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Anti-inflammatory and anti-allergic effects and underlying mechanisms of Huang-Lian-Jie-Du extract: Implication for atopic dermatitis treatment

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ABSTRACT

Ethnopharmacological relevance: Huang-Lian-Jie-Du Decoction (HLJDD), a well-known Chinese herbal formula recorded in the Tang dynasty, is composed of Coptidis rhizoma (Huang-Lian), Scutellariae radix (Huang-Qin), Phellodendri Chinensis cortex (Huang-Bai) and Gardenia fructus (Zhi-Zi). It has clinical efficacy of purging fire for removing toxin and is commonly used for the treatment of disease including Alzheimer's disease, stroke and gastrointestinal disorders. HLJDD is also frequently applied for the treatment of various skin diseases, such as atopic dermatitis (AD) and various types of eczema. The aim of this study is to investigate the anti-inflammatory and anti-allergic actions of Huang-Lian-Jie-Du ethanolic extract (HLJDE) and to elucidate underlying molecular mechanisms of action using relevant *in vitro* experimental models.

Materials and methods: The anti-inflammatory effects of HLJDE were investigated through evaluating the change of nitric oxide (NO) and the production of several cytokines and chemokines in lipopolysaccharide (LPS)-stimulated RAW264.7 cell line. Expression of mitogen-activated protein kinases (MAPKs), NF-κB p65 phosphorylation, inhibitor-κBα (IκBα) degradation were further investigated to elucidate its anti-inflammatory molecular mechanisms. Meanwhile, the anti-allergic activities of HLJDE was also evaluated using antigen-induced RBL-2H3 cell line. β-hexosaminidase and histamine release and selected cytokines and chemokines were measured to evaluate the anti-allergic activities of HLJDE. In addition, intracellular Ca^{2+} level, MAPKs and Lyn phosphorylation were further investigated to reveal its anti-allergic molecular mechanisms.

Results: HLJDE could significantly suppress the secretion of NO, IL-1β, IL-4, MCP-1 and GM-CSF in RAW264.7 cells in a dose-dependent manner. In addition, HLJDE also markedly reduced the phosphorylation of MAPKs, and inhibited the transcriptional activity of NF-κB and IκBα degradation. Furthermore, HLJDE exerted marked anti-allergic activity through inhibiting the release of β-hexosaminidase and histamine. The release of cytokines and chemokines (IL-4, TNF-α, MCP-1) from activated RBL-2H3 cells were also attenuated by pretreatment with HLJDE. The inhibitory effects on intracellular Ca^{2+} level, and reduced phosphorylation of MAPKs and Lyn are believed to be the anti-allergic mechanisms.

Conclusions: HLJDE exerted significant anti-inflammatory and anti-allergic effects through suppressing the production of allergic and inflammatory mediators via the NF-κB and MAPKs inactivation and IκBα degradation in the LPS-stimulated RAW24.7 cells, inactivation of MAPKs and Lyn pathway in antigen-induced RBL-2H3 cells. The present study provides *in vitro* experimental evidence to support the use of HLJDE for the clinical treatment of AD.

1. Introduction

Atopic dermatitis (AD) is a common relapsing chronic inflammatory skin disease characterized by erythema, pruritus, excoriation, edema, exodus and thickening of the skin (Leung et al., 2004; Tan et al., 2013). AD usually occurs during early infancy and

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childhood, but has strong tendency to recur in adulthood. It has been reported that AD affects 15–30% of children and 2–10% of adult all over the world and has high familial occurrence (Bieber, 2008; Eichenfield et al., 2012; Leung et al., 2004). In Hong Kong, the prevalence rate of school children with AD is about 20% (Leung et al., 2003). It is well-known that AD can adversely impact the quality of life of the sufferers and renders a substantial financial burden to the patients and the healthcare systems as a whole (Brown et al., 2012).

Traditionally, AD is regarded as a disease with interplay between skin barrier dysfunction and cutaneous inflammation with activation of multiple immunological and inflammatory pathways, and is regulated by a multitude of genetic and environmental factors (Bieber, 2008; Leung et al., 2004; Novak and Simon, 2011). Skin is an important interface between the host and the environment. After disruption of skin barrier, the host is becoming susceptible to numerous environmental insults, causing immune dysfunction and inflammatory response (Boguniewicz and Leung, 2011; Proksch and Brasch, 2012). Thus, multi-therapeutic approach is regarded as a suitable way for the management of AD, including the repairing of skin barrier, avoidance of various irritants and specific immunologic stimuli (foods and aeroallergens), and short-term and long-term anti-AD medications (Leung et al., 2004; Simpson, 2010). Nowadays, topical anti-inflammatory agents like corticosteroids and calcineurin inhibitors and systemic antihistamine are commonly used for AD treatment, while phototherapy and systemic immunosuppressants are often used as adjunctive treatments for managing severe disease (Saeki et al., 2009). However, the long-term nonjudicious application of corticosteroids and calcineurin inhibitors can give rise to side effects, including cutaneous atrophy, pruritus, burning sensation, and tachyphylaxis (Hsu and Wang, 2007; Simpson, 2010). For these reasons, patients frequently seek other alternative therapeutic options, and Chinese herbal medicine remains one of the most popular complementary medicines for AD.

Huang-Lian-Jie-Du Decoction (HLJDD), a well-known Chinese herbal formula first recorded in the Tang dynasty, is composed of the stem of *Coptis chinensis* Franch (Coptidis rhizoma, Huang-Lian), the root of *Scutellaria baicalensis* Georgi (Huang-Qin), the bark of *Phellodendron amurense* Rupr. (Phellodendri Chinensis cortex, Huang-Bai) and the fruit of *Gardenia jasminoide* Ellis (Gardenia fructus, Zhi-Zi) at the ratio of 3:2:2:3 (Zeng et al., 2009b). HLJDD is known to have clinical efficacy of purging fire and removing toxin and is commonly prescribed for various inflammatory diseases, infections, Alzheimer's disease, stroke and gastrointestinal disorders (Yamasaki et al., 1998; Ye et al., 2012; Yue et al., 2008; Zeng et al., 2009b). Moreover, HLJDD is also often prescribed in Chinese medicine practice for the treatment of various skin diseases, such as eczema and atopic dermatitis (Jian-hong et al., 2010; Ko and Baek, 2012).

HLJDD has been known to possess various pharmacological activities, including anti-inflammatory (Wang and Xu, 2000), antioxidative (Ohta et al., 1999), anti-microbial (Ping et al., 2007), and anti-tumor actions (Lin et al., 2013). The chemical compounds presented in this formula such as berberine, geniposide and balcalin also have many bioactivities. It has been reported that berberine has potent anti-inflammatory effect in vivo and in vitro (Mo et al., 2014) and can suppress the IgE production in human cells (Yang et al., 2014). In addition, geniposide and balcalin were found to be effective in treating allergic asthma in mouse model (Deng et al., 2013; Ma et al., 2014). Since inflammatory response plays a key role in the development and progression of AD, the anti-inflammatory activity of HLJDD is regarded as an important aspect for AD treatment. It has been reported that HLJDD could suppress certain inflammatory mediators in macrophages and inhibit carrageenan-induced paw edema in mice (Lu et al., 2011). However, the molecular signaling pathways underlying the anti-inflammatory response of HLJDD remain to be elucidated.

Besides inflammation, allergy is known to play a critical role in the initiation and exacerbation of AD. It has been known that environmental allergens could cause the relapse of AD in older children and adults (Caubet and Eigenmann, 2010). Thus, alleviation of allergy provides a worthy therapeutic strategy for AD. Up till now, the anti-allergic effect of HLJDD is unclear. The present study aimed to investigate the anti-inflammatory and anti-allergic actions of HLJDD and to elucidate the underlying molecular mechanisms of action using relevant *in vitro* experimental models.

2. Materials and methods

2.1 Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (Missouri, USA) unless otherwise specified. Geniposide, berberine, baicalein, wogonin and baicalin used in the quality control of Huang-Lian-Jie-Du ethanolic extract (HLJDE) were provided by the National Institutes for Food and Drug Control of China (Beijing, China). Antibodies against β-actin, p-38, p-p38, JNK, p-JNK, ERK, p-ERK, IκBα, NF-κB p65 and phosphorylated-NF-κB p65 were purchased from the Cell Signaling Technology (Massachusetts, USA). Polyvinylidene fluoride (PVDF) membrane was obtained form the Bio-Rad (California, USA) and ECL Chemiluminescent Substrate Reagent Kit from the Lifetechnologies (California, USA).

2.2 Preparation of the HLJDE

The crude decoction pieces of Coptidis rhizoma (Huang-Lian), Scutellariae radix (Huang-Qin), Phellodendri Chinensis cortex (Huang-Bai) and Gardenia fructus (Zhi-Zi) were purchased from Zhixin Herbal Pharmaceutical Company Ltd, a Guangzhou-based GMP accredited Chinese herbal supplier, and their identities were authenticated in accordance with the Chinese Pharmacopoeia (2010 Edition). Voucher specimens of the above 4 herbs were deposited in the Herbarium of the School of Chinese Medicine, CUHK, with reference no. AD01–04 respectively. The above 4 herbs were ground to powder or pieces and mixed in the ratio 3:2:2:3, and the mixture was then extracted with 80% aqueous ethanol in an ultrasonic bath for 30 min. The extract was filtered and the residue was further extracted twice as before. All three filtrates were combined, and concentrated in a rotary evaporator under negative pressure, and finally dried in lyophilizer to get a freeze-dried power. The resultant HLHDE was store at −20 °C for subsequent study.

2.3 Construction of HPLC fingerprinting for quality control of HLJDE

The HPLC fingerprinting of HLJDE was constructed using an ACQUITY UPLC system (Waters, USA) equipped with a PDA eλ detector, a FTN sample manager, and quaternary solvent manager. Briefly, the HLJDE was dissolved in methanol and injected into UPLC by autosampler. The chromatographic separation was achieved at a flow rate of 1 ml/min on XBridge C18 column (4.6 × 250 mm, 5 μ, Waters, USA). The mobile phase was composed of solvent A (acetonitrile) and solvent B (12 mM ammonium acetate −0.5% acetic acid). The linear gradient elution was performed from 10 to 50% A in 0–60 min, 50-10% A in 60–61 min, 10% A in 61–70 min at a flow rate of 1 ml/min. The separation temperature was set at room temperature and detection wavelength was 254 nm. The sample injection volume was 10 μl.