

# Effects of Qibaipingfei capsules on pulmonary vascular relaxation through $K_{ATP}$ channel activation by the NO/cGMP signaling pathway

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**Abstract:** This research explores the effects of Qibaipingfei (QBPF) capsules on pulmonary vascular relaxation *in vitro* and the relationship of the ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channel and nitric oxide (NO) pathway. Vasodilator effects of QBPF (0.125-2 g/kg) on rat pulmonary artery rings were observed using a multi-wire myograph system. The maximum relaxation ( $E_{max}$ ) of QBPF was detected following treatment involving endothelial denudation, N $\omega$ -nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4] oxadiazolo[4,3- $\alpha$ ]quinoxalin-1-one (ODQ), or glyburide (GLYB). Furthermore, rat models of phlegm and blood stasis syndrome combined with chronic obstructive pulmonary disease (COPD) were established using compound factors. KIR6.1 and SUR2B protein expression was analyzed by western blotting. After 9,11-dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxy-methanoprostaglandin $F_{2\alpha}$  (U46619) was used to pre-constrict endothelium-intact pulmonary artery rings, QBPF induced the effects of concentration-dependent relaxation at a concentration for 50% of maximal effect ( $EC_{50}$ ) of 0.56 g/L and  $E_{max}$  of  $84.30\% \pm 6.27\%$ . After the endothelium was denuded, the vasodilator effects reduced significantly ( $P < 0.01$ ). QBPF-induced relaxation was inhibited by L-NAME, ODQ, and GLYB ( $P < 0.01$ ). The vasodilator effect was also attenuated in the model group ( $E_{max} = 62.63\% \pm 10.02$ ,  $EC_{50} = 0.72$  g/L,  $P < 0.01$ ). In comparison with expression in the control group, SUR2B protein expression was down-regulated in the model group ( $P < 0.01$ ) but no significant difference was detected in KIR6.1 protein expression between the groups ( $P > 0.05$ ). QBPF and nicorandil (Nic) treatment up-regulated SUR2B  $K_{ATP}$  channel expression ( $P < 0.05$ ). QBPF induces endothelial-dependent relaxation in pulmonary artery rings *in vitro*, through a mechanism that potentially activates the  $K_{ATP}$  channel in pulmonary vascular smooth muscles via the NO-cyclic GMP (cGMP)-dependent pathway.

**Keywords:** Chronic obstructive pulmonary disease, Nitric oxide, ATP-sensitive  $K^+$  channel, Vascular relaxation, QBPF capsules.

## INTRODUCTION

Pulmonary arterial hypertension (PAH), a chronic and progressive disease, is a key pathological stage of chronic obstructive pulmonary disease (COPD) characterized by an increase in pulmonary vascular resistance, pulmonary vascular remodeling, and endothelial dysfunction leading to pulmonary heart disease and even death (Montani *et al.*, 2013; Shino *et al.*, 2013; Kloza *et al.*, 2014). To date, no effective treatment has been developed to adequately resolve the condition.

Traditional Chinese medicine holds that COPD belongs to the syndromes of root deficiency and branch excess, and that “Qi deficiency, phlegm and blood stasis” is the key pathogenesis of PAH development. Therapy for nourishing Qi, eliminating phlegm, and resolving stasis (Yiqi-Huatan-Quyu treatment) is the standard therapeutic strategy for COPD. The Yiqi-Huatan-Quyu recipe, the Qibaipingfei (QBPF) capsule, is composed of Huangqi (*Radix Astragali Mongolici*), Renshen (*Radix Ginseng*), Chuanxiong (*Szechwan Lovage Rhizome*), Xiebai (*Bulbus Allii Macrostemonis*), Tinglizi (*Semen Lepidii Apetali*), Wuweizi (*Fructus Schisandrae Chinensis*), and Dilong

(*Pheretima Aspergillum*), and was developed according to the therapeutic aim described above (patent no. ZL201010573274.1). Our previous studies showed that QBPF has a remarkable curative effect on COPD. QBPF effectively relieved mean pulmonary artery pressure (mPAP), inhibited the over-expression of endothelin-1 (ET-1), and increased the expression of nitric oxide (NO), through a molecular mechanism that might be related to the NO pathway.

The ATP-sensitive  $K^+$  channel ( $K_{ATP}$  channel) is a unique construction of four inward rectifier potassium channel (Kir) subunits and an equal number of sulfonylurea receptor (SUR) subunits (Kang *et al.*, 2012; Seino and Miki, 2003; Chowdhury *et al.*, 2013). The opening and closing of  $K_{ATP}$  channels is regulated by Kir inhibition or SUR subunit activation (Ma *et al.*, 2014). According to the various subtypes of SUR (SUR1, 2A, and 2B) and Kir (Kir6.x), the composition of  $K_{ATP}$  channel exhibits considerable heterogeneity across different tissues. Studies have indicated that SUR2B and Kir6.1 subunits are the main components of pulmonary artery smooth muscle potassium channels in rats or humans (Cao *et al.*, 2002; Cui *et al.*, 2002; Ko *et al.*, 2008).  $K_{ATP}$  channel openers are a therapeutic target for COPD pulmonary hypertension and other diseases (Li *et al.*, 2013; Dong *et al.*

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*al.*, 2014; Zhu *et al.*, 2015). Some studies have indicated the  $K_{ATP}$  channel as the common link to the NO pathway, as playing a key role in pulmonary vascular proliferation and reconstruction, and having a close relationship with the development and prognosis of pulmonary hypertension (Sattiraju *et al.*, 2008; Malerba *et al.*, 2010; Marinko *et al.*, 2015). In contrast, some researchers have suggested that the  $K_{ATP}$  channel-dependent effect is independent of the NO-cyclic guanosine monophosphate (cGMP)-dependent pathway (Meisheri *et al.*, 1991; Perez-Vizcaíno *et al.*, 1998). It remains unclear whether  $K_{ATP}$  channel opens function directly through or independently of cGMP in pulmonary arterial smooth muscle cells. Therefore, this study focused on the effects of endothelial NO and  $K_{ATP}$  channels on QBPF-induced vasodilatation of pulmonary artery rings to explore the regulatory mechanism of QBPF on pulmonary vasoconstriction and the correlation between  $K_{ATP}$  channels and the NO-cGMP-dependent pathway.

## MATERIALS AND METHODS

### *Animals and drugs*

One hundred and ten specific pathogen-free (SPF) Sprague-Dawley rats (50 for *in vitro* studies on pulmonary artery rings and 60 for *in vivo* studies) of weight  $200 \pm 20$  g were supplied by the Laboratory Animals Center (Anhui province, China; certificate of quality no: scxk (Wan) 2011-002). Rats were housed in the Experimental Animal Center of Anhui University of Chinese Medicine under controlled temperature ( $22 \pm 1^\circ\text{C}$ ) and humidity ( $55\% \pm 5\%$ ) conditions. QBPF was provided by the pharmacy department of the First Affiliated Hospital of Anhui University of Chinese Medicine. Nicorandil capsules (for *in vivo* use) were purchased from Chugai Pharmaceutical Company. Capsule contents were ground to an ultrafine powder and dissolved in physiological saline solution (PSS) to prepare the stock solution of 100% (i.e., 1.0g powder per milliliter of PSS).

### *In vitro pulmonary vascular ring preparation*

The three-level branch of the right pulmonary artery (100-150  $\mu\text{m}$ ) was dissected out and surrounding connective tissues were removed under stereoscopic dissecting microscopy (SMZ-168, Motic) using  $4^\circ\text{C}$  PSS solution aerated with oxygen and carbon dioxide (95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ). Each pulmonary artery was cut into 2-mm-long sections of the vascular ring, which was mounted between two parallel tungsten wires (40  $\mu\text{m}$ ) fixed in a multi-wire myograph system (DMT620M, Denmark). Changes in vascular tone were traced continuously by a polygraph chart recorder. The bathing solution at  $37 \pm 1^\circ\text{C}$  was aerated with a mixture of oxygen and carbon dioxide mixture (95% $\text{O}_2$ , 5% $\text{CO}_2$ ) as before. After a 40 min equilibration period, vascular activity of pulmonary artery rings was detected using 60 nM KPSS for pre-constriction

and 10  $\mu\text{M}$  acetylcholine (Ach, Sigma) as a vasodilator. If the vasodilation rate was more than 85%, the integrity of endothelial function was validated. When the tissue did not relax or when the diastolic amplitude difference was less than 10%, the endothelial cells were demonstrated to have completely disassociated from the vessel.

### *Pulmonary vascular tone in vitro*

The vasodilator effects of QBPF were investigated in pulmonary artery rings. First, a steady vascular tone was investigated by the addition of 10 nM 9,11-dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxy-methanoprostaglandin $\text{F}_{2\alpha}$  (U46619, Sigma), and vasodilatation effects were detected by the cumulative application of QBPF (0.125, 0.25, 0.5, 1, or 2 g/L) to pulmonary artery rings with or without endothelium in the control group. Notable reduction in relaxation was observed in endothelium-denuded pulmonary artery rings following QBPF treatment. Second, an inhibitor of NO-mediated vasodilatation was used to verify the influence of QBPF on endothelial function in endothelium intact pulmonary artery rings. Rings were incubated for 30 min with 30 $\mu\text{M}$  N $\omega$ -nitro-L-arginine methyl ester (L-NAME, Sigma), a noncompetitive inhibitor of NO synthase, or 10  $\mu\text{M}$  1H-[1,2,4]oxadiazolo[4,3- $\alpha$ ]quinoxalin-1-one (ODQ, Sigma), a selective inhibitor of guanylate cyclase, before the addition of U46619. When steady tone was established, QBPF was added cumulatively to the bathing solution. Next, the putative  $K_{ATP}$  channel blocker was added to examine its effect on QBPF-induced vasodilatation in pulmonary artery rings with or without endothelium. Pulmonary vascular rings were incubated for 30 min with 10 $\mu\text{M}$  glyburide (GLYB, Sigma), a blocker of the  $K_{ATP}$  channel, and the relaxant effects of QBPF and nicorandil (Nic, Sigma), a  $K_{ATP}$  channel opener, were studied. Finally, the vasodilator effects of QBPF on endothelium-intact rings were further compared between the control group and the model group.

### *Grouping and modeling in vivo*

Following adaptive feeding for 1 week, rats were randomly assigned to six groups by a random number table: a normal control group (Control, n=10), a model group (Model, n=20, ten of which were assigned for *in vitro* studies) that underwent COPD modeling combined with the phlegm and blood stasis syndrome model, a high dose group (H-QBPF, n=10) treated with QBPF 1 g/kg, a middle dose group (M-QBPF, n=10) treated with QBPF 0.5 g/kg, a low dose group (L-QBPF, n=10) treated with QBPF 0.25 g/kg, and a positive control group (Nicorandil, n=10) treated with 10 mg/kg Nic. Each rat in a treated group underwent intragastric administration once a day for 4 consecutive weeks.

The rat model of COPD combined with phlegm and blood stasis syndrome was established using compound factors. According to the theory of "overstrain causing qi exhaustion", rats were forced to swim in a thermostatic

water bath ( $43 \pm 1^\circ\text{C}$ ) for 30 min each day. After the fur had dried, rats were placed in a smoking box (in-house construction) of one cubic meter in volume. An exhaust of 15 cm in diameter exited the top of the box and a central processing unit fan was suspended in the middle of the box to ensure even smoke distribution; 400 g of silicon dioxide desiccant was placed in the four corners of the box. Rats were placed into the smoking box, and smoke was discharged at a rate of 20 cigarettes per hour by the smoking equipment. Finally, rats were observed for 5 hours under atmospheric pressure and hypoxic conditions with controlled temperature ( $22\text{--}24^\circ\text{C}$ ), oxygen concentration ( $11\% \pm 0.5\%$ ), and carbon dioxide ( $0.03\%$ ). These modeling processes were performed consecutively for 4 weeks, at 6 days a week.

#### Determination of pulmonary function

Rats were anesthetized intraperitoneally using 10% chloral hydrate (10 ml/kg) before dissecting lung tissues, and forced expiratory volume in 0.3 second ( $\text{FEV}_{0.3}$ ), forced vital capacity (FVC), and  $(\text{FEV}_{0.3}/\text{FVC})\%$  were determined using a small animal pulmonary function analysis system (AniRes 2005, Beijing Bestlab High-Tech Co., Ltd.).

#### Western blotting

Proteins from lung tissues were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene fluoride (PVDF) membranes, dried, blocked, and incubated at  $4^\circ\text{C}$  overnight with one of the following primary antibodies: rabbit anti-Kir6.1 (Abcam, ab80972, 1:500), goat anti-SUR2B (Santa Cruz, sc5793, 1:500), or rabbit anti- $\beta$ -actin (Santa Cruz, sc130656, 1:500). After the membranes were washed three times with TBST, they were further incubated with secondary antibody for 1 hour and then washed; bound peroxidase was detected with the ECL kit (Thermo, 34094).

#### STATISTICAL ANALYSIS

Values were expressed as means  $\pm$  standard deviation (SD). The maximum response ( $E_{\text{max}}$ ) and the concentration required for 50% of maximal effect ( $\text{EC}_{50}$ ) were estimated. Graph Pad software (version 5.0) was used to establish and analyze the concentration–response curve for each pulmonary artery ring. Differences among groups were compared by one-way analysis of variance (ANOVA) with the Student-Newman-Keuls (SNK-q) test. Moreover, two-way ANOVA followed by Bonferroni's post hoc test was used to determine the difference between two concentration response curves. Statistical analysis of data was performed using SPSS10.0 software (SPSS, Chicago, IL, USA).  $P < 0.05$  was considered statistically significant.

## RESULTS

#### Macroscopic observation of modeling

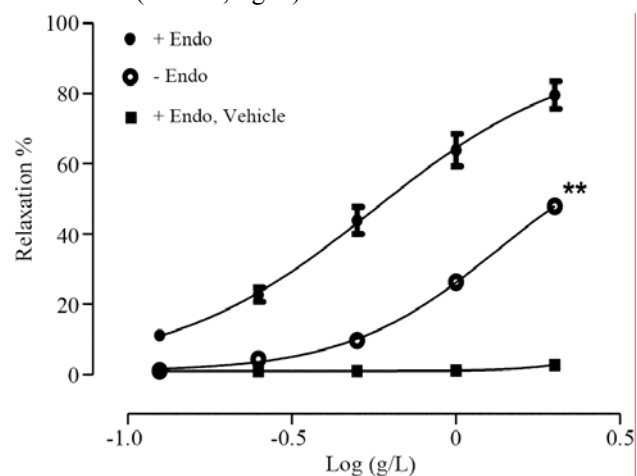
In rats that underwent modeling, respiratory symptoms such as sneezing, coughing, polypnea, and nasal and mouth secretions were observed, accompanied by reduced or no weight growth. Simultaneously, cyanosis of the lips, nose and onyx, accidie, and fur owing luster were also observed. While these symptoms were associated with rats in the H-QBPF group, animals in the M-QBPF and nicorandil groups improved after treatment. Symptoms of rats in the L-QBPF group fell somewhere between these groups.

#### Comparison of pulmonary function parameters

Compared with the control group, the values of  $\text{FEV}_{0.3}$ , FVC, and  $\text{FEV}_{0.3}/\text{FVC}$  in the model group were significantly decreased ( $P < 0.01$ ). Furthermore, the values of pulmonary function parameters significantly increased in the H-QBPF, M-QBPF, L-QBPF, and nicorandil groups compared with the model group ( $P < 0.01$ , table 1).

#### Relaxant effects of QBPF on U46619-induced pre-constriction

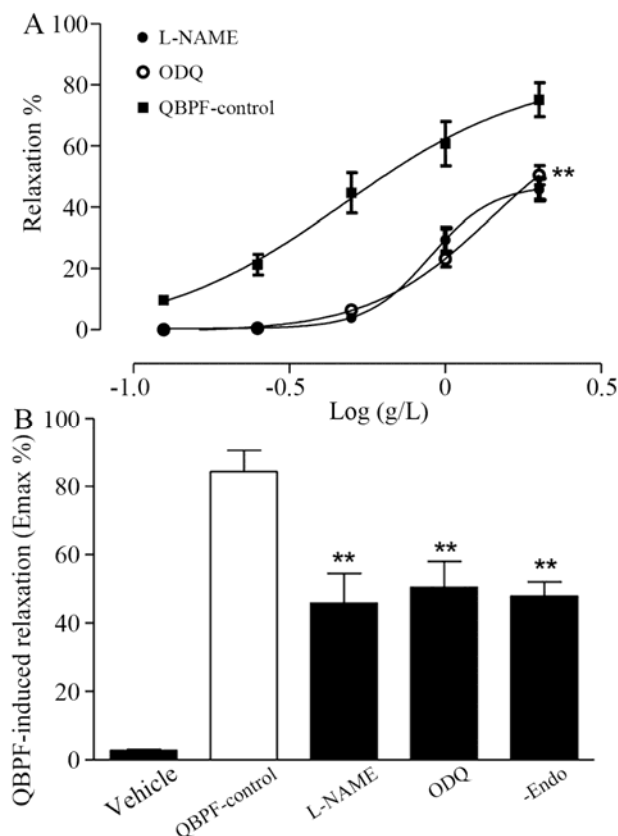
QBPF relaxed U46619-precontracted endothelium-intact pulmonary artery rings in a concentration-dependent manner with an  $\text{EC}_{50}$  of 0.56 g/L and  $E_{\text{max}}$  of  $84.30\% \pm 6.27\%$ . When pulmonary artery rings were endothelial-denuded, the magnitude of the vasorelaxation effect was significantly attenuated, with an  $\text{EC}_{50}$  of 1.33 g/L and  $E_{\text{max}}$  of  $47.91\% \pm 4.09\%$  ( $P < 0.01$ ). In addition, the vehicle had almost no vasorelaxation effect on U46619-induced vessel tone ( $P > 0.05$ , fig. 1).



**Fig. 1:** Concentration-response curves for QBPF-induced relaxation in endothelium-intact and -denuded pulmonary vascular rings pre-constricted with U46619. The effect of vehicle was used to observe on U46619-induced vessel tone. Results were expressed by mean  $\pm$  SD of 10 experiments. Vehicle had an insignificant effect on U46619-induced vessel tone ( $P > 0.05$ ). Statistical difference between curves were also demonstrated by two-way ANOVA (\*\* $P < 0.01$ ).

### Effects of L-name and ODQ on QBPF-induced vasorelaxation

To explore the role of NO-mediated vasorelaxation in pulmonary artery rings with intact endothelium, L-NAME and ODQ were used. The results indicated that L-NAME or ODQ incubation had a similar marked inhibition effect on QBPF-induced relaxation, with  $E_{max}$  of  $45.81\% \pm 8.72\%$  for L-NAME and  $50.42\% \pm 7.5\%$  for ODQ, compared with  $E_{max}$  of  $84.30\% \pm 6.27\%$  for control ( $P < 0.01$ , fig. 2a, b). Furthermore, these inhibitory effects had no notable difference compared with endothelium-denuded pulmonary artery rings ( $P > 0.05$ , fig. 2b).

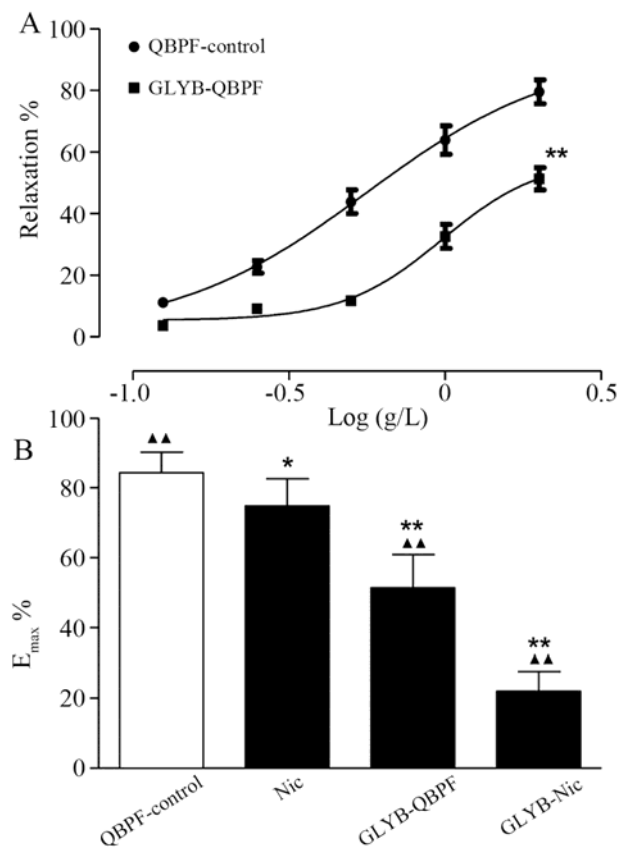


**Fig. 2:** Effects of  $30\mu\text{M}$  L-NAME or  $10\mu\text{M}$  ODQ on QBPF-induced relaxation in endothelium-intact rings pre-constricted with U46619 (a) and the maximum relaxation to QBPF under the treatment with endothelium-denudation, L-NAME or ODQ (b). Results were expressed by mean  $\pm$  SD of 6-10 experiments. Statistical difference from the control group is indicated by  $**P < 0.01$ . Statistical difference between curves were also proved by  $**P < 0.01$  (two-way ANOVA).

### Effects of $K_{ATP}$ channel blockers on QBPF-induced vasorelaxation

As a  $K_{ATP}$  channel opener, nicorandil ( $10^{-4}$  M to  $10^{-8}$  M) caused relaxation with an  $EC_{50}$  of  $3.31\mu\text{M}$  and  $E_{max}$  of  $74.81\% \pm 7.73\%$  in endothelium-intact rings. GLYB markedly attenuated nicorandil-induced vasorelaxation ( $P < 0.05$ , fig. 3b). These results validated the  $K_{ATP}$ -

mediated dilation and indicated a possible mechanism of  $K_{ATP}$  channel activation for QBPF-induced vasorelaxation. GLYB demonstrated inhibitory effects on QBPF-induced vasorelaxation in pulmonary artery rings with intact endothelium ( $P < 0.01$ ,  $E_{max} = 84.30\% \pm 6.27\%$  for control,  $51.34\% \pm 9.58\%$  for GLYB-QBPF; fig. 3).



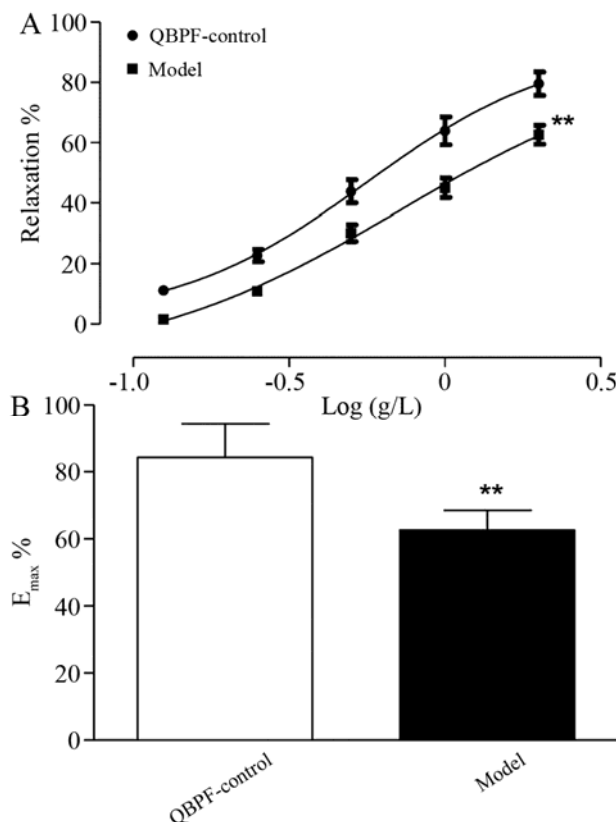
**Fig. 3:** Inhibitory effect of GLYB on QBPF-induced relaxation in endothelium-intact rings pre-constricted with U46619 (a) and the maximum relaxation to QBPF or Nicorandil under the treatment with GLYB (b). The inhibitory effect of GLYB was detected by comparison with the control group ( $*P < 0.05$ ,  $**P < 0.01$ ), and by comparison with the Nicorandil group ( $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$ ).

### QBPF-induced relaxation in model rat pulmonary vascular rings

To study vasorelaxation function in the model of phlegm and blood stasis syndrome combined with COPD, QBPF-induced vascular relaxation was used to compare differences between the control and model groups. We found that QBPF-induced vascular relaxation was diminished in pulmonary artery rings of the model group ( $P < 0.01$ ). The rate of vascular relaxation was  $22.22\%$  lower than that of the control group ( $E_{max} = 62.63\% \pm 10.02\%$  in model,  $84.30\% \pm 6.27\%$  in control;  $EC_{50} = 0.72$  g/L in model,  $0.56$  g/L in control; fig. 4).

### Expression of KIR6.1 and SUR2B in lung tissue

To further observe the effect of QBPF on  $K_{ATP}$  channels, the expression of KIR6.1 and SUR2B protein in each group was determined. The expression of SUR2B in the model group was down-regulated in comparison with that in the control group ( $P < 0.01$ ). Compared with expression in the model group, SUR2B expression was up-regulated in the QBPF and nicorandil treatment groups ( $**P < 0.01$  or  $*P < 0.05$ , fig. 5a, b). Kir6.1 expression in the model group was slightly decrease, but no significant difference was observed between each group ( $P > 0.05$ , fig. 5a, c).



**Fig. 4:** Vasorelaxation to QBPF in intact rings of model rats with phlegm and blood stasis syndrome of COPD (a) and the maximum relaxation to QBPF between the control group and the model group (b). QBPF-induced relaxation was attenuated in the model intact rings. Statistical difference between the control and model group was indicated by  $**P < 0.01$ .

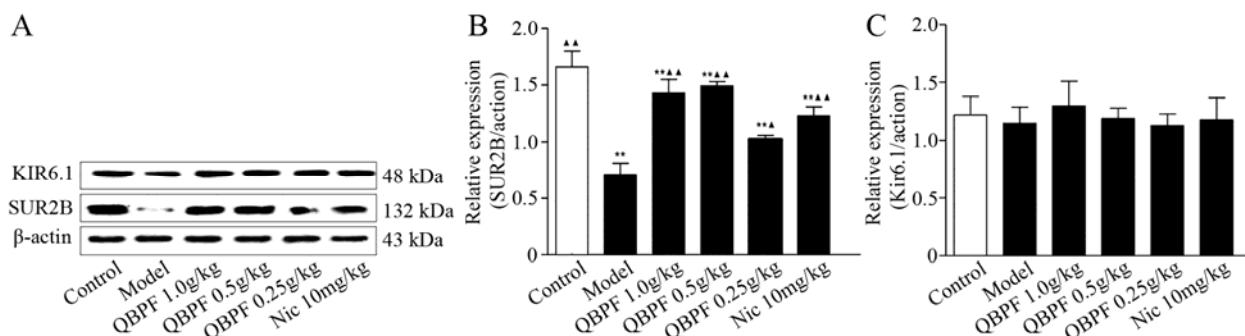
## DISCUSSION

Because of its effects of nourishing Qi, eliminating phlegm, and resolving stasis, QBPF capsules have long been used for the clinical treatment of COPD with phlegm and blood stasis syndrome. In this study, we further explored the role of endothelial NO and  $K_{ATP}$  channels in QBPF-mediated relaxation of pulmonary vessel rings and the expression of functional molecular targets for  $K_{ATP}$  channels.

Under physiological conditions, endothelial NOS (eNOS), located in the pulmonary vascular endothelium, is responsible for the majority of NO release and plays a pivotal role in maintaining endothelial morphology and function (Francis *et al.*, 2014). Reduced NO bioavailability contributes to endothelial dysfunction and leads to an increase in pulmonary artery blood pressure and the remodeling of the pulmonary vasculature (Li *et al.*, 2015; Wu *et al.*, 2015). The exogenous NO donor, L-arginine, can increase cellular cGMP levels via the activation of intracellular soluble guanylyl cyclase (sGC), thereby inhibiting cell proliferation, relaxing vascular smooth muscle, and ultimately having a critical effect on vasodilatation (Mitani *et al.*, 1997; Al-Hiti *et al.*, 2013; Ahmed *et al.*, 2014). Therefore, increase in the endogenous NO level could improve symptoms of hypoxic pulmonary hypertension and prevent pulmonary vascular remodeling (Sasaki *et al.*, 2000; Moncada and Higgs, 2006; Ahmed *et al.*, 2014). Our *in vitro* experiments in pulmonary artery rings demonstrate for the first time that QBPF plays an important role in vasodilatation in a concentration dependent manner in both endothelium-dependent and -independent artery rings. Meanwhile, relaxation by QBPF was clearly attenuated in the absence of endothelium. These results indicate the important function of endothelium in QBPF-induced relaxation of pulmonary artery rings.

An early study on NO showed that the appropriate level of NO promoted  $K_{ATP}$  channel opening in a concentration-dependent manner (de Carvalho Veloso *et al.*, 2015). As an opener of  $K_{ATP}$  channels, nicorandil has been shown to activate mitochondrial  $K_{ATP}$  channels, not only directly but also through a NO correlative pathway (Horimoto *et al.*, 2000; Kuno *et al.*, 2007; Tonelli *et al.*, 2013). In this study, similar reduction of diastolic function was verified after treatment with L-NAME, a noncompetitive inhibitor of NO synthase, and was also seen with ODQ, a selective inhibitor of guanylate cyclase (GC). Furthermore, GLYB, as a  $K_{ATP}$  channel blocker, also had an inhibiting effect on QBPF-induced vasorelaxation. These results suggest novel mechanistic links between QBPF-induced endothelium-dependent relaxation and  $K_{ATP}$  channels, primarily via the NO-cGMP dependent pathway.

Pulmonary function testing is commonly accepted as the gold standard for the diagnosis of COPD (Murphy and Panos, 2013; Mahboub *et al.*, 2014). Based on previous studies, the macroscopic observation of shortness of breath, tightness of chest, cyanosis of lips, nose and onyx, and decline in pulmonary function parameters indicated that a rat model of phlegm and blood stasis syndrome combined with COPD was established. Compared with the control group, and although the vasodilatation response of QBPF persisted in endothelium-intact pulmonary rings of the model group, a marked reduction in both vasodilatation sensitivity and potency for QBPF



**Fig. 5:** Western blot analysis of KIR6.1 and SUR2B expressions in intact rings of each group (a). The expression of SUR2B in the model group was down-regulated compared with the control group ( $**P<0.01$  or  $*P<0.05$ ), while QBPF and Nicorandil up-regulated the SUR2B expression compared to the model group ( $\Delta P<0.05$ ,  $\Delta\Delta P<0.01$ ) (b). The expression of KIR6.1 protein had no significant difference in all groups ( $P>0.05$ ) (c).

**Table 1:** Comparison of pulmonary functions

| Group      | Dose     | n  | FEV <sub>0.3</sub> (ml) | FVC (ml)    | FEV <sub>0.3</sub> /FVC (%) |
|------------|----------|----|-------------------------|-------------|-----------------------------|
| Control    | -        | 10 | 5.39±0.34**             | 5.72±0.53** | 94.64±5.88**                |
| Modeling   | -        | 10 | 1.70±0.22               | 3.02±0.14   | 56.29±7.41                  |
| H-QBPF     | 1.0g/kg  | 10 | 3.70±0.36**             | 4.71±0.45** | 78.94±8.34**                |
| M-QBPF     | 0.5g/kg  | 10 | 3.38±0.54**             | 4.54±0.40** | 75.32±15.47**               |
| L-QBPF     | 0.25g/kg | 10 | 2.76±0.34**             | 3.87±0.56*  | 67.44±10.91*                |
| Nicorandil | 10mg/kg  | 10 | 3.13±0.71**             | 4.36±0.33** | 72.06±18.07**               |

Notes: Compared with the model group, \*\* $P<0.01$ , \* $P<0.05$ .

was detected in the model group. The value of EC<sub>50</sub> in the model group also increased. Combined with data from the western blot analysis, we show that the expression of SUR2B in the model group was down-regulated in comparison with that in the control group. This indicated that long-term airway smoke exposure and hypoxic conditions lead to pulmonary vascular endothelial dysfunction and insufficient NO release that could ultimately affect  $K_{ATP}$  channel receptor expression. The effect of QBPF-induced vasodilatation was therefore reduced as a result. Furthermore, this outcome suggests that the pulmonary vasoconstriction response is closely related to pulmonary vascular remodeling in the progression of COPD to PAH, accompanied by lung small artery wall thickening and proliferation of the media layer of smooth muscle fibers.

Chronic alveolar hypoxia has been reported as an important stimulus in the progression of chronic lung disease to sustained pulmonary hypertension (Moudgil *et al.*, 2006; Lai *et al.*, 2015). Furthermore, some researchers believe that the energy metabolism pathway changes in pulmonary artery smooth muscle cells, and that the concentrations of ADP, NDP, and H<sup>+</sup> increase along with  $K_{ATP}$  channel opening. However, chronic hypoxia leads to pulmonary vascular endothelial damage and a reduction in vascular active substances of the  $K_{ATP}$  channel such as prostacyclin (PGL<sub>2</sub>) or NO. The increase in vasoactive substances, such as ET-1, 5-hydroxytryptamine, angiotensin II, inhibited the  $K_{ATP}$  channel. Ultimately,

$K_{ATP}$  channel function was inhibited, hypoxic pulmonary vascular reaction was enhanced and the gradual remodeling of pulmonary vascular was observed (Hayabuchi *et al.*, 2001; Sato *et al.*, 2000; Wang *et al.*, 2007). Our results support these findings. At the same time, an interesting phenomenon was observed whereby QBPF and nicorandil application up-regulated SUR2B expression of the  $K_{ATP}$  channel but not Kir 6.1 expression. Some researchers have reported that sulphonylureas (e.g., GLYB) inhibit or K<sup>+</sup> channel-opener drugs (e.g., nicorandil, pinacidil) activate  $K_{ATP}$  channels by interacting with the SUR subunit. However, the expression level of Kir 6.1 mRNA was unchanged (Horinaka *et al.*, 2001; Nichols *et al.*, 2006; Chen *et al.*, 2008; Yang *et al.*, 2011). Therefore, compared with the model group, the regulation of SUR2B expression by QBPF through a  $K_{ATP}$  channel mechanism was potentially achieved through the NO/cGMP signal pathway.

## CONCLUSION

The QBPF causes relaxation of endothelial-dependent *in vitro* pulmonary artery rings and is therefore, a promising drug for the clinical treatment of pulmonary hypertension associated with COPD.

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