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Vasorelaxation effect of 18β-glycyrrhetinic acid on the thoracic aorta of rats: proposed mechanism

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Received August 11, 2019, accepted September 10, 2019

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Pharmazie 74: 751-754 (2019) *doi: 10.1691/ph.2019.9724*

18β-Glycyrrhetinic acid (18β-GA) is an effective component extracted from the traditional Chinese medicine *Radix glycyrrhizae* (Leguminosae) and has various biological activities. This study was performed to investigate the vasodilatory effects of 18β-GA on isolated rat thoracic aortic rings and explore the underlying mechanisms. The rings were obtained from normal Sprague–Dawley rats and then precontracted with norepinephrine (NE) (1 μM) or KCl (60 mM). 18β-GA (1.883–11.297 mg/L) was added successively by cumulative dosing to observe and record the changes in the tension of the vascular ring. The effects of NG-nitro-l-arginine methylester (L-NAME), indomethacin (INDO), barium chloride ($BaCl_2$), 4-aminopyridine (4-AP), tetraethylammonium (TEA), and glibenclamide on the vascular diastolic function of 18β-GA were determined. 18β-GA substantially exhibited a dose-dependent vasorelaxant effect on the NE-induced and KCl-induced contractions of the rings. The integrity of the vascular endothelium had no influence on the 18β-GA-induced vasorelaxation effect in the rings. L-NAME and IDON showed no significant differences in their effects on this vasorelaxation process in the rings precontracted with NE. This result suggests that the vasorelaxation mechanism of 18β-GA may be independent of the vascular endothelium. $BaCl_2$ and 4-AP antagonized the vasorelaxation effect of 18β-GA, but TEA and glibenclamide showed no remarkable effect on the vasodilation of 18β-GA. Findings suggest that 18β-GA induces vasorelaxation in thoracic aortic rings via the receptor-operated Ca^{2+} channels and voltage-operated Ca^{2+} channels and the opening of inward rectifier potassium channels and voltage-operated potassium.

1. Introduction

Hypertension, which is considered a vital risk factor for developing other diseases such as stroke and pulmonary hypertension, is a serious threat to human health by causing cardiovascular diseases (Greenberg et al. 2005; Grubler et al. 2017; Mattson 2019). An increasing number of studies have shown that vasoconstriction and vasorelaxation play a vital role in the development of hypertension (Zicha et al. 2014; Sun et al. 2011). Although many vasodilators are commercially available, they are limited by their adverse effects and patient compliance (Krum and Pellizzer 1998; McComb et al. 2016). Therefore, the search for ideal antihypertensive drugs has far-reaching research significance.

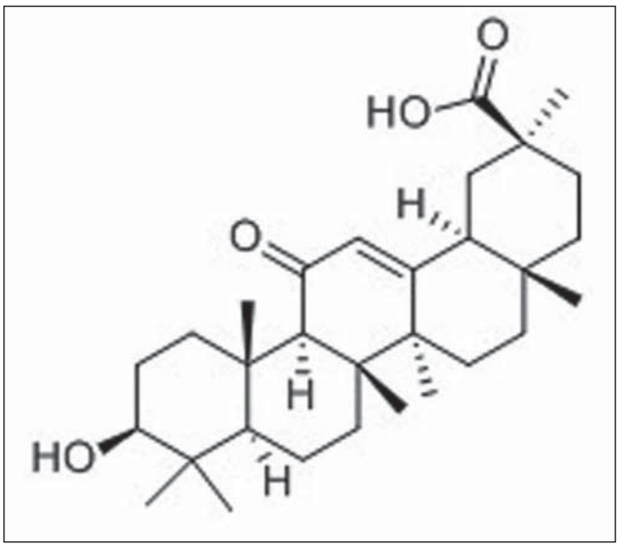

Fig. 1: Chemical structure of 18β-glycyrrhetinic acid

In the past few decades, traditional Chinese medicine (TCM) has been shown to have advantages over other forms of medicine; such advantages include its vast sources, low toxicity, few side effects, low cost, and easy acquisition (Yang et al. 2018). Therefore, TCM has become a new prospect in the exploration of effective vasodilators in natural herbs. 18β-Glycyrrhetinic acid (18β-GA, Fig. 1) is an effective component extracted from TCM *Radix glycyrrhizae* (Leguminosae) and has anti-cancer, anti-inflammation, and antioxidant effects (Zhang et al. 2019; Kong et al. 2015; Zong et al. 2013). Research proves that an ethanolic extract from glycyrrhizae has the strongest vasorelaxation activity, which relaxes the thoracic aorta rings precontracted by norepinephrine (NE) (Tan et al. 2017). On the other hand, previous studies showed that 18β-GA, which is a gap junction inhibitor, inhibited KCl- and phenylephrine(PE)-induced contraction of isolated rat interlobular arteries (Hang et al. 2014). However, the effect of 18β-GA, as a major component of glycyrrhizae, on the thoracic aorta has not been reported.

In this study, we investigated the diastolic effect of 18β-GA in isolated rat thoracic aorta rings and its related mechanism.

2. Investigations and results

2.1. Effect of 18β-GA concentration on basal tension isolated thoracic aortic rings in rats

Compared with the control group, 18β-GA significantly constricted the thoracic aorta (P<0.01; Fig. 2) in the experimental group in a dose-dependent manner.

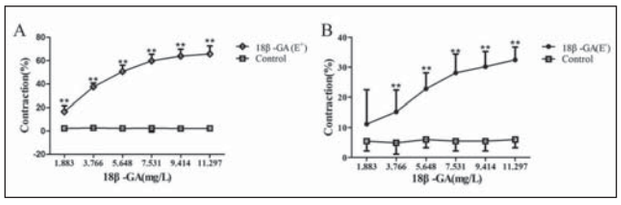

Fig. 2: Effect of various concentrations of 18β-GA on untreated isolated rat thoracic aortic rings: (A) Concentrations effect of 18β-GA on isolated rat endothcllium-intact (E+) thoracic aortic rings; (B) Concentrations effect of 18β-GA on isolated rat endothelium-denuded (E") thoracic aortic rings; Data are expressed as the mean±SEM. 18β-GA: 18β-Glycyrrhetinic acid. (n=6)

2.2. Effects of 18β-GA on the relaxation induced by NE and KCl on the isolated thoracic aortic rings of rats

In this study, 18β-GA had a dose-dependent vasorelaxant effect on NE-induced and KCl-induced contraction in the isolated thoracic aorta rings (P<0.05 and P<0.01, respectively; Fig. 3) unlike in the control group. These results indicate that 18β-GA could diastole the effects of NE-induced and KCl-induced relaxation on isolated rat thoracic aortic rings.

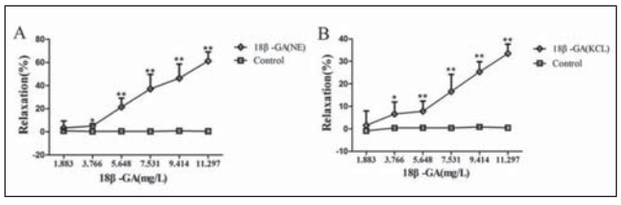

Fig. 3: Relaxation effect of 18β-GA on NE and KCl pre-contracted isolated rat thoracic aortic rings. (A) The value of tension of 18β-GA on NE pre-contracted rat thoracic aortic rings; (B) The value of tension of 18β-GA on KCl pre-contracted rat thoracic aortic rings; Data are expressed as the mean±SEM. 18β-GA: 18β-Glycyrrhetinic acid. (n=6)

2.3. Effect of 18β-GA on the relaxation induced by NE on the isolated thoracic aortic rings of endothelium-denuded rat

No significant difference was found in the effects of 18β-GA on the relaxation induced by NE between the endothelium-intact and endothelium-denuded isolated rat thoracic aortic rings (P>0.05; Fig. 4). This result illustrates that 18β-GA could simultaneously diastole the endothelium-intact or endothelium-denuded thoracic aorta after precontraction by NE.

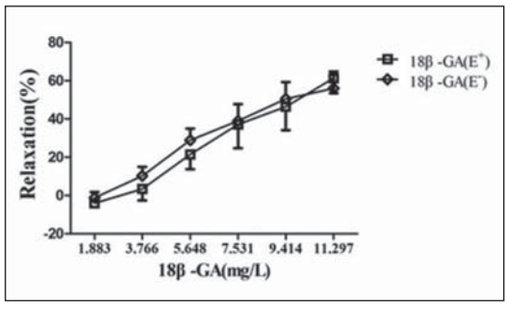

Fig 4: Relaxation effect of 18β-GA on isolated rat endothelium-intact (E^+) and endothelium-denuded (E^-) thoracic aortic rings. Data are expressed as the mean±SEM. 18β-GA: 18β-Glycyrrhetinic acid. (n=6)

2.4. Vasorelaxation effects of NG-nitro-L-arginine methylester (L-NAME) and indomethacin (INDO) on 18β-GA in the isolated thoracic aortic rings

Compared with the relaxation induced by 18β-GA without L-NAME, a NOS inhibitor, and INDO, a COX inhibitor, no significant difference was found in the relaxation induced by 18β-GA in the isolated thoracic aortic rings precontracted with NE after incubation with L-NAME and INDO for 20 min (P>0.05; Fig. 5).

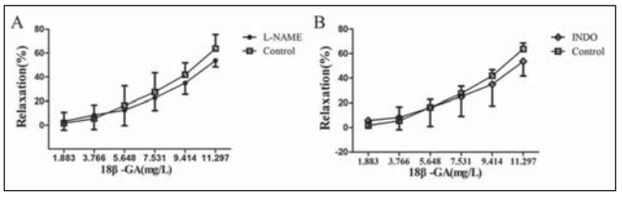

Fig 5: Relaxation effect of 18β-GA in isolated rat thoracic aortic rings in the presence of L-NAME (A) and INDO (D). Data are expressed as the mean±SEM. 18β-GA: 18β-Glycyrrhetinic acid. (n=6)

2.5. Effects of K+ channels on 18β-GA-induced relaxation in the isolated rat thoracic aortic rings

In the thoracic aortic rings, $BaCl_2$, an inward rectifier potassium (K_{ir}) channel inhibitor, and 4-aminopyridine (4-AP), a voltage-operated potassium (K_v) channel inhibitor, antagonized the vasorelaxation of 18β-GA (both P<0.05 and P<0.01; Figs. 6C and D, respectively). By contrast, tetraethylammonium (TEA), a Ca^{2+}-activated potassium (K_{ca}) channel inhibitor, and glibenclamide, an ATP-sensitive potassium (K_{ATP}) channel inhibitor, showed no significant effect on the vasodilation of 18β-GA (P>0.05; Figs. 6A and B, respectively). These results indicate that K_{ir} and K_v channels apparently play a role in vascular action, whereas the K_{ca} and K_{ATP} channels do not.

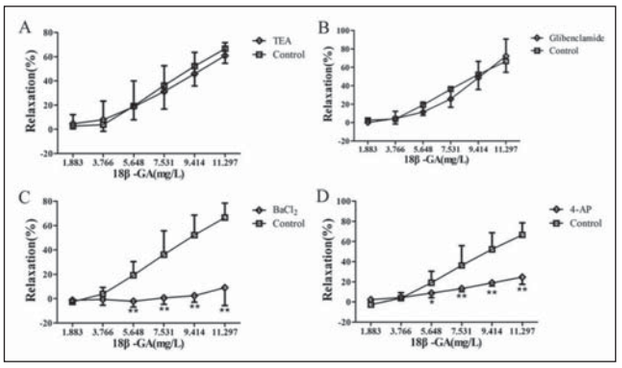

Fig 6: Relaxation effect of 18β-GA in isolated rat thoracic aortic rings in the presence of TEA (A), Glibenclamide (B), $BaCl_2$ (C) and 4-AP (D). Data are expressed as the mean±SEM. 18β-GA: 18β-Glycyrrhetinic Acid. (n=6)

3. Discussion

TCM *Radix glycyrrhizae* (Leguminosae) is widely used to treat various diseases in ningxia as a genuine TCM, because it can be planted abundantly and easily obtained (Zhang et al. 2019; Zong et al. 2013). 18β-GA is one of the main components in the commonly used *Radix glycyrrhizae*. In this study, 18β-GA showed a vasoconstriction effect on the isolated rat thoracic aorta and different degrees of vasorelaxation effect on NE-induced and KCl-induced contractions. Therefore, the role of 18β-GA in raising and stabilizing blood pressure in clinical application is related to its regulation of vascular tension. It is reported that vasorelaxation plays a vital role in treatment in the development of hypertension (Zicha et al. 2014; Sun et al. 2011; Greaney et al. 2017). Therefore, this study aimed to explore the vasodilation effect of 18β-GA on the

isolated thoracic aortic rings in rats to provide experimental data to screen for antihypertensive drugs.

At present, models for NE-induced and KCl-induced thoracic aortic contraction are currently used for drug screening and related mechanism research on cardiovascular diseases such as hypertension (Arsyad and Dobson 2016; Bai et al. 2017; Tan et al. 2017, Yan et al. 2015). The physiological activities of the vascular smooth muscle are important physiological mechanisms for regulating vasoconstriction and vasorelaxation (Bai et al. 2017; Lee et al. 2015). The vascular smooth muscle is generally believed to contract through electromechanical or pharmacomechanical coupling, and accumulation of intracellular Ca^{2+} concentration is a vital factor in these couplings (Bai et al. 2017; Lee et al. 2015; Somlyo and Somlyo 1968; Yang et al. 2018; Yin et al. 2016). Pharmacomechanical coupling is related to the activation of α-adrenergic receptors on the cell membrane to increase the extracellular Ca^{2+} influx via the receptor-operated Ca^{2+} channel (ROCC). Meanwhile, electromechanical coupling can increase extracellular Ca^{2+} influx via the voltage-operated Ca^{2+} channel (VOCC) (Ogut and Brozovich 2003; Yan et al. 2015; Yang et al. 2018). NE, an α-adrenergic agonist, contracts the vascular smooth muscle cells by inducing the extracellular Ca^{2+} influx and intracellular Ca^{2+} release through the ROCCs (Silswal et al. 2011; Takahashi and Berk 1998). KCl induces the contraction of vascular smooth muscle mainly by inducing a large amount of intracellular Ca^{2+} influx to increase the intracellular Ca^{2+} concentration, which is related to the membrane depolarization and subsequent VOCC opening. The present findings show that 18β-GA caused a dose-dependent vasorelaxant effect on the NE-induced and KCl-induced contraction in the isolated thoracic aorta rings. Thus, 18β-GA may have also inhibited the vasocontraction that was induced by selectively interfering with the ROCCs and VOCCs.

Numerous studies have shown that the stability of the endothelial function plays an important role in maintaining vascular activity and normal physiological function, such as vascular tension (Gan et al. 2016; Qu et al. 2015; Stankevicius et al. 2003). Endothelial cells regulate vascular tension by secreting vasodilating factors, such as NO and prostaglandin (PGs), and contractile factors, such as endothelin-1 (Triggle et al. 2012; Moncada et al. 1991; Yang et al. 2012). NO produced by l-arginine catalyzed by NO synthetase (NOS), an endothelium-dependent vasodilator, can activate guanylate cyclase to synthesize cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle cells. cGMP can also reduce the Ca^{2+} influx and then relax the blood vessel (Triggle et al. et al. 2012, Hoffmann et al. 2015). As a competitive inhibitor of NOS, L-NAME can block the biological effect by inhibiting the synthesis of NO to promote Ca^{2+} influx (Triggle et al. 2012; Zhang et al. 2016; Moncada et al. 1991). PGI_2 is an inhibitor of platelet aggregation and an effective vasodilator. INDO is an inhibitor of cyclooxygenase (COX), which is one of the PGI_2 synthases (Triggle et al. 2012; Salahdeen et al. 2016; Yang et al. 2012). The results suggest that 18β-GA had a vasoconstriction effect on the endothelium-intact or endothelium-denuded thoracic aorta of isolated rats. No significant difference was found in the effects of 18β-GA on the relaxation induced by NE between the endothelium-intact and endothelium-denuded isolated rat thoracic aortic rings. 18β-GA showed a dose-dependent vasorelaxation effect on the endothelium-denuded isolated rat thoracic aortic rings precontracted with NE. No significant difference was found in the effects of L-NAME and IDON on the vasorelaxation by 18β-GA in the isolated thoracic aortic rings precontracted with NE. These results illustrated that the two inhibitors did not influence the effects of 18β-GA on the tension of the vascular ring. Thus, the vasoconstriction and vasorelaxation mechanisms of 18β-GA may be independent of the vascular endothelium.

In addition to the Ca^{2+} channels, such as ROCC and VOCC, the K^+ channels are crucial factors in the regulation of the contraction and relaxation of the vascular smooth muscle, and endothelium-independent vasodilatation has been associated with K^+ channels (Khan et al. 2012; Chen et al. 2019; Gan et al. 2016; Ko et al. 2008; Silswal et al. 2011). Therefore, we investigated the vasorelaxation effect of K^+ channel-related drugs on 18β-GA in the NE preconditioning of the thoracic aorta. K^+ channels regulate muscle contraction and vascular tension not only by maintaining the resting membrane potential but also by influencing the opening and closing of the Ca^{2+} channels (Testai et al. 2016; Yang et al. 2012). Activation of the K^+ channels in vascular smooth muscle cells leads to the hyperpolarization of the cell membrane, which in turn inhibits the extracellular Ca^{2+} influx and ultimately inhibits the contraction of the smooth muscle (Chen et al. 2019; Deng et al. 2012). As is well known, four K^+ channels are existing, namely, inward rectifier potassium (K_{ir}) channels, voltage-operated potassium (K_v) channel inhibitor, Ca^2+-activated potassium (K_{ca}) channels, and ATP-sensitive potassium (KATP) channels (Stankevicius et al. 2003). These channels have been extensively investigated worldwide. The corresponding inhibitors of these channels are $BaCl_2$, 4-AP, TEA, and glibenclamide (Ko et al. 2008; Stankevicius et al. 2003; Testai et al. 2016). In this work, K_{ca} channels inhibitor TEA and K_{ATP} channels inhibitor glibenclamide did not significantly affect the vasodilation of 18β-GA. On the contrary, Kir channels inhibitor $BaCl_2$ not only has antagonized the vasorelaxation effect of 18β-GA, K_v channels inhibitor 4-AP has antagonized the vasorelaxation of 18β-GA. Therefore, the above data suggest that K_{ir} and K_v channels seem to play a role in vasodilation effect of 18β-GA.

This experiment systematically confirmed the biological effect of 18β-GA on isolated thoracic aortic rings, that is 18β-GA had a vasoconstriction effect on the isolated thoracic aortic rings and had different degrees of vasorelaxation effect on NE-induced and KCl-induced contraction. The possible mechanisms of vasodilation of 18β-GA may be the inhibition of Ca^{2+} influx by ROCC and VOCC and the opening of K_{ir} and K_v channels in the isolated thoracic aortic rings. The vasoconstriction and vasorelaxation mechanisms of 18β-GA might be independent of the vascular endothelium in the aortic rings. Although the mechanisms of 18β-GA and diastolic blood vessels still need further study, this study could provide necessary laboratory data for 18β-GA in the treatment of cardiovascular diseases such as hypertension.

4. Experimental

4.1. Animals and reagents

Sprague–Dawley (SD) rats (220–320 g) were purchased from the experimental Animal Center of Ningxia Medical University. All experimental rats had been reviewed and approved by the Animal Experimental Committee Ningxia Medical University (certificate no. SYXK Ningxia 2015-0001). The rats were kept in a temperature-controlled room (12 h light–dark cycles) and had free access to food and water.

18β-GA (with HPLC purity of 98%) was purchased from Shanghai Yuan Ye Biotechnology Chemical Company (Shanghai, China). NE was purchased from Grand Pharmaceutical China Company (Wuhan, China). L-NAME and glibenclamide were purchased from Ron Reagent Company (Shanghai, China). TEA was purchased from Bide Pharmatech Company (Shanghai, China). $BaCl_2$ was purchased from Sigma Chemical Company (MO, USA). 44-AP was purchased from Tokyo Chemical Industry (Tokyo, JP). INDO and acetylcholine (ACh) was purchased from Beijing Suo Lai Bao Technology Company (Beijing, China). KCl was supplied by Mingshen Pharmaceutical Company (Zhejiang, China). Sodium hydrogen carbonate ($NaHCO_2$), glucose, calcium chloride hexahydrate ($CaCl_2 2H_2O$), magnesium sulfate anhydrous ($MgSO_4 7H_2O$), and sodium chloride (NaCl) were supplied by Damao Chemical Reagent Factory (Tianjing, China). BL-420s Biological Functional System (Chengdu Technology & Market Company, Sichuan, China) was used to transduce the biological signals and record and analyze the experimental data.

Isolated thoracic aorta rings from the rats were retained in a Krebs–Henseleit solution (K–H solution) (6.92 g of NaCl; 0.35 g of KCl; 2.21 g of $NaHCO_2$; 0.28 g of $CaCl_2{\cdot}2H_2O$; 0.16 g of KH_2PO_4; $MgSO_4 7H_2O$; 0.29 g of glucose; pH 7.4, temperature 37 °C) with the mixed standard gas of 95% O_2 and 50% CO_2.

4.2. Preparation and screening of the isolated thoracic aortic rings

Male SD rats were euthanized by intraperitoneal injection of 20% urethane (100 mg/kg) before the removal of the isolated thoracic aortic rings. After the rats were anesthetized completely, the isolated thoracic aortic rings were immediately removed and cleaned in K–H solution at 37 °C. Then, the rings were carefully cut transversely into rings with a diameter of about 3–4 mm. The rings were placed in a 20 mL bath tank preheated to 37 °C fixed with an m-type hook at one end and the other end through the tension transducer connected with a biological acquisition system.

After stabilization for 20 min, the rings were subjected to a constant resting tension of 2.0 g for at least 60 min, with the K–H solution replaced every 20 min. The rings were challenged with 60 mM KCl to check the functional integrity of the isolated thoracic aortic rings (maximum contraction amplitudes <5%). To validate the endothelial

integrity, the rings were precontracted with NE (1 μM), followed by the relaxation with Ach (1 μM). The rings could be regarded as endothelially intact rat thoracic aorta rings with a relaxation of approximately 80%.

4.3. Vasodilatory effects of the cumulative concentration of 18β-GA on the isolated thoracic aortic rings

Endothelial and fully functional isolated rat thoracic aortic rings that were not pretreated were challenged to a cumulative concentration of 18β-GA (1.883–11.297 mg/L). The rings were contracted steadily using NE (1 μM) or KCl (60 mM), and the same volume of vehicle used in the administration with 18β-GA was added to the control group. Afterwards, the vascular tension of the endothelial intact ring was recorded.

4.4. Vasodilatory effects of 18β-GA in the endothelium-denuded rat thoracic aorta rings

To investigate the role of the endothelium in the vasorelaxation to 18β-GA, the rings with intact or without endothelium integrity were contracted steadily using NE (1 μM). Increasing concentrations of 18β-GA (1.883–11.297 mg/L) were added cumulatively to the K–H solution.

4.5. Effects of L-NAME and INDO on the vasorelaxation of 18β-GA in isolated thoracic aortic rings

After the isolated thoracic aortic rings were stabilized for 1 h in 37 °C K–H solution, the rats were divided into experimental and control groups. In the experimental group, the rings were challenged to L-NAME (0.1 mM) and INDO (1×10^{-3} mM) for 20 min, and NE (1 μM) was applied to precontract the rings. After stable vasoconstriction was reached, 18β-GA (1.883–11.297mg/L) or K–H solution was added cumulatively. The vasorelaxation rate of each group was calculated according to the amplitude of the vascular tension induced by NE or maximum contraction amplitude (100%) induced by KCl after drug administration, and the cumulative mass concentration-diastolic rate curve was drawn.

4.6. Effect of K+ channel on the vasorelaxation by 18β-GA on isolated thoracic aortic rings

To explore the vasorelaxation effect of K^+ channel on 18β-GA, the rings were incubated with TEA (10 mM), 4-AP (5 mM), $BaCl_2$ (1 mM), and glibenclamide (0.01 mM) for 20 min after precontraction with NE (1 μM). After stable vasoconstriction was reached, 18β-GA (1.883–11.297 mg/L) or K–H solution was added cumulatively.

4.7. Statistical analysis

The data were analyzed using SPSS 24.0 statistical software and presented as mean±SEM. The independent sample t-test was used to compare two samples. In all statistical tests, $P < 0.05$ was considered statistically significant.

Acknowledgements: This research was funded by "2017 Ningxia Hui Autonomous Region Science and Technology Innovation Leader Training Project, grant number KJT2017005" , "2017 Ningxia Medical University Youth Backbone Talent Cultivation Selected Project'', "Development of Glycyrrhetinic Acid on Pulmonary Hypertension (Ningxia Key R&D Program Project Foreign Science and Technology Cooperation Special, grant number 2018BFH03019)" and "Mechanism, Safety Evaluation and Preparation Development of Glycyrrhetinic Acid in the Treatment of Pulmonary Hypertension (Ningxia Key R&D Program Project Foreign Science, grant number 2019BFG02027)".

Conflict of Interest: The authors declare no conflict of interest.

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