Original Article
Glycyrrhizin combined with acitretin improve clinical symptom of psoriasis via reducing Th17 cell differentiation and related serum cytokine concentrations

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Abstract: Objective: To evaluate the effect of compound glycyrrhizin in combination with acitretin on Th17 cell and related cytokines expressions in patients with psoriasis. Methods: A total of 100 patients with psoriasis were enrolled in our study and randomized into an acitretin (Aci) group (n = 50) and a compound glycyrrhizin/acitretin combined treatment (Aci + Glyc) group (n = 50). Both groups were medicated with 3 × 10 mg acitretin per day for 8 weeks but the (Aci + Glyc) group received additionally 3 × 75 mg compound glycyrrhizin every day. A total of 50 healthy individuals were selected as the control group. The peripheral blood Th17 cell percentage as well as the IL-6, IL-17, IL-22 and TGF-β serum concentrations in addition to PASI scores were determined before and after medications. Results: The Th17 cell percentages and the serum concentrations of IL-6, IL-17, IL-22 and TGF-β in the Aci and Aci + Glyc groups were significantly higher than those in the control group before treatments (P < 0.05) and were significantly declined after the treatments (P < 0.05), but to a higher extend in the Aci + Glyc group (P < 0.05). The clinical treatment effective rates in the Aci group and the Aci + Glyc group were 76.0% and 90.0% (P < 0.05) compared to invalid events after treatments. Conclusions: Compound glycyrrhizin in combination with acitretin can improve the clinical efficacy significantly when compared with of the solely acitretin medication for psoriasis treatments via down regulating Th17 cell differentiation.

Keywords: Psoriasis, compound glycyrrhizin, acitretin, Th17

Introduction
Psoriasis is a chronic inflammatory disease affecting the skin and joints. An analysis of 53 studies suggests higher prevalence north of the equator, in lighter-skinned populations, and in older people. In the U.S., prevalence ranged from 2.2% to 3.15%; in the U.K., the range was 1.3% to 2.6% and closer to the equator (Latin America, India, Egypt, Tanzania, China, Sri Lanka, and Taiwan), prevalence rates were below 0.5% [1]. Psoriasis is now classified as an immune-mediated inflammatory disease (IMID) [2] and the interleukin 23 (IL-23)/IL-17 axis has been identified as a crucial mechanisms for psoriasis lesion development. Transforming growth factor-beta (TGF-β) and IL-6 trigger the differentiation of naive CD4+ T cell precursors into IL-10 and IL-17 producing T helper cells (Th17) cells that are important for mucosal defense, but exposure to IL-23 inhibits IL-10 expression and induces Th17 cell pathogenicity [3, 4]. These pathogenic Th17 cells are causing epidermal hyperproliferation and differentiation inhibition in psoriatic lesions mainly via IL-22 and IL-17 signaling [5-8]. Most patients diagnosed with psoriasis suffer from mild forms, for which topical medications and phototherapy are sufficient treatments. However, 20%-30% of affected patients develop moderate to severe symptoms, which usually need systemic therapies. In recent years, acitretin, a second generation synthetic retinoid, has been recommended for use in severe extensive...
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psoriasis cases, which are resistant to other forms of therapy, including topical, light as well as for palmoplantar pustular psoriasis [9]. Since its development it has been proven useful in a number of difficult-to-treat hyperkeratotic and inflammatory dermatoses [10-14]. Compound glycyrrhizin, a traditional Chinese medicine (TCM) derived from Glycyrrhiza uralensis, Glycyrrhiza glabra and Glycyrrhiza inflata is used to treat peptic ulcer, hepatitis C, as well as pulmonary and skin diseases, although clinical and experimental studies suggest that it has several other useful pharmacological properties such as antiinflammatory, antiviral, antimicrobial, antioxidative, anticancer activities, immunomodulatory, hepatoprotective and cardioprotective effects [15, 16]. It has been shown, that glycyrrhizic acid reduced the production of LPS-induced tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β in a dose-dependent manner [17] and glycyrrhizic acid treatment ameliorated abnormal glutamate-induced alterations in DPC12 cell mitochondria [18]. In addition, it has been reported, that glycyrrhizin can relieve IgE-induced allergic diseases such as dermatitis and asthma [19]. In our present study, we investigated the effect of compound glycyrrhizin in combination with acitretin on Th17 cells and related cytokine expressions of patients with psoriasis.

Patients and methods

Patients

This study was approved by the ethics committee of the Shenzhen Second Peoples’ Hospital and all patients provided informed consent. A total of 100 patients who were diagnosed with psoriasis according to the 2008 Chinese psoriasis guideline and admitted to our hospital from September, 2013 to September, 2014 were enrolled in our study. The exclusion criteria were as the following: (1) patients with malignant tumors; (2) patients who suffered from heart, liver, and kidney function disorders; (3) patients who had also autoimmune diseases; (4) patients who were medicated with glucocorticoids or immunosuppressants in the recent 3 months. The Psoriasis Area and Severity Index (PASI) scores were evaluated according to previous literature [20]. The patients were randomized into conventional treatment (Aci) (n = 50) and combined treatment (Aci + Glyc) (n = 50) groups. In addition, 50 healthy individuals, who were collected from health check examinations at the same period served as the control group.

Medications

The patients in the Aci group received 10 mg acitretin capsules (Trade name: Fangxi, produced by Chongqin Winbon Pharmaceutical Company Ltd.) three times per day. The Aci + Glyc group received the same acitretin medication plus 75 mg compound glycyrrhizin tablets (Trade name: Meineng, produced by the Japan Mino Origination Pharmaceutical Joint-stock Corporation) three times every day. The treatment period was 8 weeks for both groups.

Detection of Th17 cell percentages in peripheral blood

To 50 μl morning fasting elbow venous blood CD4-PE antibodies (5 μl) and CD8-FITC antibodies (5 μl) (Beckman Coulter, CA, USA) were added and the mixture was incubated at 4°C for 30 min in darkness. Then 2 ml NH₄Cl hemolytic agent was added, the mixture was centrifuged and the supernatant was discarded. After that 1 ml stationary liquid was added and the mixture was incubated for 20 min in darkness. After centrifugation, the supernatant was discarded and 1 ml hemolytic membrane rupture liquid was added. After 45 min incubation in darkness, the mixture was centrifuged again, the supernatant was discarded and the pellet was washed twice with PBS. Then 10 μl PE anti IL-17 monoclonal antibody (Beckman Coulter, CA, USA) were added and the mixture was incubated for 30 min in darkness. After centrifugation, the supernatant was discarded and the pellet was washed twice with PBS. Finally, the cells were suspended in 300 μl PBS and the peripheral blood Th17 percentage was determined via flow cytometry (FCM) (Navi 105, Beckman Coulter Inc., CA, USA) with PE-IgG1 (Beckman Coulter, CA, USA) as control.

Measurement of blood serum cytokine concentrations

From all participants 5 ml morning fasting venous blood was collected and placed at room temperature for 2 h. After centrifugation at 3000 rpm for 10 min the serum was separated.
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and stored at 4°C for later analyses of interleukin-6 (IL-6), interleukin-17 (IL-17), interleukin-22 (IL-22), and transforming growth factor-β1 (TGF-β1) serum concentrations with indirect ELISA kits (Beckman Coulter, CA, USA) according to the manufacturer's instructions.

Clinical efficacy

An efficacy index [(PASI score before treatment - PASI score after treatment)/PASI score before treatment] × 100% [21] was used to estimate the clinical efficacy. The scoring was cured (efficacy index ≥ 90%), excellent (efficacy index < 90% and ≥ 60%), effective (efficacy index < 60% and ≥ 20%) and invalid (efficacy index < 20%). The treatment efficacy rate was calculated as [(cured number + excellent number + effective number)/total number] × 100%.

Statistical analysis

All statistical analysis was performed using SPSS for Windows (Version 16.0, Chicago, SPSS Inc.) and normal distribution data or measurement data are expressed as mean ± standard deviation; the groups were compared using the two independent sample t-test or ANOVA test. Mann-Whitney test was used for independent variables and chi square test for group comparisons. P values < 0.05 were regarded as statistically significant.

Results

Basic demographic data of the patients

Among the 50 cases in the conventional treatment group, 28 were male and 22 were female, aged from 20 to 70 years old with an average age of (43.8 ± 9.7) years and disease courses from 1 to 15 years with an average of (5.2 ± 2.4) years. The psoriasis area and severity index (PASI) scores were from 5 to 38 with an average of (17.2 ± 4.7). Among the 50 cases in the Aci and the Aci + Glyc groups, 26 were male and 24 were female, aged from 18 to 71 years old with an average age of (44.0 ± 9.2) years and disease courses from 1 to 12 years with an average of (5.0 ± 3.1) years. PASI scores were from 6 to 37 with an average of (17.8 ± 4.8). The comparison of age, gender, disease course, and PASI scores between the Aci and the Aci + Glyc groups revealed that there was no statistically significant difference between the groups (Table 1).

The peripheral blood Th17 cell percentage was significantly more reduced after the Aci + Glyc treatment

The pretreatment Th17 cell percentages in the Aci group and the Aci + Glyc group were (4.8 ± 1.3%) and (4.7 ± 1.4%) and significantly higher than in the control group (0.8 ± 0.3%) showing that Th17 cell percentages are enhanced in psoriasis patients. The Th17 cell percentages were significantly lowered in both groups, but the post interventional Th17 percentage was significantly lower in the Aci + Glyc than in the Aci treated patients (1.0 ± 0.4% vs. 2.6 ± 1.0%, P < 0.05) and not significant different from the control values only in the Aci + Glyc group (Table 2).

Table 1. Basic information of the patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>50</td>
<td>0.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Aci group</td>
<td>50</td>
<td>4.8 ± 1.3</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Aci + Glyc group</td>
<td>50</td>
<td>4.7 ± 1.4</td>
<td>1.0 ± 0.4</td>
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</tbody>
</table>

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<td>Aci group</td>
<td>50</td>
<td>4.8 ± 1.3</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Aci + Glyc group</td>
<td>50</td>
<td>4.7 ± 1.4</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

*P < 0.05, when compared with the control group; ^P < 0.05, when compared with the Aci group; _P < 0.05, when compared with the Aci + Glyc group.

Table 2. Comparison of peripheral blood Th17 cell percentages between the groups (mean ± SD) (%)

<table>
<thead>
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<th>Number</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
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<td>50</td>
<td>0.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Aci group</td>
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<td>4.8 ± 1.3</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Aci + Glyc group</td>
<td>50</td>
<td>4.7 ± 1.4</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

*P < 0.05, when compared with the control group; ^P < 0.05, when compared with the Aci group; _P < 0.05, when compared with the Aci + Glyc group.
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The expression of peripheral blood Th17 cell related cytokines was significantly elevated in the psoriasis patients. IL-6, IL-17, IL-22 and TGF-β expression levels in the psoriasis patients were significantly higher than those in the control group ($P < 0.05$). After the Aci and the Aci + Glyc treatments the blood serum concentrations of IL-6 were reduced by 12.6% and 36.0%, that of IL-17 by 37.5% and 67.9%, that of IL-22 by 32.8% and 57.9% and TGF-β serum concentrations were reduced by 13.8% and 34.9%, respectively. In both treatment groups the indicated cytokine concentrations dropped significantly, but in the Aci + Glyc group they were significantly lower than in the Aci group after the medications (Figure 1).

Glycyrrhizin could effectively enhance the curative effects of acitretin in the treatment of psoriasis

The clinical efficacy rates were superior in the Aci + Glyc than in the Aci group, since from the former patients 25 were cured and from the latter patients only 18 cases. Also in the excellent scored patients the number in the Aci + Glyc group was 1.5 times higher than in the Aci group. A Chi-square test comparing Aci and Aci + Glyc group trends revealed a significant difference ($P = 0.0204$) and the treatment efficacy rates in the Aci and the Aci + Glyc groups were 76.0% and 90.0% ($P < 0.05$) (Table 3).

Discussion

In our study, the treatment efficacy rates in the Aci and the Aci + Glyc groups were 76.0% and 90.0% ($P < 0.05$) indicating that compound glycyrrhizin can effectively relieve patients from psoriasis lesions. On the other hand, the lower efficacy of acitretin might be due to the applied dose of 30 mg/day, which is less than dosages recommended in other studies [13, 22]. The psoriasis patients had significantly enhanced serum cytokine concentrations, which is in accordance with previous literature, that IL-6 [23], IL-17 and IL-22 [24, 25] as well as TGF-β [26] blood serum concentrations are increased in psoriasis affected persons. Both treatments led to significant reductions of Th17 cell differentiation and IL-6, IL-17, IL-22 and TGF-β expressions. Previous literature reported, that retinoic acids inhibited the development Th17 cells induced by TGF-β and IL-6 [27-29], which is in accordance with our data, that acitretin significantly reduced the percentage of Th17 cells in

Table 3. Comparison of the clinical efficacy between the two groups (N, %)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Cured</th>
<th>Excellent</th>
<th>Effective</th>
<th>Invalid</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aci group</td>
<td>50</td>
<td>18 (36.0)</td>
<td>10 (20.0)</td>
<td>10 (20.0)</td>
<td>12 (24.0)</td>
<td>0.0204*</td>
</tr>
<tr>
<td>Aci + Glyc group</td>
<td>50</td>
<td>25 (50.0)</td>
<td>15 (30.0)</td>
<td>5 (10.0)</td>
<td>5 (10.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test for evaluation of Aci and Aci + Glyc group trend differences.

Figure 1. Expression levels of peripheral blood Th17 cytokines in the indicated groups group (mean ± SD) (ng/L). Pre = pre-treatment, Post = post-treatment. Note: In the pre-treatment group, *$P < 0.05$ compared to control; in the post-treatment group, *$P < 0.05$ compared to control and $\Delta P < 0.05$ compared to pre-treatment.

The expression of peripheral blood Th17 cell related cytokines was significantly elevated in the psoriasis patients.
psoriasis patients. Th17 cells play a central role in the pathogenesis of tissue inflammation in autoimmune diseases [30, 31]. Mease (2015) recently reviewed the efficacy and safety of new medications targeting the Th17 pathway, including inhibition of IL-17 and IL-23 particularly in psoriasis patients who have not gained benefit from or could not use anti-tumor necrosis factor (TNF) medications for safety or tolerability reasons [32]. Th17 cells secrete IL-22 in addition to IL-17 and also increase serum and cutaneous eruption site IL-23 expression in psoriatic patients [33]. Cytokines, such as IL-22 inhibit epidermal differentiation and induce pro-inflammatory gene expression and migration of human keratinocytes [5, 7]. The expression levels of TGF-β, and IL-6, are the key factors of Th cell differentiation and several studies showed, that glycyrrhizic acid reduced IL-6 and IL-17 expressions [17, 34, 35], which might block the differentiation of Th17 cells or inhibit IL-17 signaling. Another study showed that glycyrrhizic acid induced the generation of burn-induced CD8+ type 2 T cell activity counteracting anti-type 2 T cells (anti-BI2T cells) [36, 37] and an enhanced frequency of CD8+ type 2 T cells in psoriasis patients has been detected [38].

Taken together, our study revealed that compound glycyrrhizin effectively could suppress Th17 differentiation and signaling, but explanations for molecular mechanisms need further research.

In conclusion, we found that the peripheral blood cytokine concentrations of IL-6, IL-17, IL-22 and TGF-β as well as the Th17 cell percentage were significantly more reduced to levels close to those in normal healthy individuals by addition of compound glycyrrhizin to an acitretin medication, suggesting the combined use of compound glycyrrhizin and acitretin can produce a synergistic effect by antagonizing Th17 cell related mechanisms.

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**Disclosure of conflict of interest**

None.

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