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Polyphenol-rich blue honeysuckle extract alleviates silica particle-induced inflammatory responses and macrophage apoptosis via NRF2/HO-1 and MAPK signaling

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ABSTRACT

The blue honeysuckle is a rich source of bioactive compounds. In this study, we obtained a polyphenol-rich blue honeysuckle extract (BHE) and found that cyanidin 3-glucoside and chlorogenic acid accounted for most of its phenolic content. Administration of BHE to mice alleviated silica particle (SP)-induced lung inflammation and reduced the recruitment of macrophages to lungs. BHE also reduced the pro-inflammatory cytokine levels in the bronchoalveolar lavage fluid. *In vitro* study showed that BHE reduced pro-inflammatory cytokine secretion by macrophages and reduced apoptosis in macrophages. Cell signaling analysis revealed that BHE inhibited p38 and c-Jun N-terminal kinase phosphorylation and decreased the nuclear expression of nuclear factor-κB. BHE treatment also down-regulated the expression of inducible nitric oxide synthase and up-regulated two anti-oxidant mediators, nuclear factor (erythroid-derived 2)-like 2 and heme oxygenase-1, in macrophages. The findings demonstrated that BHE may serve as a complementary and alternative functional food to prevent SP-induced pulmonary diseases.

1. Introduction

The blue honeysuckle (BH) has been gaining attention and interest not only because of the pleasant taste of its berries but also because of its health-promoting benefits. The plant grows mainly as a wild shrub in Russia, Japan, and northeastern China or in horticultural farms and plantations in some other countries (Oszmianski & Kucharska, 2018). BH berries are a rich source of antioxidant compounds, including ascorbic acid and polyphenolic constituents, particularly anthocyanins and phenolic acids (Caprioli et al., 2016; Hummer, 2006), which have been reported to have multiple bioactive properties. Some previous investigations of nutritional and phytochemical properties of BH extracts (BHEs) have reported that BHEs exhibit multiple health-promoting benefits, including antimicrobial (Puupponen-Pimia et al., 2001; Raudsepp et al., 2013), anti-inflammatory (Martin et al., 2014; Zdařilová, Svobodová, Chytilová, Šimánek, & Ulrichová, 2010), anti-diabetic (Jurgoński, Juśkiewicz, & Zduńczyk, 2013; Svarcova, Jan, & Valentova, 2007), cardioprotective (Svarcova et al., 2007), and hepatoprotective effects (Wang, Li, Ma et al., 2016; Wang, Li, Zhu et al., 2016).

The process of industrialization increases the exposure of workers to respiratory particles. It is estimated that tens of millions of workers are exposed to silica particles (SPs) worldwide, many of whom are exposed to high concentrations (Leung, Yu, & Chen, 2012). There are a number of processes leading to occupational exposure to SPs, such as sand-blasting, silica milling, rock drilling, and tunneling, while new sources of occupational exposure continue to emerge, e.g., in the jeans industry (Barmania, 2016; Steenland & Ward, 2014). The invaded SPs lead to pulmonary inflammation. The role of macrophages in the lung is to clear the inhaled debris, and macrophages are believed to be the first cells of the body to have significant contact with inhaled SPs (Hamilton, Thakur, & Holian, 2008). Inhaled SPs lead to the production of reactive

Abbreviations: BALF, bronchoalveolar lavage fluid; BAX, B-cell lymphoma 2-associated X protein; BH, blue honeysuckle; BHE, blue honeysuckle extract; C3G, cyanidin-3-glucoside; CGA, chlorogenic acid; DAD, diode-array detector; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; H&E, hematoxylin and eosin; HO-1, heme oxygenase-1; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; MS/MS, tandem mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, nuclear factor-kappa B; NO, nitric oxide; NRF2, nuclear factor (erythroid-derived 2)-like 2; PARP, poly(ADP-ribose) polymerase; PBS, phosphate-buffered saline; PCA, protocatechuic acid; ROS, reactive oxygen species; SP, silica particle; TBST, Tris-buffered saline with Tween 20; TNF, tumor necrosis factor

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oxygen species (ROS), either directly by acting on freshly cleaved particle surfaces or indirectly through their effect on phagocytic cells. Macrophages engulf SPs but also increase oxygen consumption, nitric oxide (NO) synthesis, and ROS production, and the expression of NO synthase. SPs can induce pulmonary inflammation and the release of cytokines by macrophages as a result of excessive oxidative stress (Fubini and Hubbard, 2003; Vallyathan & Shi, 1997). SP-induced oxidative stress has also been tightly linked to nuclear factor-kappa B (NF-κB) and activator protein 1 transcription activation via p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling (Chen, Sun, Kuh, Gaydos, & Demers, 1995). The inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and monocyte chemoattractant protein-1 (MCP-1) are most commonly associated with SP-induced free radicals (Barrett, Johnston, Oberdorster, & Finkelstein, 1999; Gossart et al., 1996; Liu, Zhang, and Forman, 2007).

Currently, BH berries are included in the group of so-called superfoods, which are much appreciated for their health benefits (Caprioli et al., 2016). However, to the best of our knowledge, there has been no study on the effects of BHE on SP-induced lung inflammation and on cytokine release by macrophages exposed to SPs. In the current study, we obtained a polyphenol-rich BHE and analyzed its bioactive constituents by high-performance liquid chromatography (HPLC). Furthermore, we evaluated anti-inflammatory effects of BHE on SP-induced lung inflammation and performed an *in vitro* study to test the effects of the polyphenol-rich BHE on macrophages exposed to SPs. Moreover, the implicated cytokines and molecular mechanisms were explored.

2. Materials and methods

2.1. Preparation of blue-berried honeysuckle extract

A detailed description of the preparation and purification of BHE has been presented in our previous study (Wang, Zhu et al., 2016). Briefly, frozen BH berries were homogenized and extracted in an ultrasonic bath (40 °C) for 90 min with 0.1% HCl-acidified ethanol in a ratio of 1:25. The crude extract was filtered. Ethanol was removed from the concentrated solution by rotary evaporation. Next, the concentrate was purified through a glass column loaded with a nonionic polystyrene-divinylbenzene resin (D101, Dingguo, Shanghai, China). The loading speed of the sample was 4 BV/h with a 12-h adsorption time. After sufficient adsorption, ethanol was used for elution at a speed of 5 BV/h. A concentrated liquid was obtained after rotary evaporation. Finally, the concentrate was freeze-dried into a powder using a vacuum freeze-dryer (Labconco, Kansas City, MO, USA). The powder was stored at −20 °C until use.

2.2. Identification and quantification of anthocyanins, flavonoids, and phenolic acids in blue honeysuckle extract

The extract powder (10 mg) was dissolved in 2 mL of methyl alcohol. The sample was filtered through a 0.22-μm filter before identification and quantification of anthocyanins and flavonoids using an HPLC–electrospray ionization (ESI)–tandem mass spectrometry (MS/MS) system (Agilent 1100; Palo Alto, CA, USA) at 520 nm and 350 nm. The detailed conditions have been described in our previous study (Wang, Zhu et al., 2016). Individual compounds were quantified based on calibration curves of structurally related external standards [cyanidin-3-glucoside (C3G) and quercetin-3-glucoside]. The concentrations of the standards ranged from 1 to 50 μg/mL.

Phenolic acids were identified and quantified using an HPLC system (Agilent 1100) equipped with a diode-array detector (DAD; G4212B), and their retention times and peak areas were compared with those of standards. In the present study, calibration curves of three external standards [chlorogenic acid (CGA), caffeic acid, and ellagic acid] were used (Wang, Li, Ma et al., 2016). The concentrations of the standards ranged from 1 to 100 μg/mL. The detailed conditions were as follows: a Thermo Acclaim™ organic acid column (4.0 × 250 mm, 5 μm, 120 A) was used. The optimized mobile phase consisted of methanol (A) and phosphate-buffered saline (PBS), pH 2.6 (B). The elution gradient was as follows: 0–5 min, 10% A; 5–60 min, 10%–65% A; 60–62 min, 65%–10% A; 62–67 min, 10% A. The injection volume was 10 μL, and the flow rate was maintained at 0.8 mL/min. The detection wavelength was 280 nm.

2.3. Animal treatment

C57BL/6 mice (5–7 weeks old) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Liaoning, China). All animals were housed in a specific pathogen-free environment at a temperature of 23 ± 1 °C and a 12-h light/dark cycle, with free access to a standard mouse chow and water.

After acclimation for one week, the mice were randomly divided into five groups (n = 8 each). The pulmonary inflammation mouse model was induced according to previously published methods (Li et al., 2016, 2017; Liu et al., 2018). Briefly, all mice were anesthetized with an injection of 10% chloral hydrate. SP solution in saline (5 mg in 50 μL, mean diameter = 1.5 μm) was directly administered by a single intra-tracheal instillation on day 0 in all mice; the SP solution was replaced with saline for mice in group 1. Group 1 (saline control) and group 2 (model group) mice were administered distilled water by gavage daily for seven days. Mice in groups 3, 4, and 5 were intra-tracheally instilled the SP solution but received BHE (100, 200, and 400 mg/kg body weight in distilled water, respectively) by gavage daily for seven days. All animals were weighed daily. After seven days, the animals were anesthetized by intraperitoneal infusion with 10% chloral hydrate (5 mL/kg body weight). The lungs were excised, and samples of lung tissues were snap-frozen in liquid nitrogen and stored at −80 °C for western blot analysis, while the remaining tissue was fixed in 4% paraformaldehyde for histological analysis.

All the experimental protocols were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and were approved by the Institutional Animal Care and Use Committee at Shenyang Agricultural University, Shenyang, China (License No. SYXK < Liao > 2011-0001).

2.4. Isolation of bronchoalveolar lavage fluid (BALF) and differential cell counting

For bronchoalveolar lavage fluid (BALF), the trachea was cannulated and lavaged twice with 1 mL of sterile saline at room temperature. The samples were centrifuged at 1500 rpm for 5 min, and the cell-free supernatants were stored for cytokine detection (Li et al., 2016). The BALF cell pellet was got after lysis of red blood cells (RBCs). Cells were washed and re-suspended in PBS solution. Total cell counts were determined by standard hematologic procedures. After that, total cells were stained using the Wright-Giemsa method. Macrophages, lymphocytes and neutrophils were identified in 200 cells using standard morphologic criteria.

2.5. Cell culture

The mouse macrophage MH-S cell line was purchased from the National Infrastructure of Cell Line Resource (Beijing, China). MH-S cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified incubator with 95% air/5% CO_2.