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Protective effect of HwangRyunHaeDok-Tang water extract against chronic obstructive pulmonary disease induced by cigarette smoke and lipopolysaccharide in a mouse model

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ABSTRACT

Ethnopharmacological relevance: Hwangryunhaedok-tang is an oriental herbal formula treated to cure inflammation and gastric disorders in China, Japan, and Korea. We explored the protective effects of Hwangryunhaedok-tang water extract (HRWE) against airway pathophysiological changes caused by cigarette smoke (CS) and lipopolysaccharide (LPS) in a mouse.

Materials and methods: We performed quantitative analyses of five marker components, namely geniposide, baicalin, coptisine, plamatine, and berberine, using high-performance liquid chromatography. Animals were received CS exposure (1 h per day) for 7 days. LPS was administered intranasally on day 4. Mice were received HRWE at dose of 100 or 200 mg/kg for 1 h before CS exposure.

Results: Treatment with HRWE significantly suppressed the increased inflammatory cell count induced by CS and LPS exposure. In addition, reduction in IL-6, TNF-α and IL-1β in broncho-alveolar lavage fluid (BALF) was observed after HRWE treatment. HRWE not only decreased inflammatory cell infiltration in lung, but also decreased the expression of iNOS, NF-κB and matrix metallopeptidase (MMP)-9 in lung tissues.

Conclusion: This study showed that HRWE can attenuate respiratory inflammation caused by CS and LPS exposure. Therefore, HRWE has potential for treating airway inflammatory disease.

1. Introduction

Cigarette smoke (CS) consists of a complex mixture of more than 7000 chemical compounds and oxidants. It is a crucial inflammatory cause in the progression of chronic obstructive pulmonary disease (COPD) (Wahl et al., 2016; Nesi et al., 2016). COPD features recruitment of inflammatory cells into lung tissues and increased production of pro-inflammatory cytokines and proteases (Roh et al., 2010; Bucher et al., 2016). Since these inflammatory mediators exacerbate COPD, suppressing inflammatory responses is considered an important strategy for controlling COPD (Vaidyanathan and Damodar, 2015).

NF-κB transcription factor is considered as a key player in modulation of gene expression related with physiological responses induced by various injuries (Roh et al., 2010). In inflammatory responses, NF-κB is associated with induction of inflammatory mediator such as matrix metallopeptidase (MMP)-9, inducible-nitric oxide synthase (iNOS), interleukin (IL)-1β and tumor necrosis factor receptor (TNF)-α (Cha et al., 2016). These inductions ultimately produce nitric oxide, destroy normal alveolar structure, and cause infiltration of inflammatory cells into damaging lesions (O'Sullivan et al., 2014; Pang et al., 2015). In particular, pro-inflammatory mediators have been found in sputum and lavage samples of COPD patients (Mercer et al., 2005).

Herbal-based therapies have become increasingly popular among patients and physicians owing to their effectiveness and fewer side effects (Lee et al., 2014; Shin et al., 2011). Hwangryunhaedok-tang (orengedokuto in Japanese and huang-lian-jie-du-tang in Chinese) is an oriental herbal formula consisting of 4 herbs (*Gardeniae Fructus*, *Coptidis Rhizoma*, *Phellodendri Cortex*, and *Scutellariae Radix*). It has been used in China, Japan, and Korea to treat fever, inflammation,

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gastritis, and hypertension (Kwak et al., 2013; Kim et al., 2013; Seo et al., 2015). Recent studies have demonstrated that Hwangryunhaedok-tang possesses various pharmacological properties such as anti-inflammatory, antioxidant, anti-atherosclerotic, and anti-proliferative effects (Kwak et al., 2013; Seo et al., 2015). However, thus far, no study has investigated its protective effect against neutrophilic airway inflammation induced by CS.

The goal of experiment was to explore protective effects of Hwangryunhaedok-tang water extract (HRWE) against airway inflammatory responses caused by CS and lipopolysaccharide (LPS) exposure. To determine the possible mechanism of HRWE, the expression and production levels of inflammatory mediators were examined in CS and LPS-exposed mice.

2. Materials and methods

2.1. Plant materials

Scutellaria baicalensis Georgi (*Scutellariae Radix*, Labiatae), *Phellodendron amurense* Ruprecht (Phellodendri Cortex, Rutaceae) and *Coptis japonica* Makino (*Coptidis Rhizoma*, Ranunculaceae) were purchased from HMAX (Jecheon, Korea). *Gardenia jasminoides* Ellis (*Gardeniae Fructus*, Rubiaceae) was purchased from Omniherb (Yeongcheon, Korea). The 4 herbs were obtained from the Korea Institute of Oriental Medicine (2008–KE20–1, 2008–KE20–2, 2008–KE20–3, and 2008–KE20–4).

2.2. Chemicals and materials

Five reference standards (geniposide, baicalin, coptisine, palmatine, and berberine) with purity ≥98.0% were obtained from Wako (Osaka, Japan). High-performance liquid chromatography (HPLC)-grade water, methanol and acetonitrile were obtained from commercial company (J.T. Baker, NJ, USA). Phosphoric acid and sodium dodecyl sulfate (SDS) were obtained from commercial company (Daejung Chemicals & Metals, Daejeon, Korea and MP Biomedicals, OH, USA).

2.3. Preparation of Hwangryunhaedok-tang decoction

Hwangryunhaedok-tang decoction was prepared at KIOM. Briefly, four raw medicinal herbs (*Scutellariae Radix*, 2.5 kg; *Phellodendri Cortex*, 2.5 kg; *Coptidis Rhizoma*, 2.5 kg; and *Gardeniae Fructus*, 2.5 kg) were mixed and extracted in 100 L of distilled water using an electric extractor (Kyungseo Machine, Incheon, Korea). The solution was filtered through a standard sieve and freeze-dried using a vacuum controlled freeze dryer (PVT100, IlShinBioBase, Yangju, Korea). The amount of lyophilized Hwangryunhaedok-tang powder obtained was 1713.6 g (yield: 17.1%). Water was added to the powder to prepare the decoction.

2.4. HPLC analysis of Hwangryunhaedok-tang decoction

Simultaneous determination of the five markers in Hwangryunhaedok-tang decoction was conducted using a Shimadzu Prominence LC-20A system (Kyoto, Japan) equipped with DGU-20A3 online degasser, LC-20AT pumps, CTO-20A column oven, SIL-20AC auto sample injector, and SPD-M20A photodiode array (PDA) detector. Data were recorded and processed with LCsolution software. To separation of five marker components, we used a Phenomenex Gemini C18 column (250×4.6 mm, 5 μm, Torrance, CA, USA). The column temperature was maintained at 35 °C. The mobile phase consisted of 10% (v/v) acetonitrile, 0.2% SDS, phosphoric acid 200 μL/L (A), and acetonitrile (B). The gradient flow was set as follows: 10–40% B (0–20 min), 40–50% B (20–40 min), 50–100% B (40–50 min), 100–10% B (50–55 min), and 10% B (55–70 min). The flow rate and injection volume were 1.0 mL/min and 10 μL, respectively. For quantitative analysis, lyophilized Hwangryunhaedok-tang extract (250 mg) was dissolved in 25 mL of distilled water. Water extraction was performed at room temperature for 20 min by using an ultrasonicator (Branson 8510; Branson Ultrasonics, Danbury, CT, USA). Quantitative analysis of baicalin was performed after a 10-fold dilution of the sample solution. Each solution was filtered using a 0.2-μm membrane filter before injecting into HPLC system.

2.5. Animals

C57BL/6N male mice (Specific-pathogen-free, 6 weeks old and 20–25 g) were obtained from Samtako (Osan, Korea). They were divided into five groups under standard conditions with water and food provided *ad libitum*. All experimental protocols were granted by the Institutional Animal Care and Use Committee of Chonnam National University.

2.6. Establishment of the CS and LPS-caused airway inflammation mouse

To produced CS, we used 3R4F research cigarette (Kentucky reference cigarettes; Center for Tobacco Reference Products, University of Kentucky, USA). CS exposure was performed according to previously described (Shin et al., 2014). The animals were received CS exposure for 1 h per day in exposure chamber. The exposure periods is 7 days. LPS (10 μg per mouse) was exposed to animals by intranasal instillation under anesthesia on day 4. HRWE was treated to animals at doses of 100 or 200 mg/kg by oral gavage 1 h before CS exposure for 7 days.

2.7. Collection of broncho alveolar lavage fluid (BALF)

The animals were anesthetized by Zoletil 50 (25 mg/kg; Virbac Korea, Seoul, Korea) at 48 h after LPS instillation,. A tracheostomy was conducted as previously study (Shin et al., 2014). The cells were resuspended in PBS after collecting the supernatant. The suspended cells were spread onto glass microscope slides; each slide was air-dried and then stained with Diff-Quik® reagent (IMEB Inc., San Marcos, CA, USA).

2.8. Measurement of proinflammatory mediators in BALF

The TNF-α, IL-1β and IL-6 were analyzed by ELISA kits (R & D System, CA, USA) according to instructions of manufacturer. The absorbance (450 nm) was measured using a microplate reader (Bio-Rad, CA, USA).

2.9. Immunoblotting

Immunoblot was performed as previously study (Shin et al., 2014). The primary antibodies were anti-pNF-κB (Abcam, MA, USA), anti-NF-κB (Abcam), anti-β-actin (Cell Signaling, MA, USA) and anti-iNOS (Santa Cruz, MA, USA).

2.10. Gelatin zymography

Gelatin zymography was conducted as previously studies (Shin et al., 2014) to determine gelatinase activity. The white band on a gelatin gel was represented as proteolytic areas induced by MMP-9.

2.11. Lung tissue histopathology and immunohistochemistry

The lungs from each animal were collected, immersed, embedded in paraffin and sliced into 4-μm thick, which were then stained with hematoxylin and eosin solution (Sigma-Aldrich, CO, USA) and MMP-9 antibody (Abcam).