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Paeoniflorin augments systemic *Candida albicans* infection through inhibiting Th1 and Th17 cell expression in a mouse model

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ABSTRACT

Paeoniflorin (PF), a Chinese herbal medicine, has been widely used in clinical practice in China because of its dual immunoregulatory effects. A previous study found that PF inhibited the biofilm formation of *Candida albicans* (*C. albicans*) *in vitro*; however, whether PF plays an antifungal role *in vivo* is still unexplored. In this study, we sought to examine the effect of PF alone or in combination with an antifungal agent, fluconazole (FCZ), using a mouse model of systemic candidiasis. The results showed that the survival time of mice treated with PF alone or PF + FCZ decreased compared with the Infected alone and FCZ treated groups, respectively (8.20 ± 1.75 *vs* 10.40 ± 2.50 days, $P < 0.05$; 24.60 ± 6.55 *vs* 29.00 ± 3.16 days, $P < 0.05$). The fungal burden in the kidney of mice increased in the PF alone and PF + FCZ treated groups compared with the Infected alone or FCZ treated group. Furthermore, it was found that the PF and PF + FCZ treated groups showed significantly decreased levels of serum interferon gamma (IFN-γ), interleukin (IL)-17, and IL-22, and an increased level of serum IL-4; PF had no effect on the production of tumor necrosis factor alpha (TNF-α). PF alone or in combination with FCZ decreased the proliferation of Th1 (IFN-γ^+CD4$^+$) and Th17 cells (IL-17$^+$CD4$^+$) and increased the expression of Th2 cells (IL-4$^+$CD4$^+$). These results suggested that PF treatment could be detrimental to the host response to systemic *C. albicans* infection in mice. Thus, caution might be required for clinical use of PF in patients with fungal infection.

1. Introduction

Candida albicans (*C. albicans*), as a common fungal pathogen of humans, may colonize asymptomatically in skin, gastrointestinal tract, and oral and vaginal mucosa in 30%–70% of individuals. Overgrowth of *C. albicans* often results in candidiasis [1] and even severe systemic *C. albicans* infection in immunocompromised patients, thus leading to high morbidity and mortality [2,3]. Although antifungal agents, such as fluconazole (FCZ), ketoconazole, and voriconazole, have been used widely and effectively, resistance to these azole drugs is emerging as a problematic issue in clinical practice [4]. Further, in addition to the virulence of *Candida* and the efficacy of antifungal agents, host defense is implicated in the progression and prognosis of disseminated candidiasis (systemic *C. albicans* infection) [5]. It has been advocated that modulating the host's immune system may be an optional strategy to treat infectious diseases including fungal infection [6].

Paeoniflorin (PF), the principal bioactive component of total glucosides of peony, has been reported to have different pharmacological effects, such as antioxidative, anti-inflammatory, and hepatoprotective effects [7]. Because of its safety and immunoregulatory effects, PF has been widely used in treating various inflammatory and autoimmune diseases, such as systemic lupus erythematosus [8], rheumatoid arthritis [9], Sjogren's syndrome [10], allergic contact dermatitis [11], and psoriasis [12]. PF may also prevent acute lung injury, renal dysfunction, and cardiac systolic impairment in the lipopolysaccharide (LPS)-induced endotoxemia mouse model [13,14]. Previously, PF was found to inhibit noninfectious inflammatory response in the allergic contact dermatitis models both *in vivo* and *in vitro* [11,15]. A study found that PF inhibited the biofilm formation of *C. albicans in vitro* [16]. It was speculated that PF *per se* or in combination with FCZ might inhibit fungal infection or reduce inflammation *in vivo*.

T cells and their cytokines are known to play a central role in antifungal infection. It has been suggested that CD4$^+$ T cell subsets, such as Th1 [interferon gamma (IFN-γ)$^+$CD4$^+$] and Th17 [interleukin (IL)-

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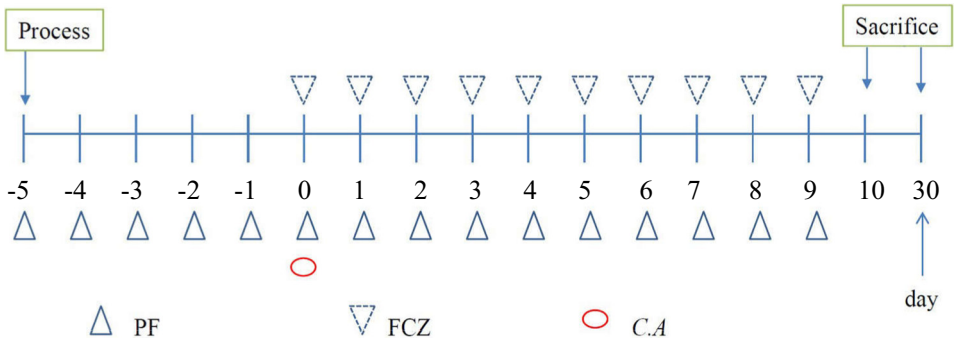

Fig. 1. BALB/c mice were randomly assigned into five groups and treated intragastrically with normal saline, PF, or FCZ. Design of systemic *C. albicans* infection model and experimental groups: Sixe groups of BALB/c mice aged 4 to 8 weeks, each containing 10 mice, were used to monitor survival of mice with PF or PF + FCZ treatment and systemic *C. albicans* infection. In the second part, each group contained 20 mice, which were used to monitor the immune responses of treating mice.
The mice were pretreated by gastro gavage with normal saline or PF for five consecutive days (from day −5 today −1) before infection of *C. albicans*. Systemic infection model was established by injecting the standard strain of *C. albicans via* caudal vein on day 0. Infected alone and Vehicle groups were administrated normal saline continually each day. On day 0, FCZ and PF + FCZ groups were treated with FCZ (1.0 mg/kg) for 10 consecutive days (from day 0 to day 9). The PF and PF + FCZ groups were also treated with PF by gastro gavage for 10 consecutive days (from days 0 to 9). On days 10 and 30, five mice were randomly chosen from every group and sacrificed by cervical dislocation.

17^+CD4^+/IL-22^+CD4^+] cells, are important in immune response against fungal infection [17]. In contrast, Th2 cells ($CD4^+$IL-4^+) were found to play a detrimental role in most fungal diseases [18]. Furthermore, cytokines are critical in both the innate and adaptive arms of the immune response to candidiasis. Of note, IFN-γ is involved in regulating the immune system and controlling the infection process, including fungal infection [19]. Besides, subsequent signaling through both the IL-17 and IL-22 receptors results in the cooperative upregulation of neutrophil chemokines, antimicrobial peptides, and other pro-inflammatory cytokines. Collectively, these downstream effector molecules provide a means by which the Th17 pathway mediates the clearance of extracellular pathogens [20]. In contrast, the susceptibility of a mouse to primary systemic *C. albicans* infection is associated with anti-inflammatory cytokines such as IL-10 and IL-4. It is known that IL-10 and IL-4 sustain a Th2 response, suppress the induction of a Th1 response, and downregulate phagocyte candidacidal activity [21].

The present study sought to investigate the effects of PF alone or PF + FCZ on fungal infection in a mouse model. It was found that applying PF solely could shorten the survival of systemic *C. albicans* infection and increase fungal burden in the mouse model. Meanwhile, an antagonistic effect of PF + FCZ was also observed with decreased survival and increased kidney fungal burden. Furthermore, PF could decrease the serum level of IFN-γ, IL-17, and IL-22 and promote the secretion of IL-4 in the infected mouse model. In accordance with these results, PF could decrease the proliferation of Th1 and Th17 and induce the expression of Th2 in mice with systemic *C. albicans* infection.

2. Materials and methods

2.1. Regents and chemicals

PF was purchased from Mansite Technology Ltd. (Chengdu, China), with a purity of > 98.5% determined by high-performance liquid chromatography. PF was dissolved in sterilized saline as a stock solution at 8 mg/mL concentration (75 mg dissolved in 9 mL of sterile saline and 375 μL of ethyl alcohol). Fluconazole and sodium chloride were produced by Hui Rui Pharmaceutical Factory.

All the mice were aged 4–8 weeks, weighing 18–20 g. The animals were housed with five mice per cage at 22 °C with a 12-h light-dark cycle. Water and standard diet were available *ad libitum*.

According to the government guidelines for animal care, all animal experiments were approved by the Animal Study Committee of the Institute of Dermatology at Chinese Academy of Medical Sciences (CAMS) in Nanjing, Jiangsu, China. Our experiment mainly consisted of two parts: part one aimed to determine survival rate, and part two sought to observe the immune responses of treating mice. Six groups of BALB/c mice aged 4 to 8 weeks, each including 10 mice, were used to monitor survival of mice on PF or PF + FCZ treatment and systemic *C. albicans* infection. In the second part, we have provided detailed description of the numbers; six groups of BALB/c mice aged 4 to 8 weeks, each including 20 mice, the total numbers of mice in second part were 120, which were used to monitor the immune responses of treating mice.

C. albicans strain (ATCC5314) was provided by the Department of Mycology, CAMS. It was routinely grown at 30 °C on Sabouraud dextrose agar. The concentration was then adjusted to the appropriate values for the experiment by the bacterial turbid-metric method.

2.2. Experimental design and C. albicans animal model

BALB/c mice were randomly assigned into six groups and treated intragastrically with normal saline, PF, or FCZ. Mice in the FCZ treated group were infected with *C. albicans* and received FCZ (from day 0 to day 9), the mice in the PF treated group received PF each day for 15 days (from day −5 to day 9) prior to and after infection with *C. albicans*, and the mice in the PF + FCZ treated group were infected with *C. albicans* and received PF (from day −5 to day 9) and FCZ (from day 0 to day 9). The mice in the Infected alone group, which were infected with *C. albicans* without being treated with PF or FCZ, were included as a control group along with a group of mice that only received normal saline. The mice in the only PF group received PF each day for 15 days (from day −5 to day 9) without infecting with *C. albicans*. The mice were challenged *via* the lateral tail vein with 0.5 mL of a *C. albicans* suspension containing 1.25×10^6 colony-forming units. The dosage of PF solution and FCZ was 100 mg/kg and 1.0 mg/kg, respectively. The mice in the Vehicle group were given only normal saline without being infected with *C. albicans* and treated with PF or FCZ (Fig. 1).

2.3. Survival of mice in different treatment groups

Once the mice were successfully infected with *C. albicans*, the general state of the experimental animals was observed, including breathing, appetite, spirit, activity stimulus-response, and hair condition. The survival status of mice in each group was monitored and recorded for 30 days.

2.4. Determination of fungal burden

After establishing the aforementioned *C. albicans*-infected mouse model again (Fig. 1), all six groups were sacrificed by cervical dislocation 10 and 30 days after infection. The kidney, liver, and spleen were collected, weighed, and homogenized in 5 mL of sterile saline. Then, 50 μL of this bacterial suspension was placed on Sabouraud dextrose agar (SDA) plates and incubated for 48 h at 30 °C for CFU/g to determine the fungal burden in the kidney, spleen, and liver.