SHORT COMMUNICATION

Topical application of *Scutellaria baicalensis* suppresses 2,4-dinitrochlorobenzene-induced contact dermatitis

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(Received 16 February 2015; final version received 3 April 2015)

Allergic contact dermatitis (ACD) is a prototypic T-cell-mediated cutaneous inflammatory response. In the present study we describe the anti-allergic effect of topically applied *Scutellaria baicalensis* aqueous extract (WSBE) in suppressing 2,4-dinitrochlorobenzene (DNCB)-induced ACD in BALB/c mice. Topically applied WSBE attenuated the epidermal thickness and mast cell infiltration into the skin in DNCB-induced contact dermatitis. Furthermore, WSBE suppressed DNCB-induced production of serum IgE as well as IL-4, IFN-\(\gamma\), and TNF-\(\alpha\) in the skin. Topical application of WSBE also ameliorated the significant decrease in dermal glutathione and superoxide dismutase levels. Moreover, present results demonstrated that the baicalin, bioactive compound of WSBE, was able to penetrate into the skin following topical application, which was confirmed by the HPLC analysis using rat model. Taken together, topical application of WSBE exerts beneficial effects in contact dermatitis model, suggesting that WSBE might be a candidate for the treatment of contact dermatitis.

**Keywords:** 2,4-dinitrochlorobenzene; contact dermatitis; mouse; *Scutellaria baicalensis*

1. Introduction

Allergic contact dermatitis (ACD) is one of the most common occupational diseases in industrialised countries with a great socioeconomic impact (Kadyk et al. 2004). ACD is a T cell-mediated inflammatory reaction occurring at the site of challenge with a contact allergen in

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sensitised individuals (Saint-Mezard et al. 2004). Following contact with the skin, haptens cause a variety of adverse health effects including IgE-mediated (immediate hypersensitivity) and T cell-mediated (delayed hypersensitivity) responses (Martin et al. 2011).

_Scutellaria baicalensis_ Georgi (_S. baicalensis_) contains various bioactive components of flavonoids and its roots have been used for the treatment of inflammatory and allergic diseases and smooth muscle relaxer (Kim et al. 2005). Among the flavonoids, the four major of bioactive components are baicalin, its aglycone baicalein, wogonoside and its aglycone wogonin (Gao et al. 2001). It is known that they have anti-inflammatory, antioxidant and antitumour activities (Shieh et al. 2000; Chuang et al. 2005). There have been reported that the oral administration of extracts from _S. baicalensis_ prevents chemicals-induced hypersensitivity (Kim et al. 2005; Kimura & Sumiyoshi 2011; Jung et al. 2012). However, in comparison with topical therapy, systemic treatment with medicinal plant derived extracts usually requires higher doses to show therapeutic efficacy, which increase both cost and the probability of toxicity. Therefore, topical application is especially attractive for dermatologists, because of the potential for efficacy with relatively mild systemic adverse effects during topical application (Chiu & Tsai 2011). Although the beneficial effect of _S. baicalensis_ on chemical induced allergic dermatitis using various haptens is widely studied, little is known about preventative effect of _S. baicalensis_ on contact allergic dermatitis following topical application.

The aim of the current study was to investigate the anti-allergic effects of the standardised _S. baicalensis_ aqueous extract (WSBE) on DNCB-induced contact dermatitis in female BALB/c mice. Furthermore, the skin absorption of WSBE was assessed in rats after topical application using baicalin reference standard.

2. Results and discussion

In the present study, we demonstrate that topical application of WSBE, which contains baicalin, wogonoside, baicalein, and wogonin, attenuates ACD by decreasing inflammatory cytokines in the DNCB-treated BALB/c mice. It was further supported by histopathological analysis in showing that DNCB-induced increase in epidermal thickness and infiltration of leukocytes into dermis was suppressed by WSBE treatment. As shown in Figure S1, the epidermal thickness is significantly thicker in the DNCB alone treated positive group than in the vehicle-treated negative group (Figure S1C; \( p < 0.05 \)). The positive group distinctly showed severe epidermal hyperplasia, hyperkeratosis, and infiltration of inflammatory cells and mast cell in the dermis. In contrast, the epidermal thickness, dermal mast cell number and inflammatory infiltration are slightly decreased in the WSBE treated group (Figure S1).

Topical agents such as glucocorticosteroids, non-steroid anti-inflammatory drugs, and immunosuppressants are currently used for the treatment of various cutaneous inflammatory diseases, but these agents are often associated with severe adverse effects (Yuan et al. 2010). In this aspect, there is a great need for the development of new and effective therapies for ACD. In a safety aspect, the dermal safety of WSBE in topical application has been confirmed in the both skin reaction and skin sensitisation models (Kim et al. 2013). Recently, Jung et al. (2012) reported that _S. baicalensis_ may effectively suppress inflammation by down-regulating the expression of various inflammatory mediators and reducing the production of inflammatory cytokines in human mast cells. However, it shows a weak inhibition of the passive cutaneous anaphylaxis reaction after oral administration of _S. baicalensis_ extract at 280 mg kg\(^{-1}\) (Jung et al. 2012). In the present study, we found that topically applied WSBE produced dose-dependent inhibitory effects on DNCB-induced epidermal hyperplasia, infiltration of mast cell in dermis together with IFN-\(\gamma\), IL-4, and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) production of the dorsal skin of BALB/c mice. Compared with the vehicle group, the serum IgE and skin IFN-\(\gamma\), IL-4, and TNF-\(\alpha\) levels were markedly increased in the DNCB-treated group. However,
dexamethasone and WSBE are significantly decreased inflammatory cytokines in the DNCB sensitised mice (Figures S2 and S3). Differences in skin absorption and oral administration of WSBE could contribute to the differences of efficacy in contact dermatitis and differences in the experimental protocol could account for the discrepancy.

Mast cell-derived pro-inflammatory cytokines, particularly TNF-α, play a key biological role in allergic reactions (Saint-Mezard et al. 2004). Production of TNF-α plays a detrimental role in allergies and asthma, as a mediator of the late-phase inflammatory reaction (Broide 2001). TNF-α in mast cells is stored in cytoplasmic granules and is released with histamine and other preformed mediators within minutes of antigenic stimulation. This suggests a potential role in early inflammation as well (Broide 2001). In this study, WSBE significantly inhibited TNF-α level in DNCB-sensitised back skin, leading to the suppression of immune function under this allergic condition. This result from in vivo is consistent with previous result from in vitro using human mast cells (Jung et al. 2012).

Oxidative stress and inflammation have been associated with contact dermatitis and some skin irritant known to generate free radicals and reactive oxygen species (ROS) (Briganti et al. 2001). Inhibition of nitric oxide (NO) over-production repressed leucocyte accumulation and oedema formation in the guinea pig skin, indicating that NO implicates in skin inflammation (Briganti & Picardo 2003). Moreover, it is generally accepted that the excessive NO implicate in inflammation and could lead Cu/Zn superoxide dismutase (SOD), potent antioxidants, up-regulation (Guzik et al. 2003). Shao et al. (2004) observed that S. baicalensis shows a potent radical scavenging effect in the in vitro ROS generation system. Furthermore, baicalein attenuated mitochondrial oxidative stress by scavenging ROS, including superoxide anions and hydroxyl radicals, and by induction of nuclear factor erythroid-related factor 2 transcription factor-mediated SOD (Lee et al. 2011). In the present study, the WSBE applied group showed decreased NO and thiobarbituric acid along with recovered glutathione and SOD levels, which indicate that WSBE could regulate intracellular redox homeostasis in skin (Figure S4). Berardesca et al. (1997) reported that topically applied α-hydroxyacids having antioxidant properties modulated stratum corneum barrier function and prevented skin irritation by reducing the oxidant products such as 4-hydroxy-2-nonenal or malonaldehyde, which are able to induce protein damage, apoptosis or release of pro-inflammatory mediators, such as cytokines.

Pharmacokinetic studies on the main flavonoids of S. baicalensis following oral administration have been fully carried out (Li et al. 2011). It is generally assumed that baicalin itself is poorly absorbed from the gastrointestinal tract, but is hydrolysed to its aglycone, baicalein by intestinal microflora and then restored to its original form from the absorbed baicalein in the body (Akao et al. 2000). In our previous study, the WSBE showed negligible irritation of the skin after in vivo topical application, indicating their feasibility for dermal use (Kim et al. 2013). However, no evidence currently exists to support the notion that significant amounts of flavonoids of S. baicalensis can be delivered into the skin for topical use. Our results demonstrated that baicalin was able to penetrate into the skin, which was confirmed by the mean plasma concentration of baicalin after topical application (Figure S5). Results from the present study imply that a topically applied WSBE is a plausible strategy to counteract poor systemic absorption.

3. Conclusion
Our results suggest that WSBE could be a potential therapeutic candidate for contact dermatitis. Further investigations of the molecular mechanism should be necessary to understand the anti-hypersensitivity of topically applied WSBE.
Supplementary material

Experimental details relating to this article are available online, alongside Figures S1–S5.

Disclosure statement

No potential conflict of interest was reported by the authors.

Note

1. These authors contributed equally to this work.

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