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Icariin attenuates glucocorticoid insensitivity mediated by repeated psychosocial stress on an ovalbumin-induced murine model of asthma

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ABSTRACT

Evidence shows that psychosocial stress exacerbates asthma, but there is little intervention to alleviate negative effects of psychosocial stress on asthma. We investigated the role of icariin in anti-inflammation and anti-anxiety potential in a murine model combined psychosocial stress with allergic exposure. The results indicated that icariin administered remarkable increased activity in the center of the open field, reversed airway hyperresponsivenesss, reduced inflammatory cytokine infiltration to the lung and whole body and also in part recovered glucocorticoid responsiveness. Furthermore, our data also showed that icariin significantly inhibited increases of corticosterone and markedly increased glucocorticoid receptor mRNA and protein expression in the lungs of mice exposed to both stress and allergen. Collectively, we speculate that inducing glucocorticoid receptor modulation might be the potential mechanisms of icariin to facilitate corticosteroid responsiveness of cytokine production.

1. Introduction

Although physicians and scientists have long recognized and investigated the underlying mechanisms that chronic psychosocial stress contributes to exacerbations of asthma [1–5], there is little intervention to alleviate the negative effects of psychosocial stress on asthma. Evidences had showed that altered innate immune and inflammatory cell function through neural and hormonal pathways played an important role in how psychosocial stress aggravates asthma [6–9]. Experiment studies had demonstrated that physical and psychological stressors activated the hypothalamic–pituitary–adrenal (HPA) axis and increased levels of circulating glucocorticoid hormones [10,11]. However, raised endogenous circulating glucocorticoid had not eased the airway inflammatory, but enhanced and prolonged airway inflammation and altered corticosterone responsiveness [12]. Researchers speculated that these effects were mediated at least in part by impaired glucocorticoid receptor (GR) expression and function [13], which was also confirmed in our previous study [14]. In our previous study, we used social disruption, a well-characterized psychological stressor, to imitate psychosocial stress and established three murine models: ovalbumin (OVA)-induced only model, social disruption stress (SDR) model, combined OVA-induced and SDR model, and mice without any interference as the control. By comparison between models and the control mice in aspects of animal behaviors in the open field test (OFT), airway hyperresponsiveness (AHR), airway inflammation, glucocorticoid sensitivity and the expression of GR in lung tissue, we found that SDR can promote anxiety behavior, activate the HPA axis, increase AHR and inflammation, and also impair glucocorticoid sensitivity and its function in a murine model of OVA-induced asthma. The down-regulation of GR expression induced by SDR is in part associated with glucocorticoid insensitivity, which leads to asthma exacerbation.

Icariin(2-(4′-methoxylphenyl)-3-rhamnosido-5-hydroxyl-7-glucosido-8-(3′-methyl-2-butylenyl)-4-chromanone) (Fig. 1) is a flavonoid extracted from the traditional Chinese herb *Epimedium brevicornum* Maxim which has been used as a tonic, aphrodisiac and an anti-rheumatic in traditional Chinese medicine for centuries [15]. Numerous studies from our group and others showed that icariin exhibited a wide range of pharmacological and biological activities, including estrogenic activity[16], anti-tumor effects [17], immunoregulation [18] and protective effects against neurotoxicity [19,20] and it was widely used to treat various diseases, such as coronary heart disease, osteoporosis, menopause syndrome, rheumatism, bronchitis and hypogonadism [21]. Notably, we recently studied the mechanism of icariin's effect on rat asthmatic model [18]. The date showed that icariin could regulate the imbalance of helper T (Th) 1/Th2 cytokines and associated transcription factors T-bet and GATA-3 in asthmatic rat pulmonary tissue. Moreover, icariin could inhibit the activation of NF-κB p65 protein in asthmatic rat pulmonary tissue. Another study from our group demonstrated that icariin could attenuate social defeat-induced down-regulation of glucocorticoid receptor in mice and its derivative, ICT, exert anti-inflammatory, anti-tumor effects,

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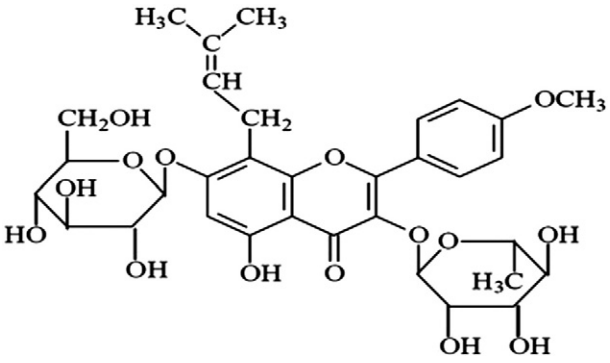

Fig. 1. Chemical structure of icariin (C33H40O15; molecular weight = 676.67).

and modulate myeloid derived suppressive cell (MDSC) functions [22,23].

In order to investigate the role of icariin in protective effect of psychosocial stress on asthma, we used a unique murine model that combined SDR and OVA challenge, which had been established in our previous study [14]. We hypothesized that icariin could alleviate the airway inflammation and anxiety behaviors in the murine model of combined SDR and OVA challenge by modifying HPA-axis function, regulating innate immune cell production of cytokines, and that this effect was mediated by an altered corticosteroid action.

2. Materials and methods

2.1. Animals

54 specific pathogen-free male BALB/c (test mice) and 20 male C57BL/6J (aggressors) [24] were purchased from Shanghai SLAC Co (Shanghai, China) and used in this study. BALB/c studied for their stress and allergic responses (aged 6–8 weeks, weighed 18–20 g, 3 per cage) were allowed to acclimate to their new environment for 1 week before experiment and were maintained on a 12-h light/dark schedule (lights on at 6:00 a.m.) with food and water available ad libitum. Then, the total of 54 mice were randomly assigned to six groups (n = 9 per group): A—no manipulation (control); B—sensitized, antigen challenged, and social disruption model (asthma-SDR); C—administered dexamethasone (DEX) 1 mg/kg and sensitized, antigen challenged, social disruption model (DEX 1 mg/kg-Asthma-SDR); D—administered icariin 10 mg/kg and sensitized, antigen challenged, social disruption model (Icariin 10 mg/kg-Asthma-SDR); E—administered icariin 50 mg/kg and sensitized, antigen challenged, social disruption model (Icariin 50 mg/kg-Asthma-SDR); F—administered icariin 100 mg/kg and sensitized, antigen challenged, social disruption model (Icariin 100 mg/kg-Asthma-SDR). In addition, C57BL/6J mice arrived in the animal facility at 8–9 weeks old and were allowed 1 week habituation before being singly-housed in cages for 4 weeks in order to reduce inter-individual variability and increase the likelihood of social interaction-induced stress without creating overt phenotypical changes. All animal experiments were approved by the Huashan Hospital, Fudan University Animal Care and Use Committee (permit number: 11-1002).

2.2. Selection of aggressive intruders

Prior to the social disruption procedure, C57BL/6J mice were screened in a preliminary study for aggressive behaviors towards a separate cohort of BALB/c mice to ensure the defeat of the resident experimental mice, as previously described [25]. The heaviest C57BL/6 mice were selected for the aggression test. Latency of first attack and dominance status of mice were visually determined by observing key behaviors such as tail rattling, chasing or biting for dominance and escape and upright postures or immobility for submission [25–27]. Mice being aggressive, dominant and presenting the lowest latency to attack were then selected to be aggressive intruders for the stress procedure [28]. Five C57BL/6J mice were excluded in this study.

2.3. Social disruption model

Social disruption model has been extensively used and has been previously described by our laboratory [14,22] and others with slight modulation in this study. Briefly, an older and larger male C57BL/6J (the intruder) was placed into the cage containing an established social hierarchy of three young male BALB/c (the residents) for a 2-h confrontation beginning at 4:30 p.m. Animals underwent six SDR cycles over a week: three nightly cycles, one night off, and three more cycles. Observations were made to ensure that the aggressors defeated the experimental subjects. To avoid habituation to the intruder, aggressors were rotated daily. An intruder displaying no aggressive behavior within the first 5 min towards resident mouse was replaced by another one. Different aggressors were used on consecutive nights. To make sure that mice in the control groups were left undisturbed, they were put in another room during the SDR cycles. Researchers observed carefully in order to avoid serious injury to happen. In the whole experiment process, no severe injury happened.

2.4. Allergic sensitization and challenge

Asthma-SDR mice were sensitized and challenged by OVA according to the modified protocol reported previously [29]. Briefly, the mice were immunized at 1 and 8 days (7 days apart) by a peritoneal injection of sterile saline 0.5 ml containing OVA 10 μg (Grade V, Sigma, St. Louis, MO, USA) and aluminum hydroxide 4 mg. Fourteen days after the first sensitization, the mice were challenged continuously for 2 weeks (30 min each day) by an inhalation of 1% OVA through an ultrasound aerification inhaler (Buxco, USA). Half an hour before each challenge, DEX (Roche Pharmaceutical Ltd., Shanghai, China) at a dose of 1 mg/kg, icariin (Sichuan Chengdu Pharmaceutical Co., Chengdu, China) at the dose of 10 mg/kg, 50 mg/kg, 100 mg/kg were orally administered into the stomachs of mice in the DEX-Asthma-SDR group, Icariin 10 mg/kg-Asthma-SDR group, Icariin 50 mg/kg-Asthma-SDR group and Icariin 100 mg/kg-Asthma-SDR.

2.5. Sensitization, antigen challenge, and social stress schedule

The challenge schedule is presented in Fig. 2 and briefly summarized below: day 1 and day 8—sensitization (ip); days 15–28—antigen challenge (inhalation); days 22–24—psychosocial stress; and days 26–28—psychosocial stress; and days 15–28—icariin or DEX administration (orally).

2.6. The open field test

Spontaneous activity under novelty stress was measured for 5 min, approximately on the morning immediately following the final cycle of social disruption. As previously described [30], all mice were acclimatized to the test room for 1 h and thereafter placed in a clear Plexiglas chamber (Med Associates Inc., St. Albans, VT, USA) measuring 270 × 270 × 200 mm with arrays of 16 × 16 photo detector, positioned 2.5 cm and 10 cm above the floor of the chamber. The software (Activity Monitor version 5, Med Associates) allowed a distinction to be made between repetitive interruptions of adjacent photo beams. As indexes of ambulatory counts (number of individual horizontal movements registered when the mice walked on all four feet) and vertical counts (rearing counts registered when mice's body inclined vertically with hind paws on the floor and forepaws on the wall of the activity cage) were evaluated. The time spent at the center of the chamber served as an index of anxiety [31]. The equipment allowed four mice to test at