International Immunopharmacology 25 (2015) 432–439

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Oral administration of paeoniflorin attenuates allergic contact dermatitis by inhibiting dendritic cell migration and Th1 and Th17 differentiation in a mouse model

Dongmei Shi[a,b,1], Xuefeng Li[c,1], Dongmei Li[d], Quanjing Zhao[e], Yongnian Shen[a], Hongxia Yan[b], Hongjun Fu[b], Hailin Zheng[a], Guixia Lu[a], Ying Qiu[b,*], Weida Liu[a,**]

[a] Department of Mycology, Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College, Nanjing, Jiangsu, PR China
[b] Department of Dermatology, Jining No. 1 People's Hospital, Shandong, PR China
[c] Department of Ultrasonography, Jining No. 1 People's Hospital, Shandong, PR China
[d] Georgetown University Medical Center, Washington D.C., USA
[e] Department of Pathology, Jining No. 1 People's Hospital, Shandong, PR China

ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 14 February 2015
Accepted 18 February 2015
Available online 3 March 2015

Keywords:
Allergic contact dermatitis
Paeoniflorin
1-chloro-2,4-dinitrobenzene
Contact sensitivity
Dendritic cells

ABSTRACT

Allergic contact dermatitis (ACD) is a hapten-specific $CD4^+$ T-cells mediated inflammatory response of the skin. Its pathomechanism involves 2 phases, an induction phase and an elicitation phase. Langerhans cells (LCs) and dendritic cells (DCs) in the skin play key roles in presenting low molecular weight chemicals (haptens) to the lymph nodes. Therefore, inhibition of the migration of LCs or DCs and T-cell proliferation is each expected to control ACD disease. To explore the effectiveness of paeoniflorin (PF) on the migration of LCs and T-cell proliferation *in vivo*, we establish a murine model of ACD, promoted by repeated exposure to an allergen (specifically 1-Chloro-2,4-dinitrobenzene (DNCB)). Administration of PF inhibits DC migration in this DNCB-induced model in the induction phase. As a result, epidermal LC density in the elicitation phase increased in PF-treated mice when compared to PF-untreated mice. At the same time, PF reduced IFN-γ^+CD4^+ and IL-17^+CD4^+ T cells proliferation (but not IL-4^+CD4^+ T cells proliferation), leading to an attenuated cutaneous inflammatory response. Consistent with this T-cell proliferation profile, secretions of IFN-γ and IL-17 were reduced and IL-10 secretion increased in PF-treated mice, but production of IL-4 and IL-5 remained unchanged in the skin and blood samples. These results suggest that oral administration of PF can treat and prevent ACD effectively through inhibition of DC migration, and thus decrease the capacity of DCs to stimulate Th1 and Th17 cell differentiation and cytokine production.

1. Introduction

Allergic contact dermatitis (ACD) is a skin disease caused by an allergic reaction to the allergens in contact with the skin, characterized by redness, papules, vesicles, followed by scaling and dryness [1,2]. Approximately, 15–20% of the population worldwide is affected by this disease [3,4], leading to a loss of 4 million work-days per year and almost $400 million in the United States alone [5–7]. Although therapeutic application of steroids can often effectively reduce skin inflammation, patients with ACD suffer from chronic disease in some cases.

ACD is a T cell delayed-type hypersensitivity (type IV) response initiated by skin contact with haptens (low-molecular weight chemicals). Haptens penetrate the skin and covalently bind to carrier proteins to become fully immunogenic [8]. Therefore, models of ACD are often employed for studying Ag-specific T cell-mediated immune responses [9,10]. In the sensitization phase, a hapten (*i.e.* an allergen, drug, or metal) is applied to the skin of an animal, usually on the abdomen. Consequentially, the elicitation phase is attained by a subsequent exposure to the same hapten at a remote skin site, such as ear skin [11].

Dendritic cells (DCs) play key roles in the initiation and elicitation of ACD [12]. Sensitization of the skin to allergens occurs when allergens are taken up by antigen-presenting cells in the skin, such as Langerhans cells (LCs) or dermal DCs, and brought to the draining lymph nodes to prime allergen-specific T cells. In lymphoid tissue, hapten-bearing antigen-presenting cells 'educate' naïve T lymphocytes, and in response naïve T lymphocytes become allergen-specific memory or effector T

Abbreviations: DCs, dendritic cells; LCs, Langerhans cells; ACD, allergic contact dermatitis; DNCB, 1-chloro-2,4-dinitrobenzene; PF, paeoniflorin

* Correspondence to: Y. Qiu, Department of Dermatology, Jining No. 1 People's Hospital, No. 6 Jiankang Road, Jining 272011, Shandong, P. R. China. Tel.: +86 537 2253401.
** Correspondence to: W. Liu, Department of Mycology, Institute of Dermatology, No. 12 Jiang Wangmiao Street, Nanjing 210042, Jiangsu, P. R. China. Tel.: +86 25 85470580; fax: +86 25 85414477.
E-mail addresses: qiou_ying@sohu.com (Y. Qiu), liumyco@hotmail.com (W. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.intimp.2015.02.031
1567-5769/

cells [4]. When the patient encounters the same hapten again, the hapten diffuses through the skin and is taken up by LCs or dermal DCs, which in turn present the hapten to specific T cells [13]. These specific T cells are then activated and induce cytokine production that further activates the inflammatory response to elicit the typical clinical symptoms of ACD; redness, papules and vesicles.

Experiments for ACD control have focused on prevention of DC migration, inhibition of Th1/Th2/Th17 responses and reduction of local inflammatory cytokine products [12,14]. Attempts to modulate the immune response to ACD have been conducted in a number of clinical trials but have achieved only limited success [15]. Today, topical glucocorticoids still remain an effective remedy for the treatment of ACD. However, prolonged use of high doses of glucocorticoids can cause a variety of side effects.

Paeoniflorin (PF), a water-soluble monoterpene glycoside found in the root of *Paeonia lactiflora Pall* (the peony), has been suggested to possess a wide range of pharmacological features, including anti-oxidant, anti-inflammatory, and anti-cancer effects [16]. As both Th1 and Th2 immune responses are implicated in the development of ACD skin lesions, PF has been suggested to inhibit inflammatory responses via modulating the balance of inflammatory and anti-inflammatory cytokines in ACD [17]. In our previous study *in vitro*, we also demonstrated that PF inhibited DC maturation and migration, and inhibited Th1 cell proliferation in response to hapten (1-Chloro-2,4-dinitrobenzene (DNCB)) [18].

In this study, we focus on the capacity of PF to influence migration of DCs and T cell proliferation in an *in vivo* DNCB-induced ACD model. The influence of orally administered PF on DC migration, Th1, Th2 and Th17 cell differentiation and cytokine secretions are clearly demonstrated.

2. Materials and methods

2.1. Regents and chemicals

1-Chloro-2,4-dinitrobenzene (DNCB), acetone and olive oil were obtained from Sigma Chemical Co. (USA). RPMI 1640 medium, Hank's balance salt solution, penicillin, streptomycin, L-glutamine, and fetal bovine serum (FBS) were purchased from Gibco (USA).

PF (powder compound, purity determined by HPLC is >98%) was purchased from Silida Technology Ltd. (Tianjin, China) in Fig. 1. PF was dissolved in 50 ml of sterilized PBS to make a 2000 mg/ml stock solution. Working solutions were further diluted in saline.

2.2. Mice and ethics statement

Female BALB/c mice were purchased from the Experimental Center of Yangzhou University (Jiangsu, China). The mice were bred in the animal experimental center of the Institute of Dermatology, Chinese Academy of Medical Sciences (CAMS) in Nanjing, Jiangsu. Animals were housed with six mice per cage at 22 °C with a 12-hours light–dark cycle. Experiments were conducted with 8 week old mice with body weight 18–20 g. Water and standard diet were available *ad libitum*. The experiments were performed in a pathogen-free environment. The protocols for animal experiments were all approved by the Animal Study Committee of the Institute of Dermatology, CAMS, according to the government guidelines for animal care.

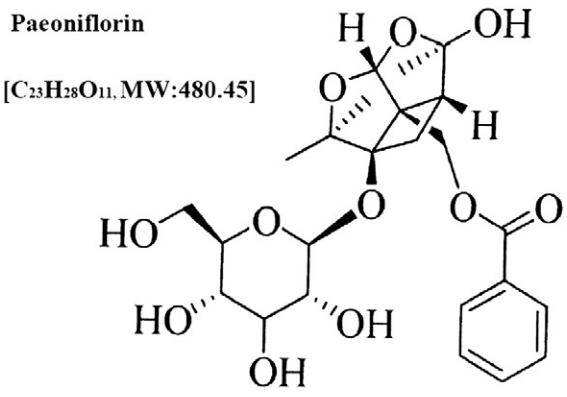

Fig. 1. The chemical structure of paeoniflorin.

2.3. Sensitization and challenge procedures to induce ACD and local lymph node assay

DNCB-induced ACD was achieved as previously described [19,20]. After complete removal of hair in an abdominal area of approximately 4 cm^2, 100 μl of 1% DNCB solution (dissolved in a 4:1 mixture of acetone: olive oil) was applied to the stripped epidermis for three consecutive days (days 0–2) to sensitize mice. Seven days later (day 9), the elicitation phase was induced by single application of 0.2% DNCB solution (acetone: olive oil; 4:1) to the right ear of each mouse. From day 0 mice were administered PF orally (100 or 200 mg/kg per day) or PBS as control for the subsequent 10 days. At 24 h post-challenge, blood was collected by orbital puncture, and swelling of the ear was measured by a comparison of the ear thickness (cutometer, Mitutoyo, Neuss, Germany). The ear was removed for histopathological and RT-PCR examination, as described below.

To assess the effects of PF on the sensitization phase, a local lymph node assay was performed as previously described, with minor modifications [21,22]. Over a period of 7 days (days 0–7), mice were administrated PF or PBS orally. From day 4 mice were sensitized by application of 100 μl of 1% DNCB (acetone: olive oil; 4:1) onto the dorsum of both ears for three consecutive days (days 4–6). On day 7, the mice were

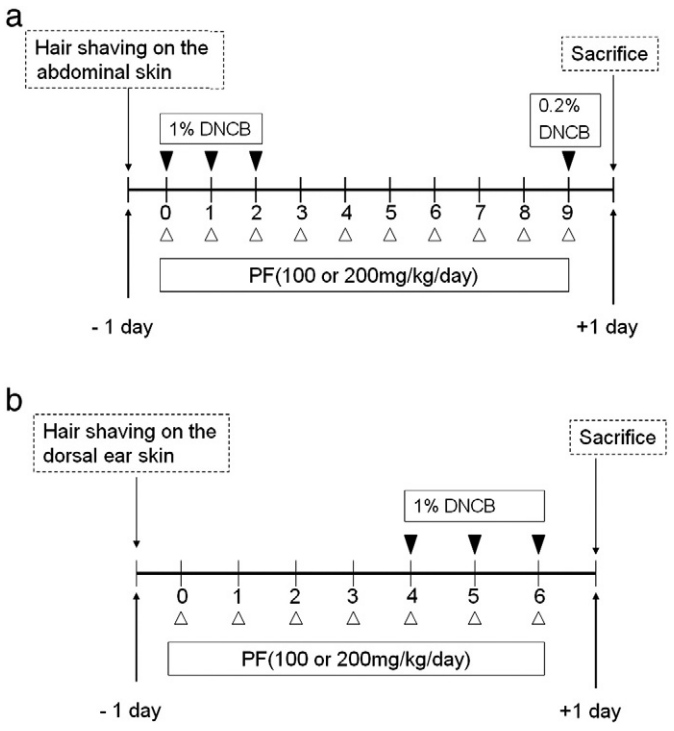

Fig. 2. Design of allergic contact dermatitis model and lymph node assay. (a) Schematic diagram of establishment of a mouse model of allergic contact dermatitis using Th1 contact sensitizers (2,4-dinitrochlorobenzene, DNCB). Mice were administered paeoniflorin (PF) (100 or 200 mg/kg/day) or PBS orally from day 0 to 10. 100 μl of 1% DNCB in 4:1 (v/v) acetone/olive oil was administered daily to the stripped abdominal skin to sensitize mice from day 1 to 3. On day 10 the elicitation phase was induced by administration of 0.2% DNCB (acetone: olive oil; 4:1) to the right ear. (b) To assess the effects of PF on the sensitization phase, a local lymph node assay was performed. On days 0–7 mice were administrated PF or PBS orally. From day 4, mice were sensitized by application of 100 μl of 1% DNCB (Sigma, USA) (DMSO: olive oil; 4:1) onto the mouse ears for 3 consecutive days (days 4–6). On day 7, mice were sacrificed by cervical dislocation and auricular draining lymph nodes were obtained for the experiment as follows.