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Effects of San-Huang-Xie-Xin-Tang on U46619-induced increase in pulmonary arterial blood pressure

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ABSTRACT

Ethnopharmacological relevance: San-Huang-Xie-Xin-Tang (SHXT), composed of *Coptidis rhizoma*, *Scutellariae radix* and *Rhei rhizoma*, is traditionally used to treat hypertension.
Aim of the study: Our aim was to investigate the pharmacology effect of SHXT on a thromboxane A_2 analogue U46619-induced increase in pulmonary hypertension and protein expression in primary pulmonary smooth muscle cells (PASMCs).
Materials and methods: Arterial blood pressure and isometric tension in the aorta and pulmonary artery of rats were measured by pressure and force transducers, respectively. Protein expressions on PASMCs were detected by Western blotting.
Results: SHXT significantly attenuated U46619-induced increase in arterial blood pressure. The inhibitory effect of SHXT on pulmonary arterial pressure was greater than systemic arterial pressure in U46619 treated rats. Similarly, the inhibitory effect of SHXT on U46619-induced vasoconstriction in rat pulmonary arterial rings was greater than that in aortic rings. In U46619 treated PASMCs, SHXT down-regulated expression of phosphodiesterase type 5 (PDE5), Rho-kinase (ROCK) II, cyclooxygenase-2 (COX-2) and up-regulated expression of soluble guanylyl cyclase (sGC) α_1 and sGCβ_1.
Conclusions: SHXT attenuated U46619-induced increase in systemic and pulmonary arterial blood pressure. Inhibition of PDE5, ROCK-II, COX-2 and stimulation of sGC may play important roles in the cardiovascular effects of SHXT.

1. Introduction

San-Huang-Xie-Xin-Tang (SHXT, also named San'o-Shashin-to in Japan), composed of *Coptidis rhizoma*, *Scutellariae radix* and *Rhei rhizoma*, is clinically used to treat hypertension and various simultaneous symptoms (Chen and Hsieh, 1986a,b; Sanae et al., 2001). Berberine, one of the main components in *Coptidis rhizoma*, has been used for the treatment of dysentery, hypertension, inflammation and liver disease (Bova et al., 1992; Chiou et al., 1991) in China and Japan. Berberine inhibits angiotensin converting enzyme and directly release NO/cGMP in the vascular tissues (Kang et al., 2002). The dried root of *Scutellaria baicalensis* has been widely used as popular antibacterial and antiviral agents, and are reported to lower blood pressure (Huang et al., 2005), but its main components baicalein and baicalin exert endothelium-dependent contraction and direct relaxation in rat mesenteric artery (Chen et al., 1999). *Rhei Rhizoma* extracts reportedly have beneficial biological effects such as lowering serum cholesterol and improving diabetic nephropathy as well as protecting pro-oxidation (Kim et al., 2002; Park et al., 2002). However, the pharmacological effects of SHXT on cardiovascular disease are poorly understood. Pulmonary arterial hypertension (PAH) is a severe cardiovascular disease and contributes to the morbidity and mortality of adult and pediatric patients (Carbone et al., 2005; Naeije, 2005; Roy and Couriel, 2006).

PAH is a progressive disease of pulmonary arteries characterized by a sustained increase in pulmonary pressure and vascular remodeling (Runo and Loyd, 2003). Phosphodiesterase Type 5 (PDE5) plays an important role in the modulating nitric oxide (NO)/cGMP-mediated pulmonary vasodilation (Rabe et al., 1994). Rho-kinase has been recognized as important in cellular signaling in processes including cell growth, differentiation, gene expression, adhesion, actin cytoskeleton rearrangement, migration, and contraction (Fagan et al., 2004). Rho signaling alters expression of several genes known to be important in regulating pulmonary vascular tone and structure (Fu et al., 1998; Gong et al., 1996; Uehata et al., 1997). Moreover, studies have shown that increased formation of COX-dependent vasoconstricting factors, especially thromboxane (TX) A_2, is involved in abnormal vasomotor function in pulmonary hypertension (Mikhail et al., 1998). Increase of TXA_2 production is related to the pathogenesis of numerous

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pulmonary, inflammatory, cardiovascular and thromboembolic diseases (Dogne et al., 2004; Maruyama et al., 2005; Said, 2006). U46619 (9, 11-dideoxy-11α-epoxymethano-prostaglandin $F_{2\alpha}$), a TXA_2 mimetic analogue, is commonly used to contract pulmonary vascular smooth muscle cells and induce pulmonary arterial hypertension in experimental animal studies (Hall, 1991; Lambermont et al., 2003; Schermuly et al., 2005; Zamora et al., 1993).

SHXT has been used to treat gastritis, gastric bleeding, peptic ulcers (Lin and Tan, 1994) and hypertension (Chen and Hsieh, 1986a,b; Sanae et al., 2001). Post-treatment with SHXT attenuated LPS-induced inflammatory responses (Lo et al., 2005a), lung edema and lethality (Lo et al., 2005b). Furthermore, our report also showed that SHXT reduced *H. pylori*-induced inflammation on AGS cells (Shih et al., 2007). In this study, we investigated the pharmacological effects of SHXT on U46619-induced increase in systemic and pulmonary arterial hypertension and protein expression in primary pulmonary smooth muscle cells (PASMCs) of rats.

2. Materials and methods

2.1. Materials

The voucher specimen and method for extraction and analysis of SHXT were described previously (Lo et al., 2005b). Briefly, the blended mixture of *Coptidis rhizome*, root of *Scutellariae radix* and rhizome of *Rhei officinale* Baill was prepared in a ratio of 1:1:2, respectively. DMEM culture medium, fetal bovine serum (FBS) and gentamicin were purchased from GIBCO/BRL Life Technologies (USA). Anti-sGCα$_1$ rabbit polyclonal IgG, anti-sGCβ$_1$ polyclonal IgG and anti-β-actin mouse polyclonal IgG were obtained from Sigma–Aldrich (USA). Anti-PDE5 mouse polyclonal IgG was obtained from BD (USA). Anti-ROKα/ROCK-II polyclonal IgG were obtained from upstate (USA). Anti-COX-2 goat polyclonal IgG, goat anti-mouse IgG horseradish peroxidase, donkey anti-goat IgG horseradish peroxidase and goat anti-rabbit IgG horseradish peroxidase were purchased from Santa Cruze Biotechnology (USA). All other chemicals were purchased from Sigma Chemical Company (USA).

2.2. Animal preparation

This study was approved by the Animal Care and Use Committee at the Kaohsiung Medical University. Male Wistar rats, weighing 250–350 g, were provided by the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). They were housed under conditions of constant temperature and controlled illumination (lights on between 7:30 and 19:30). Rats were allowed food and water ad libitum.

2.3. Measurement of mean pulmonary arterial pressure (MPAP) and mean systemic arterial pressure (MSAP)

Rats were anesthetized with pentobarbital sodium (40 mg/kg, ip) and following tracheal cannulation, a polyethylene catheter (PE-50) was inserted into the femoral artery to measure mean systemic arterial pressure (MSAP) and heart rates. A femoral vein was cannulated for intravenous injection of drugs. Once the blood pressure reached steady state, a parasternal thoracotomy was performed and a catheter (PE-10) was inserted into the main pulmonary artery via the right ventricular outflow tract to measure mean pulmonary arterial pressure (MSAP). The body temperature was maintained at 37 °C by a heating pad. MSAP and MPAP were monitored continuously using a PowerLab (ADInstruments) four-channel data recorder with a pressure transducer (Spectramed, Model P10EZ, U.S.A.).

2.4. U46619-induced pulmonary arterial hypertension

PAH is defined as a sustained elevation of pulmonary arterial pressure of more than 25 mmHg at rest or of more than 30 mmHg with exercise, with a mean pulmonary-capillary wedge pressure and left ventricular end-diastolic pressure of less than 15 mmHg (Gaine and Rubin, 1998). U46619 (9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) was dissolved in 0.9% saline at a stock concentration of 15.5 μg/ml and administered at a rate of 2.5 μg/kg/min and titrated to achieve an increase of pulmonary vascular resistance of at least 100%. Once a stable infusion rate had been achieved (between 30 and 45 min following the starting of the infusion), the rats were given a bolus dose of SHXT, followed by continuous administration of U46619 for 30 min. The lungs were mechanically ventilated with a mixture of oxygen and room air to maintain normocapnia and normoxia. After a homodynamic steady state was present for 15 min, all homodynamic measurements were repeated. The experimental protocol was completed within 2 h.

2.5. Preparation of pulmonary artery (PA) and thoracic aortic (AO) rings

Isolated aterial rings were harvested and prepared as previously described (Lo et al., 1997; Pulido et al., 2000). Rats anesthetized with pentobarbital sodium (40 mg/kg, ip) then sacrificed. Median sternotomy was performed, and heparin sulfate (500 USP units) was injected into the right ventricular outflow tract. After the removal of the heart and lungs en bloc, the AO and the left and right PAs were quickly excised. The AO and the right and left main branch PAs were cleaned of fat and connective tissues; then cut into 3–4 mm wide rings; two PA and two AO rings were obtained from each rat. Care was taken during this process to avoid endothelial injury.

2.6. Isometric tension experiments

The PA and AO rings were placed on stainless steel wires and suspended in individual 10-ml organ bath and filled with Krebs solution of the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24 and glucose 11; The solution was maintained at 37 °C and aerated with 95% O_2 plus 5% CO_2. Isometric tension was measured with a force-displacement transducer (Model FT03, Grass, Quincy, MA, U.S.A.) and recorded on a highspeed videograph (Model L1969, Coulbourn, Lehigh Valley, PA, U.S.A.). Rings were stretched at 10-min intervals in increments of 0.25 g to reach optimal resting tension. The optimal resting tension was defined as the minimum level of stretch required to achieve the largest contractile response to 60 mM KCl and was determined in the preliminary experiments to be 0.75 g and 1 g for the size of PA rings and AO rings used in these experiments, respectively. After the rings had been stretched to their optimal resting tension, the contractile response to 60 mM KCl was measured. After a washing out of KCl from the organ bath and the return of isometric tension to prestimulation values, each ring was precontracted with the thromboxane analogue U46619. Rings were suspended at 0.75 g for PA rings or 1 g for AO rings and allowed to equilibrate for 60 min, during which time Krebs solution was changed every 30 min. After equilibration, U46619 (0.5 μM) was added to the tissue bath. Cumulative response curves to SHXT were then generated over the concentration range of 0.0005 to 10 mg/ml. For determination of the concentration-response curve, the ring was allowed to reach a steady state before advancing to the next higher concentration. Four to six rats (8–12 rings) were studied in each group.