org/10.1152/physiolgenomics.00169.2007

[10] Macchia, A., Marchioli, R., Tognoni, G., Scarano, M., Marfisi, R., Tavazzi, L. and Rich, S. (2010) Systematic Review of Trials Using Vasodilators in Pulmonary Arterial Hypertension: Why a New Approach is Needed. *American Heart Journal*, **159**, 245-257. https://doi.org/10.1016/j.ahj.2009.11.028

[11] Ivy, D. and Saji, B.T. (2010) A New Era in Medical Management of Severe Pediatric Pulmonary Arterial Hypertension. *Nihon Shoni Junkanki Gakkai Zasshi*, **26**, 206-218.

[12] Kumar, R., Mickael, C., Chabon, J., Gebreab, L., Rutebemberwa, A., Garcia, A.R., Koyanagi, D.E., Sanders, L., Gandjeva, A., Kearns, M.T., Barthel, L., Janssen, W.J., Mauad, T., Bandeira, A., Schmidt, E., Tuder, R.M. and Graham, B.B. (2015) The Causal Role of IL-4 and IL-13 in Schistosoma Mansoni Pulmonary Hypertension. *American Journal of Respiratory and Critical Care Medicine*, **192**, 998-1008. https://doi.org/10.1164/rccm.201410-1820OC

[13] Ng, T.B. (2006) Pharmacological Activity of Sanchi Ginseng (Panax Notoginseng). *Journal of Pharmacy and Pharmacology*, **58**, 1007-1019. https://doi.org/10.1211/jpp.58.8.0001

[14] Nah, S.Y., Park, H.J. and McClesky, E.W. (1995) A Trace Component of Ginseng that Inhibit Ca^{2+} Channels through a Pertussis Toxin-Sensitive G Protein. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 8739-8743. https://doi.org/10.1073/pnas.92.19.8739

[15] Xu, Q.H., Fang, X.L. and Chen, D.F. (2003) Pharmacokinetics and Bioavailability of Ginsenoside Rb1 and Rg1 from Panax Notoginseng in Rats. *Journal of Ethnopharmacology*, **84**, 187-192. https://doi.org/10.1016/S0378-8741(02)00317-3

[16] Chen, S.W., Li, X.H., Ye, K.H., Jiang, Z.F. and Ren, X.D. (2004) Total Saponins of Panax Notoginseng Protect Rabbit Iliac Artery against Balloon Endothelial Denudation Injury. *Acta Pharmaceutica Sinica*, **25**, 1151-1156.

[17] Wang, C.Z., McEntee, E., Wicks, S., Wu, J.A. and Yuan, C.S. (2006) Phytochemical and Analytical Studies of Panax Notoginseng (Burk.). *Journal of Natural Medicines*, **60**, 97-106. https://doi.org/10.1007/s11418-005-0027-x

[18] Ma, W.P., Yang, Y.N., Zhou, D.B. and Zhou, W.Q. (2004) The Preventive Effect of Panax Notoginseng Saponins (PNS) on Chronic Hypoxic Pulmonary Hypertension in Rats. *Chinese Journal of Pathophysiology*, **20**, 1074-1077.

[19] Mortimer, H.J., Peacock, A.J., Kirk, A. and Welsh, D.J. (2007) P38 MAP Kinase: Essential Role in Hypoxia-Mediated Human Pulmonary Artery Fibroblast Proliferation. *Pulmonary Pharmacology & Therapeutics*, **20**, 718-725. https://doi.org/10.1016/j.pupt.2006.08.007

[20] Welsh, D.J., Scott, P.H. and Peacock, A.J. (2006) P38 MAP Kinase Isoform Activity and Cell Cycle Regulators in the Proliferative Response of Pulmonary and Systemic Artery Fibroblasts to Acute Hypoxia. *Pulmonary Pharmacology & Therapeutics*, **19**, 128-138. https://doi.org/10.1016/j.pupt.2005.04.008

[21] Qiu, X., Zheng, M., Song, D., Huang, L., Tang, L., Ying, L. and Wang, W. (2016) Notoginsenoside Rb1 Inhibits Activation of ERK and p38 MAPK Pathways Induced by Hypoxia and Hypercapnia. *Experimental and Therapeutic Medicine*, **11**, 2455-2461. https://doi.org/10.3892/etm.2016.3217

[22] Panzhinskiy, E., Zawada, W.M., Stenmark, K.R. and Das, M. (2012) Hypoxia Induces Unique Proliferative Response in Adventitial Fibroblasts by Activating PDGFβ Receptor-JNK1 Signalling. *Cardiovascular Research*, **95**, 356-365. https://doi.org/10.1093/cvr/cvs194

[23] Welsh, D., Mortimer, H., Kirk, A. and Peacock, A. (2005) The Role of p38 Mitogen-Activated Protein Kinase in Hypoxia-Induced Vascular Cell Proliferation: An Interspecies Comparison. *Chest*, **128**, 573S-574S. https://doi.org/10.1378/chest.128.6_suppl.573S

[24] Welsh, D.J., Peacock, A.J., MacLean, M. and Harnett, M. (2001) Chronic Hypoxia Induces Constitutive p38 Mitogen-Activated Protein Kinase Activity that Correlates with Enhanced Cellular Proliferation in Fibroblasts from Rat Pulmonary but Not Systemic Arteries. *American Journal of Respiratory and Critical Care Medicine*, **164**, 282-289. https://doi.org/10.1164/ajrccm.164.2.2008054

[25] Goncharova, E.A. (2013) mTOR and Vascular Remodeling in Lung Diseases: Current Challenges and Therapeutic Prospects. *The FASEB Journal*, **27**, 1796-1807. https://doi.org/10.1096/fj.12-222224

[26] Church, A.C., Martin, D.H., Wadsworth, R., Bryson, G., Fisher, A.J., Welsh, D.J. and Peacock, A.J. (2015) The Reversal of Pulmonary Vascular Remodeling through Inhibition of p38 MAPK-Alpha: A Potential Novel Anti-Inflammatory Strategy in Pulmonary Hypertension. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **309**, L333-347. https://doi.org/10.1152/ajplung.00038.2015

[27] Welch, L.C., Lecuona, E., Briva, A., Trejo, H.E., Dada, L.A. and Sznajdera, J.I. (2010) Extracellular Signal-Regulated Kinase (ERK) Participates in the Hypercapnia-Induced Na, K-ATPase Downregulation. *FEBS Letters*, **584**, 3985-3989. https://doi.org/10.1016/j.febslet.2010.08.002

[28] Dipp, M. and Evans, A.M. (2001) Cyclic ADP-Ribose Is the Primary Trigger for Hypoxic Pulmonary Vasoconstriction in the Rat Lung in Situ. *Circulation Research*, **89**, 77-83. https://doi.org/10.1161/hh1301.093616

[29] Morio, Y. and McMurtry, I.F. (2002) Ca^{2+} Release from Ryanodine-Sensitive Store Contributes to Mechanism of Hypoxic Vasoconstriction in Rat Lungs. *Journal of Applied Physiology*, **92**, 527-534.

[30] Dipp, M., Nye, P.C. and Evans, A.M. (2001) Hypoxic Release of Calcium from the Sarcoplasmic Reticulum of Pulmonary Artery Smooth Muscle. *American Journal of Physiology*, **281**, L318-325.

[31] Robertson, T.P., Ward, J.P. and Aaronson, P.I. (2001) Hypoxia Induces the Release of a Pulmonary-Selective, Ca^{2+}-Sensitising, Vasoconstrictor from the Perfused Rat Lung. *Cardiovascular Research*, **50**, 145-150. https://doi.org/10.1016/S0008-6363(01)00192-4

[32] Archer, S.L., Huang, J., Henry, T., Peterson, D. and Weir, E.K. (1993) A Redox-Based O_2 Sensor in Rat Pulmonary Vasculature. *Circulation Research*, **73**, 1100-1112. https://doi.org/10.1161/01.RES.73.6.1100

[33] Firth, A.L., Yuill, K.H. and Smirnov, S.V. (2008) Mitochondria-Dependent Regulation of KV Currents in rat Pulmonary Artery Smooth Muscle Cells. *American Journal of Physiology*, **295**, L61-70. https://doi.org/10.1152/ajplung.90243.2008

[34] Platoshyn, O., Yu, Y., Ko, E.A., Remillard, C.V. and Yuan, J.X. (2007) Heterogeneity of Hypoxia-Mediated Decrease in I(K(V)) and Increase in $[Ca^{2+}]$(cyt) in Pulmonary Artery Smooth Muscle Cells. *American Journal of Physiology*, **293**, L402-416. https://doi.org/10.1152/ajplung.00391.2006

[35] Si, Y.C., Zhang, J.P., Xie, C.E., Zhang, L.J. and Jiang, X.N. (2011) Effects of Panax Notoginseng Saponins on Proliferation and Differentiation of Rat Hippocampal Neural Stem Cells. *The American Journal of Chinese Medicine*, **39**, 999-1013. https://doi.org/10.1142/S0192415X11009366

[36] Wang, C.Z., Xie, J.T., Fishbein, A., Aung, H.H., He, H., Mehendale, S.R., He, T.C., Du, W. and Yuan, C.S. (2009) Antiproliferative Effects of Different Plant Parts of Panax Notoginseng on SW480 Human Colorectal Cancer Cells. *Phytotherapy Research*, **23**, 6-13. https://doi.org/10.1002/ptr.2383

[37] Guo, J., Li, L., Wu, Y.J., Yan, Y., Xu, X.N., Wang, S.B., Yuan, T.Y., Fang, L.H. and Du, G.H. (2013) Inhibitory Effects of Brazilin on the Vascular Smooth Muscle Cell Proliferation and Migration Induced by PDGF-BB. *The American Journal of Chinese Medicine*, **41**, 1283-1296. https://doi.org/10.1142/S0192415X13500869