Protective Effects of Isoliquiritigenin on Hypoxia-induced Pulmonary Artery Endothelial Cells Dysfunction

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Abstract: Hypoxia-induced pulmonary endothelial dysfunction plays critical roles in the pathological process of pulmonary arterial hypertension (PAH). Isoliquiritigenin (ISL), a member of the flavonoid family, possesses numerous pharmacological properties and has beneficial effects on the cardiovascular system. In this study, rat primary pulmonary artery endothelial cells (PAECs) were divided into a total 5 groups: normoxia, hypoxia, hypoxia+25μM ISL, hypoxia+50μM ISL, and hypoxia+100μM ISL. To determine the effect of ISL on the regulation of hypoxia-induced secretion of inflammatory cytokines and vasoactive factors in PAECs, the levels of ET-1, TNF-α and IL-6 were measured in cell culture supernatant. In addition, HIF-1α, ET-1, TNF-α, and IL-6 mRNA levels were also assessed. The results showed that hypoxia increased the levels of ET-1, TNF-α and IL-6 in cell culture supernatant and upregulated HIF-1α, ET-1, TNF-α and IL-6 mRNA levels in PAECs. However, ISL reduced the levels of ET-1, TNF-α and IL-6 in cell culture supernatant and prevented hypoxia-induced HIF-1α, ET-1, TNF-α and IL-6 mRNA induction. Our results showed that ISL might have protective effects on hypoxia-induced PAECs dysfunction.

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive and life-threatening disease characterized by pulmonary artery structural remodeling, elevated pulmonary artery pressure, and right ventricular hypertrophy [1]. These pathophysiological features typically lead to right ventricular failure and even death [2]. The pathobiology of PAH is not fully understood and a specific treatment has not been found. Therefore, finding novel effective pharmacotherapy for PAH is urgent. In the pathogenesis of PAH, factors such as hypoxia that are known to induce and exacerbate PAH can target pulmonary artery endothelial cells (PAECs) and induce their dysfunction [3]. Hypoxia has significant effects on the regulation of synthesis and secretion of inflammatory cytokines and vasoactive factors in PAECs [4].

Isoliquiritigenin (ISL), a bioactive ingredient of flavonoids, is extracted from the roots and rhizomes of licorice [5]. It has been confirmed that ISL possesses numerous pharmacological properties such as anticancer, anti-microbial, antioxidant, and anti-inflammatory activities, as well as immunoregulatory, neuroprotective, and cardioprotective effects [6-10]. In the present study, we investigated the effects of ISL on the secretion of inflammatory cytokines and vasoactive factors in PAECs under hypoxia conditions to explore the potential effects of ISL on PAH.

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2. Materials and Methods

2.1 Reagents.

ISL>99% purity verified by HPLC was purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). DMEM, FBS, Trizol reagent, and trypsin solution were from Invitrogen (Carlsbad, CA, USA).

2.2 PAECs Hypoxia.

Rat primary PAECs were divided into a total 5 groups: normoxia, hypoxia, hypoxia+25μM ISL, hypoxia+50μM ISL, and hypoxia+100μM ISL. PAECs were exposed either in 21% oxygen or 3% oxygen condition for 24 h.

2.3 Assay of ET-1, TNF-α and IL-6.

The levels of endothelin-1 (ET-1), tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6) in cell culture supernatant were measured with ELISA kits (Beyotime Institute of Biotechnology Shanghai, China) following the instructions of the manufacturer, and were analyzed with a spectrophotometer.

2.4 Quantitative Real-Time RT-PCR Analysis.

RNA was extracted from cells by using Trizol agent. Quantitative real-time RT-PCR was performed to assess mRNA expression of the following genes. The relative amount of the mRNA expression for hypoxia-inducible factor-1α (HIF-1α), TNF-α, IL-6 or ET-1 was represented using the $2^{-\Delta\Delta CT}$ value. The primers for genes were HIF-1α: forward: 5′-TGAGCTCACATCTTGATAAAGCTTCT-3′, reverse: 5′-GGGCTTTCAGATAAAAACAGTCCAT-3′; TNF-α: forward: 5′-CCCTCACACTCAGATCATCTTCT-3′, reverse: 5′-GCTACGACGTGGGCTACAG-3′; IL-6: forward: 5′-GACAAAGCCAGAGTCCTTCA-3′, reverse: 5′-ACTAGGTTTGCCGAGTAGAC-3′; ET-1: forward: 5′- CTCGCTCTATGTAAGTCATGG-3′, reverse: 5′- GCTCCTGCTCCTCCTTGATG-3′; housekeeping gene GAPDH: forward: 5′-AGGTCGGTGTGAACGGATTTG-3′, reverse: 5′- TGTAGACCATGTAGTTGAGGTCA -3′, respectively.

2.5 Statistical Analyses.

Results were expressed as means ± S.E.M. Statistical analysis was performed by SPSS (Version 11.5, SPSS Inc., Chicago, USA). Comparisons between groups were performed using ANOVA with a Holms–Sidak post hoc test. Significant difference was accepted at $P < 0.05$.

3. Results

3.1 ISL Reduced Hypoxia-Induced Secretion of ET-1, TNF-α and IL-6 in PAECs.

To determine the potential effect of ISL on the regulation of hypoxia-induced secretion of inflammatory cytokines and vasoactive factors in PAECs, the levels of ET-1, TNF-α and IL-6 were measured in cell culture supernatant. Compared with normoxia group, the levels of ET-1, TNF-α and IL-6 in the hypoxia group were elevated significantly. ISL administration reduced the levels of ET-1, TNF-α and IL-6 in cell culture supernatant. Furthermore, the effect of ISL on PAECs was enhanced by increasing ISL concentrations (as shown in Table 1).

Table 1 The levels of ET-1, TNF-α and IL-6 in cell culture supernatant

Group	ET-1 (pg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)
normoxia	12.78±0.72	87.66±6.75	13.39±0.86
hypoxia	20.63±1.28*	122.72±10.21*	22.13±1.36*
hypoxia+25μM ISL	17.68±1.12$^{\#}$	106.69±10.02$^{\#}$	19.51±1.17$^{\#}$

hypoxia+50μM ISL	15.34±0.97#	101.76±8.37#	15.98±1.01#
hypoxia+100μM ISL	14.96±0.86#	96.16±8.78#	15.03±0.98#

Values are means ± SEM, *P < 0.05 compared with normoxia group. #P < 0.05 compared with hypoxia group (n = 3).

3.2 ISL Attenuated Hypoxia-Induced High Levels of HIF-1α, ET-1, TNF-α, and IL-6 mRNA in PAECs.

To determine whether hypoxia modulates HIF-1α, ET-1, TNF-α, and IL-6 gene expression and the potential effects of ISL on HIF-1α, ET-1, TNF-α, and IL-6, we assessed HIF-1α, ET-1, TNF-α, and IL-6 mRNA levels (as shown in Table 2). Results confirmed that exposure to hypoxia increased the levels of HIF-1α, ET-1, TNF-α, and IL-6 mRNA in PAECs, and treatment with ISL prevented hypoxia-mediated HIF-1α, ET-1, TNF-α, and IL-6 mRNA induction.

Table 2 Relative Expression of HIF-1α, ET-1, TNF-α, and IL-6 mRNA in PAECs

Group	HIF-1α mRNA	ET-1 mRNA	TNF-α mRNA	IL-6 mRNA
normoxia	1.000±0.000	1.000±0.000	1.000±0.000	1.000±0.000
hypoxia	1.891±0.230*	3.120±0.438*	2.763±0.469*	3.806±0.504*
hypoxia+25μM ISL	1.617±0.245#	2.580±0.397#	2.016±0.396#	3.011±0.402#
hypoxia+50μM ISL	1.324±0.189#	1.826±0.348#	1.735±0.390#	2.172±0.521#
hypoxia+100μM ISL	1.312±0.143#	1.459±0.382#	1.546±0.248#	2.014±0.361#

Values are means ± SEM, *P < 0.05 compared with normoxia group. #P < 0.05 compared with hypoxia group (n = 3).

4. Discussion

Multiple mechanisms promoting the pulmonary vascular remodeling in PAH include pulmonary endothelial dysfunction, sustained inflammation, inhibition of cell death, and excessive activation of signaling pathways and transcription factors [11]. It has been suggested that hypoxia-induced pulmonary endothelial dysfunction may result in the exposure of underlying smooth muscle cells to proliferative mediators resulting in their abnormal proliferation [12]. Hypoxia causes PAECs to induce a vasoconstrictive environment through increased production of vasoconstrictor factor including ET-1 and decreased production of vasodilator factor such as nitric oxide (NO) [4]. ET-1 is a potent vasoconstrictor and promoter of pulmonary arterial smooth muscle cells proliferation with a crucial role in PAH [13]. It has been demonstrated that the levels of ET-1 are increased in the peripheral and pulmonary circulations of patients with PAH [13]. In addition, in vitro studies also demonstrate that exposure of PAECs to hypoxia causes the synthesis and secretion of inflammatory cytokines such as TNF-α and IL-6 [4]. Patients with PAH and animal models of PAH, increased circulating levels of TNF-α and IL-6 are observed. Moreover, the higher levels of TNF-α and IL-6 are associated with an increased risk of death in PAH patients [14]. It has been suggested that IL-6 promoted the development and progression of pulmonary vascular remodeling through pro-proliferative mechanisms [15]. Hypoxic IL-6 deficient mice showed less inflammatory cell recruitment in the lungs and reduced pulmonary vascular remodeling [16]. In addition, Rats treated with TNF-α blocker showed amelioration in pulmonary hemodynamics and right ventricular hypertrophy [17]. In the present study, we found that hypoxia increased the levels of ET-1, TNF-α and IL-6 in cell culture supernatant and upregulated ET-1, TNF-α and IL-6 mRNA levels in PAECs. However, ISL reduced the levels of ET-1, TNF-α and IL-6 in cell culture supernatant and prevented hypoxia-mediated ET-1, TNF-α and IL-6 mRNA induction.

HIF-1α as a key regulator in the cellular adaptation to hypoxia participates in many pathological processes including PAH. Exposure of PAECs to hypoxia causes increased HIF-1α protein levels and HIF-1α DNA-binding activity [18]. During the development of PAH, HIF-1α plays a critical by

transactivating its target genes, such as ET-1, IL-6, vascular endothelial growth factor (VEGF), endothelial and inducible nitric oxide synthase, and erythropoietin [19]. In the present investigation, we observed that HIF-1α was up-regulated by hypoxia at the mRNA levels in PAECs. However, ISL attenuated the increased expression of HIF-1α mRNA levels caused by hypoxia.

Our data show that ISL attenuates hypoxia-induced synthesis and secretion of inflammatory cytokines and vasoactive factors in PAECs. These findings indicate that ISL might have protective effects on hypoxia-induced PAECs dysfunction.

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Conflicts and Interest: The authors have declared that no competing interests exist.

References

[1] Bhogal S, Mukherjee D, Banerjee S, Tan W, Paul TK. Current Trends and Future Perspectives in the Treatment of Pulmonary Arterial Hypertension. Current problems in cardiology. 2017.

[2] Bazan IS, Fares WH. Pulmonary hypertension: diagnostic and therapeutic challenges. Therapeutics and clinical risk management. 2015;11:1221-33.

[3] Helan M, Aravamudan B, Hartman WR, Thompson MA, Johnson BD, Pabelick CM, et al. BDNF secretion by human pulmonary artery endothelial cells in response to hypoxia. Journal of molecular and cellular cardiology. 2014;68:89-97.

[4] Pugliese SC, Poth JM, Fini MA, Olschewski A, El Kasmi KC, Stenmark KR. The role of inflammation in hypoxic pulmonary hypertension: from cellular mechanisms to clinical phenotypes. American journal of physiology Lung cellular and molecular physiology. 2015;308:L229-52.

[5] Peng F, Du Q, Peng C, Wang N, Tang H, Xie X, et al. A Review: The Pharmacology of Isoliquiritigenin. Phytotherapy research : PTR. 2015;29:969-77.

[6] Wang Z, Wang N, Han S, Wang D, Mo S, Yu L, et al. Dietary compound isoliquiritigenin inhibits breast cancer neoangiogenesis via VEGF/VEGFR-2 signaling pathway. PloS one. 2013;8:e68566.

[7] Lee SH, Kim JY, Seo GS, Kim YC, Sohn DH. Isoliquiritigenin, from Dalbergia odorifera, up-regulates anti-inflammatory heme oxygenase-1 expression in RAW264.7 macrophages. Inflammation research : official journal of the European Histamine Research Society [et al]. 2009;58:257-62.

[8] Park SJ, Song HY, Youn HS. Suppression of the TRIF-dependent signaling pathway of toll-like receptors by isoliquiritigenin in RAW264.7 macrophages. Molecules and cells. 2009;28:365-8.

[9] Lee HK, Yang EJ, Kim JY, Song KS, Seong YH. Inhibitory effects of Glycyrrhizae radix and its active component, isoliquiritigenin, on Abeta(25-35)-induced neurotoxicity in cultured rat cortical neurons. Archives of pharmacal research. 2012;35:897-904.

[10] Wang L, Yang R, Yuan B, Liu Y, Liu C. The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb. Acta pharmaceutica Sinica B. 2015;5:310-5.

[11] Guignabert C, Dorfmuller P. Pathology and Pathobiology of Pulmonary Hypertension. Seminars in respiratory and critical care medicine. 2017;38:571-84.

[12] Burton VJ, Holmes AM, Ciuclan LI, Robinson A, Roger JS, Jarai G, et al. Attenuation of leukocyte recruitment via CXCR1/2 inhibition stops the progression of PAH in mice with genetic ablation of endothelial BMPR-II. Blood. 2011;118:4750-8.

[13] Ventetuolo CE, Kawut SM, Lederer DJ. Plasma endothelin-1 and vascular endothelial growth

factor levels and their relationship to hemodynamics in idiopathic pulmonary fibrosis. Respiration; international review of thoracic diseases. 2012;84:299-305.

[14] Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, et al. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. Circulation. 2010;122:920-7.

[15] Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. Circulation research. 2009;104:236-44, 28p following 44.

[16] Savale L, Tu L, Rideau D, Izziki M, Maitre B, Adnot S, et al. Impact of interleukin-6 on hypoxia-induced pulmonary hypertension and lung inflammation in mice. Respiratory research. 2009;10:6.

[17] Wang Q, Zuo XR, Wang YY, Xie WP, Wang H, Zhang M. Monocrotaline-induced pulmonary arterial hypertension is attenuated by TNF-alpha antagonists via the suppression of TNF-alpha expression and NF-kappaB pathway in rats. Vascular pharmacology. 2013;58:71-7.

[18] Shimoda LA, Semenza GL. HIF and the lung: role of hypoxia-inducible factors in pulmonary development and disease. American journal of respiratory and critical care medicine. 2011;183:152-6.

[19] Voelkel NF, Mizuno S, Bogaard HJ. The role of hypoxia in pulmonary vascular diseases: a perspective. American journal of physiology Lung cellular and molecular physiology. 2013;304:L457-65.