

# Oxymatrine therapy inhibited epidermal cell proliferation and apoptosis in severe plaque psoriasis

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**Linked Comment:** Groma. *Br J Dermatol* 2019; **181**:891–892.

## Summary

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### Accepted for publication

28 February 2019

### Funding sources

The Natural Science Foundation of Ningxia (NZ17154).

### Conflicts of interest

None declared.

H.-J.S. and H.Z. contributed equally to this work.

DOI 10.1111/bjd.17852

**Background** Psoriasis is a chronic skin disorder that manifests as epidermal keratinocyte hyperplasia.

**Objectives** We examined the effect of oxymatrine treatment on cell proliferation and apoptosis in skin lesions of psoriasis.

**Patients and methods** Patients with severe plaque psoriasis were treated with oxymatrine or with acitretin. The skin lesions were stained with proliferating cell nuclear antigen (PCNA), Ki-67 and Bcl-2, as well as examined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL). We performed correlations of the Psoriasis Area and Severity Index (PASI) and the proliferation and apoptosis index.

**Results** Oxymatrine significantly reduced the psoriasis lesions as demonstrated by the reduced PASI score after treatment [6.91; 95% confidence interval (CI) 5.00–8.81,  $P < 0.001$ ]. In the oxymatrine group, the mitotic index was 26.15 (95% CI 24.80–27.49) before oxymatrine treatment, decreasing to 14.52 (95% CI 13.82–15.25;  $P < 0.001$ ) after treatment, but remained higher than the normal group (6.24; 95% CI 5.87–6.61,  $P < 0.001$ ). Oxymatrine also inhibited the proliferation of epidermal cells in the skin lesion as indicated by the reduced proliferation index after treatment ( $P < 0.01$ ). In addition, oxymatrine treatment reduced cellular apoptosis as shown by increased Bcl-2 expression and a decrease in TUNEL-positive cells. The PASI score was positively correlated with mitotic index, proliferation index and apoptotic index (TUNEL), but negatively correlated with Bcl-2 expression.

**Conclusions** Oxymatrine treatment reduced proliferation but inhibited apoptosis of cells in the skin lesion. The balance between cell proliferation and turnover may contribute to the significant alleviation of psoriasis by oxymatrine.

### What's already known about this topic?

- Psoriasis manifests as epidermal keratinocyte hyperplasia with proliferation, keratinocyte maturation and turnover rates.
- Current drugs for psoriasis may inhibit cell proliferation but could not adjust the balance of cell division, differentiation and apoptosis.

### What does this study add?

- We studied the efficacy of oxymatrine in the treatment of psoriasis and analysed the correlation of skin lesions, proliferation and apoptosis index before and after oxymatrine treatment.

### What is the translational message?

- Our study has demonstrated that oxymatrine is effective in the treatment of severe plaque psoriasis.
- It has comparable efficacy with acitretin.
- Because acitretin treatment was sometimes associated with metabolic abnormalities, our study suggests oxymatrine therapy as an alternative treatment for psoriasis in the context of acitretin allergy or adverse reactions.

Psoriasis is a common chronic inflammatory disease of the skin characterized by erythematous plaques with hyperkeratosis that produce the classic silvery scales. The pathogenesis of psoriasis involves a complex cutaneous inflammatory response and abnormal proliferation and differentiation of keratinocytes.<sup>1</sup> Epidermal keratinocyte hyperplasia with proliferation, maturation and turnover are important mechanisms in the development of psoriasis.<sup>2,3</sup> Current treatments of psoriasis include retinoic acid-based regimens, immunosuppressors (e.g. acitretin,<sup>4</sup> methotrexate<sup>5</sup>), vitamin D3,<sup>6</sup> phototherapy,<sup>7</sup> topical applications of corticosteroids<sup>8</sup> and other biological agents.<sup>9,10</sup> To date, there is no cure for psoriasis and no single psoriasis treatment works universally. The side-effects of current treatments also underscore the need for new pharmacological therapies for psoriasis.

Oxymatrine is an alkaloid extracted from the leguminous plant *Sophora flavescens* Ait, *S. alopecuroides* or *S. subprostrata* Chun et T. Chen. Oxymatrine has been shown to have anti-inflammatory and antioxidant properties. Due to its inhibitory activity on cell proliferation, oxymatrine has been used to treat tumours, hepatitis and cirrhosis.<sup>11,12</sup> A clinical trial using oxymatrine to treat patients with hepatitis has demonstrated the safety of oxymatrine with minimal adverse effects.<sup>13</sup> Our preclinical study showed that oxymatrine was effective in improving psoriatic skin lesions. In the present study, we examined the underlying mechanism of action of oxymatrine on cell proliferation and apoptosis in psoriatic lesions. The skin tissues stained with proliferating cell nuclear antigen (PCNA), Ki-67, Bcl-2 and apoptotic cells were identified by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay.

## Patients and methods

### Patients

Patients were recruited from December 2012 to July 2016 at the General Hospital of Ningxia Medical University. They were diagnosed with psoriasis by clinical and/or histopathological examination in the outpatient department. The research project was approved by the Ethics Committee of Ningxia Medical University and all patients gave informed signed consent (Clinical trial registration number: ChiCTR-TRC-14004301).

The inclusion criteria were severe plaque psoriasis; Psoriasis Area and Severity Index (PASI) score  $\geq 12$ ;<sup>14,15</sup> disease course  $\geq 6$  months; and at least one previous course of systemic treatment.

The exclusion criteria were allergy to oxymatrine or acitretin; guttate psoriasis; erythrodermic psoriasis; psoriasis arthropathica; pustular psoriasis; presence of severe liver and kidney damage, mental illness, haematopoietic dysfunction, or other serious organic disease; treatment with immunosuppression or high doses of glucocorticoids or retinoid in the past 8 weeks; pregnancy; and lactation.

Criteria for removal from the trial: not using the drugs or using the drug without following instructions; stopping with inadequate treatment; noncompliance; severe adverse reactions, complications, or special physiological changes; refusing biopsy.

### Oxymatrine or acitretin treatment and skin lesions

Oxymatrine has been used to treat solid tumours, with a dose of 1000–1500 mg intravenously, once daily for 30–45 days.<sup>16,17</sup> Moreover, our preliminary clinical findings indicated that oral 0.6 g daily oxymatrine had little effect but intravenous administration produced a significant effect.<sup>18</sup> Furthermore, studies have shown that the absolute bioavailability of oxymatrine by the oral route was very low.<sup>19</sup> We therefore chose 0.6 g daily through intravenous drip (in outpatient clinics). Patients in the oxymatrine group were treated with 0.6 g/100 mL of oxymatrine (Tianqingfuxin; Zhengdianqing Company Ltd, Jiangsu, China) intravenously once daily for 8 weeks. Patients in the acitretin group were treated with oral acitretin capsules (Fangxi; Chongqing Huabang Co. Ltd, Chongqing City, China) at 0.75 mg kg<sup>-1</sup> once daily as a starting dose, and the dosage was reduced to 20–30 mg daily according to the patient's weight as a maintenance dose after 2 weeks when the drug took effect, and acitretin was administered orally for 8 weeks. No other topical ointment was used as adjuvant therapy. Full-thickness skin lesions (about 0.8 × 1.0 cm before and after treatment) were acquired with local anaesthesia. Normal, nonpsoriatic skin samples were obtained from abandoned healthy skin of patients in the operating room of the Department of Burn and Plastic Surgery. Informed consent was obtained from the patients.

## Clinical evaluation

Psoriatic skin lesions were observed during the course of treatment, and PASI scores were calculated accordingly.<sup>14,15</sup>

## Immunohistochemistry

Skin specimens were sectioned and stained with haematoxylin and eosin. Cell mitosis was observed under light microscopy and the number of cells in mitotic division out of 300 epithelial basal layer cells was counted. At high magnification, increase in mitotic cell volume, nuclear concentration, and irregular splits or multicore states were counted. The mitotic index (%) was calculated in terms of number of mitotic cells per 100 basal cells.

Cell proliferation or apoptosis was assessed using sections of skin specimens stained with mouse antihuman PCNA monoclonal antibody (mAb), mouse antihuman Ki-67 mAb or mouse antihuman Bcl-2 mAb. All antibodies were purchased from Zhongshanjinjiao Company Ltd, Beijing, China. The stained slides were observed under a BX51 Olympus biological microscope (Olympus; Tokyo, Japan). PCNA or Ki-67 positive cells were stained with intranuclear granular deposits whereas Bcl-2 staining was accomplished with intracytoplasmic granular deposits.

According to the scoring methods of Garcia *et al.*,<sup>20</sup> two indicators were observed: (i) score of 1–3 according to positive cell percentage of 0–20%, 21–50%, 51–100%; (ii) Light stain was recorded as 1 and dark stain was recorded as 2. Combining the two indicators, a score of 2 was recorded as (+), 3 was (++) and  $\geq 4$  was (+++).

## Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling method assay

Skin specimens were also stained with the TUNEL assay using the Dead End™ Colorimetric TUNEL System kit (Promega, Madison, WI, U.S.A.) following the manufacturer's instructions. We used Image-Pro Plus Version 6.0 image analysis software (Media Cybernetics, Rockville, MD, U.S.A.) to analyse the images. Cells within five fields of each slide were analysed with high magnification ( $\times 400$ ) and the apoptosis index (AI) was calculated as the number of apoptotic cells out of every 100 cells.

## Statistical analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, U.S.A.). Quantitative data were expressed as mean  $\pm$  SD. The difference between two groups was compared using an unpaired Student's *t*-test. A chi-square test was used for comparison of categorical data. The analysed samples showed a normal distribution. Correlations were assessed by calculation of Pearson's correlation coefficient. A *P*-value  $< 0.05$  was considered statistically significant.

## Results

### Patients and clinical Psoriasis Area and Severity Index scores

A total of 79 patients were recruited into our study: 42 received oxymatrine and 37 received acitretin treatment. During the course of therapy, two and four participants were lost to follow-up in the oxymatrine and acitretin groups, respectively. As for the participants who finished the treatments, 12 of 40 patients in the oxymatrine group and nine of 33 patients in the acitretin group received both pre- and post-treatment biopsies. Other participants refused skin biopsy at either one or both time points. We included normal skin from 10 healthy participants without related skin lesions as normal controls (Table 1; Fig. 1).

In the oxymatrine group, PASI scores were not statistically significant between the 12 patients before treatment [23.6; 95% confidence interval (CI) 18.26–28.94, *P* = 0.701] and the 28 patients who refused skin biopsy (24.9; 95% CI 20.91–28.91) (Fig. 2b). There remains no statistical significance in the PASI scores between patients who refused skin biopsy and those who agreed to skin biopsy after treatment (*P* = 0.562). Therefore, the 12 patients from whom we obtained skin biopsies are an adequate representation of the total 40 patients of the group. In the acitretin group, PASI scores were not statistically significant between the nine patients before treatment (23.4; 95% CI 20.04–30.72, *P* = 0.900) and the 24 patients who refused skin biopsy (24.9; 95% CI 20.50–29.30). After treatment, there was still no statistical significance between patients who refused skin biopsy and those who agreed to skin biopsy after treatment (*P* = 0.794). After 8 weeks of treatment with intravenous oxymatrine, psoriatic skin lesions improved significantly compared with pretreatment, as revealed by the change of erythema to dark, reduced scales and thinner lesions (Fig. 2a). The PASI score after oxymatrine treatment was 6.91 (95% CI 5.00–8.81), a significant decrease relative to pretreatment values (24.5; 95% CI 21.41–27.62, *P*  $< 0.001$ ). The PASI score after acitretin treatment (7.41, 95% CI 5.74–9.08) was also significantly reduced compared with pretreatment values (25.03, 95% CI 21.66–28.40, *P*  $< 0.001$ ) (Fig. 2c).

**Table 1** Patient characteristics

Groups	n	Age, years <sup>a</sup>	Sex (F : M)	Disease course, years <sup>a</sup>
Oxymatrine group	40	32.1 $\pm$ 11.71	21 : 19	6.5 $\pm$ 6.72
No biopsy	28	32.1 $\pm$ 12.17	14 : 14	5.8 $\pm$ 7.04
Biopsy	12	32.1 $\pm$ 11.06	7 : 5	8.2 $\pm$ 5.81
Acitretin group	33	33.9 $\pm$ 10.64	17 : 16	6.7 $\pm$ 7.01
No biopsy	24	33.5 $\pm$ 10.63	11 : 13	5.9 $\pm$ 7.25
Biopsy	9	35.1 $\pm$ 11.22	6 : 3	8.8 $\pm$ 6.18
Control	10	31.8 $\pm$ 12.05	6 : 4	–

<sup>a</sup>Mean  $\pm$  SD

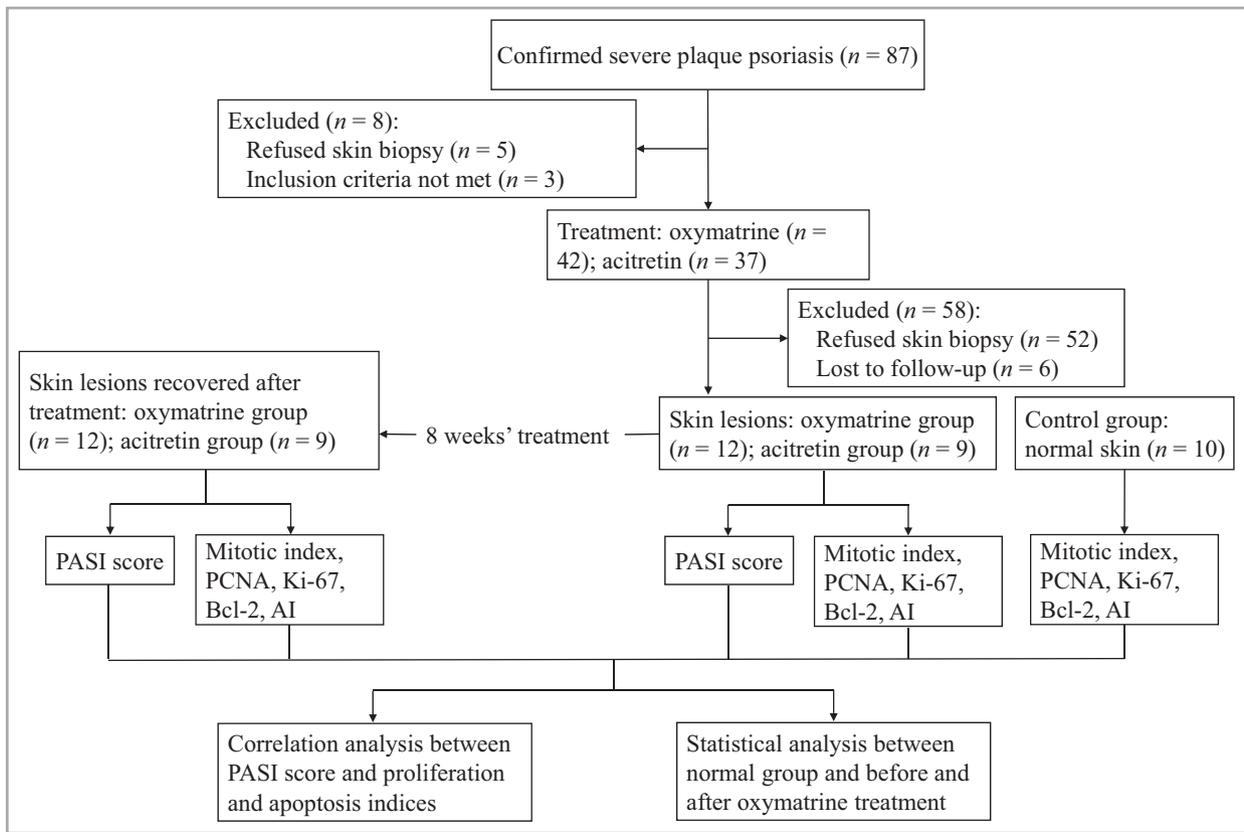


Fig 1. Study flowchart. AI, apoptosis index; PASI, Psoriasis Area and Severity Index; PCNA, proliferating cell nuclear antigen.

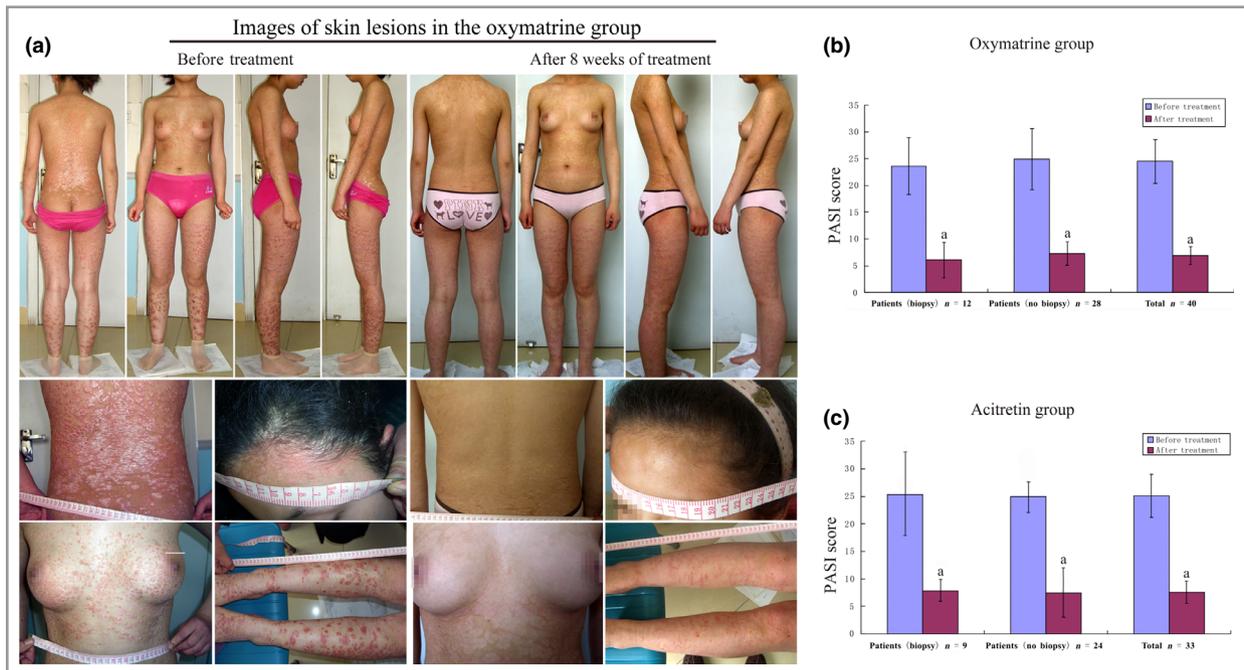


Fig 2. (a) Clinical photographs of skin lesions in the oxymatrine group, before and 8 weeks after treat. (b, c) Psoriasis Area and Severity Index (PASI) score before and after treatment, with (b) oxymatrine and (c) acitretin. <sup>a</sup>P-values vs. before treatment (95% confidence interval); <sup>a</sup>P < 0.001.

## Staining results

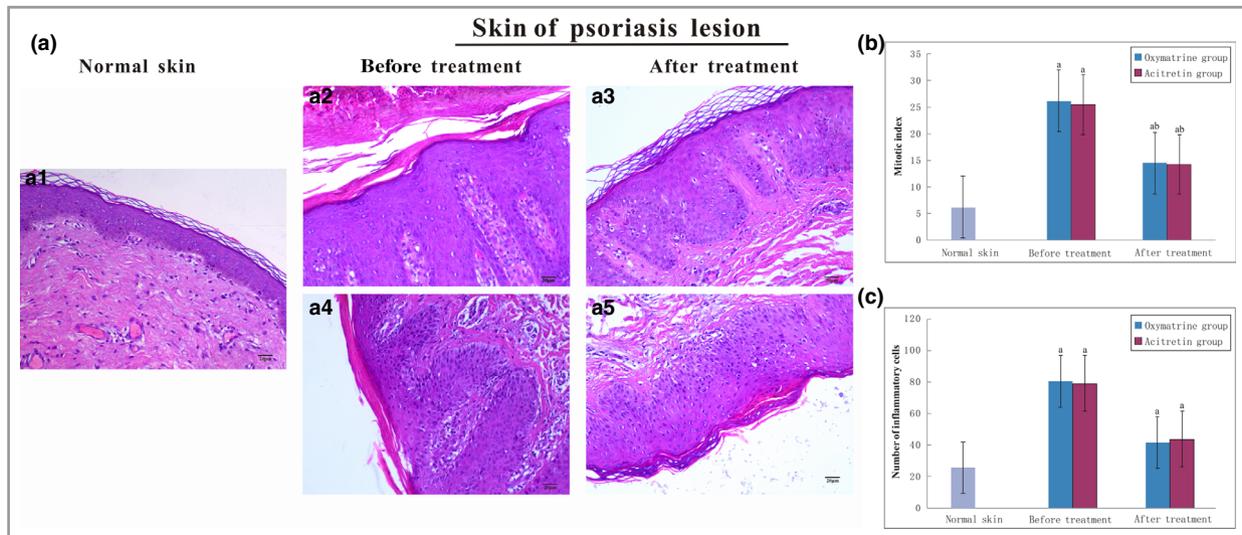
Compared with normal skin, the epithelial cells in the psoriatic skin lesions before treatment were multilayered with obvious parakeratosis and hyperkeratosis. Munro microabscesses were found in hyperkeratotic areas. The granular layer was thin and disappearing. The elongated rete ridges of the epidermis were associated with acanthosis, clubbing of the dermal papillae, dilation and oedema of the capillaries in the superficial dermis. Many of the cells were hyperchromatic with enlarged nuclei. In contrast, oxymatrine and acitretin treatment significantly decreased skin hyperkeratosis and eliminated parakeratosis. The prickle layer was thinner with less dilation of the capillaries (Fig. 3a).

There were fewer cells in active mitosis within the basal layer of the normal skin while the epidermis was well differentiated (Fig. 3a1). In contrast, skin lesions from patients with psoriasis were undergoing active mitosis and cell proliferation (Fig. 3a2, a4) and the number of cells in mitosis within the basal layer decreased dramatically after treatment (Fig. 3a3, a5). In the oxymatrine group, the mitotic index was 26.15 (95% CI 24.80–27.49) before oxymatrine treatment, decreasing to 14.52 (95% CI 13.82–15.25;  $P < 0.001$ ) after treatment. In the acitretin group, the mitotic index was 25.46 (95% CI 23.55–27.37) before acitretin treatment, decreasing to 14.27 (95% CI 12.79–15.75;  $P < 0.001$ ) after treatment. The mitotic index of both the oxymatrine and acitretin groups remained higher than that of normal skin (6.24; 95% CI 5.87–6.61,  $P < 0.001$ ) (Fig. 3b). Inflammatory cell infiltration was also observed in the epithelial basal cell layer and the superficial dermis in the skin lesions before treatment. The number of inflammatory cells in the basal layer of the oxymatrine group was 80.42 (95% CI 76.93–83.90) before treatment and

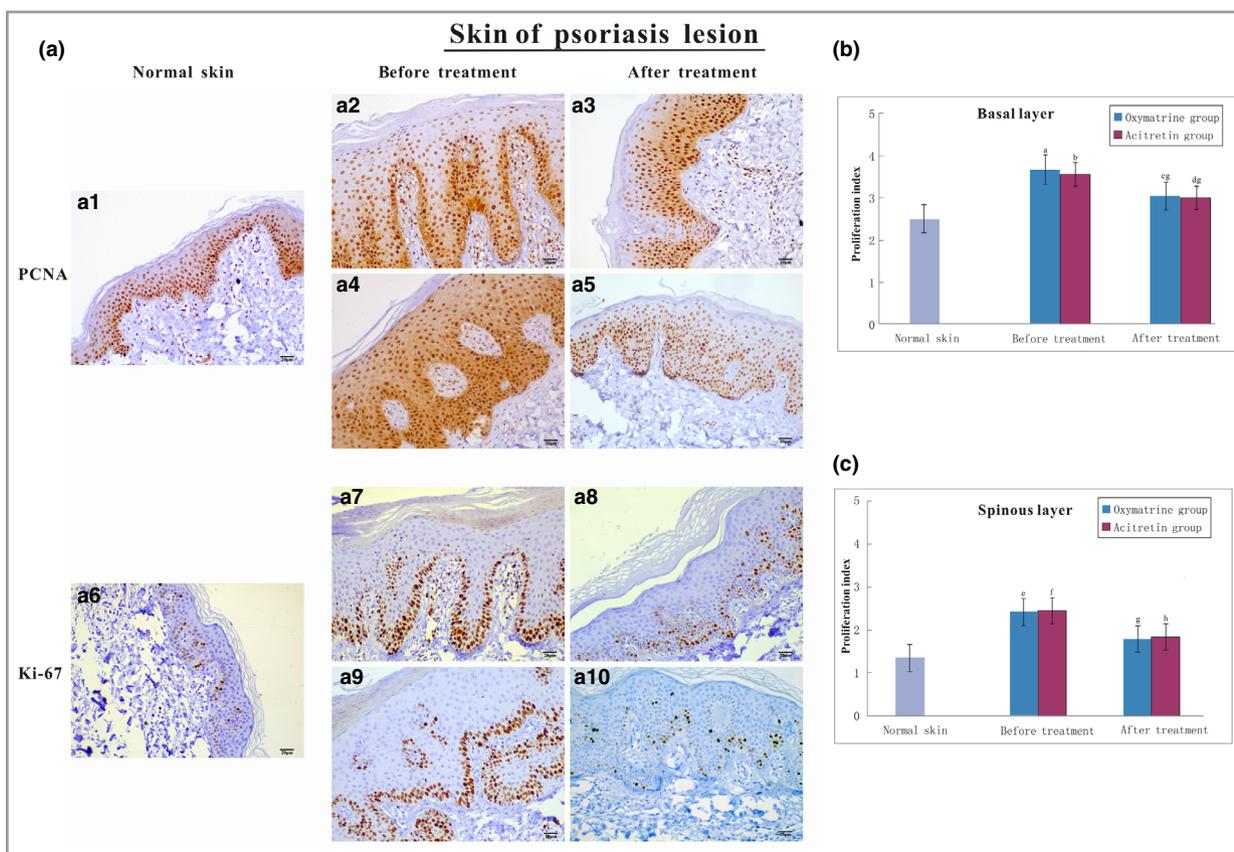
dropped to 41.75 (95% CI 37.35–46.15,  $P < 0.001$ ) after 8-week oxymatrine treatment. In the acitretin group, the number of inflammatory cells was 79.00 (95% CI 74.00–84.00) before treatment and dropped to 43.78 (95% CI 39.54–48.02,  $P < 0.001$ ) after the 8-week acitretin treatment (Fig. 3c).

Compared with limited PCNA and Ki-67 staining at the basal and spinous layers of the normal skin (Fig. 4a1, a6), psoriatic skin prior to oxymatrine treatment showed a significant increase in PCNA and Ki-67 at the basal layer (3.67; 95% CI 3.22–4.11,  $P < 0.001$ ) and spinous layer (2.42; 95% CI 1.83–3.00,  $P = 0.009$ ) (Fig. 4a2, a7). Similarly, skin samples from the acitretin group prior to treatment also showed an increase in the expression of PCNA and Ki-67 at the basal layer (3.56; 95% CI 3.04–4.07,  $P = 0.001$ ) and spinous layer (2.44; 95% CI 1.74–3.15,  $P = 0.014$ ) (Fig. 4a4, a9). After 8 weeks of oxymatrine treatment, the proliferation index (PCNA and Ki-67 expression) decreased significantly (Fig. 4a3, a8) in the basal layer (3.04; 95% CI 2.72–3.36,  $P < 0.001$ ) and spinous layer (1.79; 95% CI 1.24–2.35,  $P < 0.001$ ) compared with pretreatment levels. In the acitretin group, the proliferation index (PCNA and Ki-67 expression) decreased significantly in the basal layer (3.00; 95% CI 2.62–3.38,  $P < 0.001$ ) and spinous layer (1.83; 95% CI 1.19–2.48,  $P = 0.002$ ) compared with pretreatment levels (Fig. 4a5, a10). The proliferation index in the basal layer of both groups was still significantly higher than normal, healthy controls after treatment ( $P = 0.017$  in the oxymatrine group and  $P = 0.041$  in the acitretin group), but the proliferation index in the spinous layer was not significantly different compared with the normal group (Fig. 4b, c).

The expression of Bcl-2 in the normal skin samples was relatively high with moderate-to-strong positive staining of the basal layer (Fig. 5a1). In skin lesions of patients before



**Fig 3.** (a) Histopathological changes, (b) mitotic index and (c) number of inflammatory cells in normal skin and psoriatic skin before and after treatment. Representative haematoxylin and eosin staining of the skin lesion: (a1) normal skin; (a2, a4) before treatment; (a3, a5) after 8 weeks of treatment (scale bars represent 20  $\mu$ m). All bar graphs display mean fold enrichment  $\pm$  SEM. <sup>a</sup> $P$ -values vs. normal group (95% CI); <sup>b</sup> $P$ -values vs. before treatment (95% CI). <sup>a</sup> $P < 0.001$ ; <sup>b</sup> $P < 0.001$ . CI, confidence interval.



**Fig 4.** The proliferation index (total) in normal skin and psoriatic skin before and after treatment. (a1–a5) IHC staining for PCNA. (a1) normal skin; (a2, a4) before treatment; (a3, a5) after 8 weeks of treatment. (a6–a10) IHC staining for Ki-67; (a6) normal skin; (a7, a9) before treatment; (a8, a10) after 8 weeks of treatment (scale bars represent 20  $\mu$ m). All bar graphs display mean fold enrichment  $\pm$  SEM. <sup>a–f</sup>*P*-values vs. normal group (95% CI); <sup>g,h</sup>*P*-values vs. before treatment (95% CI); <sup>a</sup>*P* < 0.001; <sup>b</sup>*P* = 0.001; <sup>c</sup>*P* = 0.017; <sup>d</sup>*P* = 0.041; <sup>e</sup>*P* = 0.009; <sup>f</sup>*P* = 0.014; <sup>g</sup>*P* < 0.001; <sup>h</sup>*P* = 0.002. CI, confidence interval; IHC, immunohistochemistry; PCNA, proliferating cell nuclear antigen.

treatment, the expression of Bcl-2 in the oxymatrine group (2.67; 95% CI 2.25–3.08, *P* = 0.021) and acitretin group (2.67; 95% CI 2.12–3.21, *P* = 0.041) were significantly lower compared with that of normal skin (3.60; 95% CI 2.83–4.37; Fig. 5a2, a4). After oxymatrine treatment, Bcl-2 expression (3.50; 95% CI; 2.93–4.07) in these original skin lesions increased significantly (*P* < 0.001), and recovered to levels not significantly different from normal skin (*P* = 0.815; Fig. 5a3, b). Acitretin treatment also resulted in a significant increase in Bcl-2 expression (3.44; 95% CI 2.67–4.22) in these original skin lesions (*P* = 0.001) and recovered to levels not significantly different from normal skin (*P* = 0.750; Fig. 5a5, b). TUNEL staining revealed a few positive reactions in the superficial layer of the epidermis and a negative reaction in the dermis of normal skin. There was no obvious cellular apoptosis, as indicated by pyknosis, deformation and dissociation (Fig. 5a6). Psoriatic lesions before oxymatrine treatment showed a large number of scattered positive cells in the different layers of the epidermis. The AI values (0.25, 95% CI 0.23–0.27) of the oxymatrine group and acitretin groups (0.25, 95% CI 0.23–0.28) were significantly higher than that of normal skin (0.13, 95% CI 0.11–0.14, *P* < 0.001) (Fig. 5a7, a10). After oxymatrine treatment, the AI

(0.18; 95% CI 0.15–0.21; *P* < 0.001) decreased significantly compared with pretreatment levels, but remained higher than that of normal skin (*P* = 0.001; Fig. 5a8, a9, c). After acitretin treatment, the AI (0.15; 95% CI 0.13–0.17; *P* < 0.001) decreased significantly compared with pretreatment levels, but remained higher than that of normal skin (*P* = 0.014; Fig. 5a11, a12, c).

### Correlation analysis between clinical Psoriasis Area and Severity Index scores and laboratory index

We next examined correlations between clinical PASI scores and the laboratory data. All PASI scores were positively correlated with the overall mitotic index, proliferation index (PCNA and Ki-67 staining) and apoptotic index but negatively correlated with Bcl-2 expression in the different layers of the skin before and after treatment (Fig. 6).

### Discussion

Normal skin tissue has a well-differentiated epidermis with low levels of mitoses<sup>21</sup> whereas psoriasis involves hyperkeratosis of epidermal cells and inflammation.<sup>22,23</sup> Dermal pathology of

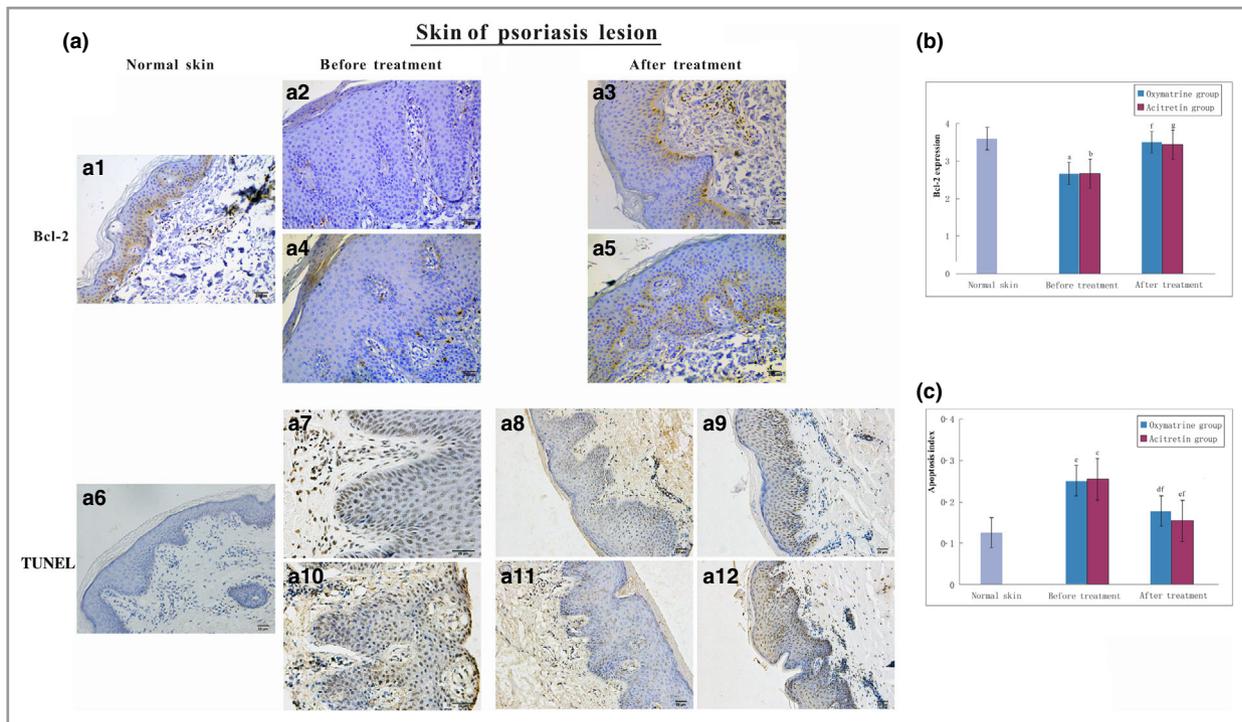


Fig 5. The apoptosis index (total) in normal skin and psoriasis skin before and after treatment. (a1–a5) IHC staining for BCL-2; (a1) normal skin; (a2, a4) before treatment; (a3, a5) after 8 weeks of treatment. (a6–a12) TUNEL staining; (a6) normal skin; (a7, a10) before treatment; (a8, a9, a11, a12) after 8 weeks of treatment (scale bars represent 20  $\mu$ m). All bar graphs display mean fold enrichment  $\pm$  SEM. <sup>a</sup> $P$ -values vs. normal group (95% CI); <sup>f,8</sup> $P$ -values vs. before treatment (95% CI). <sup>a</sup> $P$  = 0.021; <sup>b</sup> $P$  = 0.041; <sup>c</sup> $P$  < 0.001; <sup>d</sup> $P$  = 0.001; <sup>e</sup> $P$  = 0.014; <sup>f</sup> $P$  < 0.001; <sup>8</sup> $P$  = 0.001. CI, confidence interval; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling method.

psoriatic skin lesions reveals active proliferation at the basal layer, with a higher mitotic index relative to healthy skin.<sup>24</sup> Oxymatrine has been shown to have antiproliferative and anticancer capacities and has been used mainly to treat various types of solid tumours.<sup>11</sup> Oxymatrine has antiproliferative activity that can restore the normal differentiation process.<sup>25–27</sup> Previously, we demonstrated that oxymatrine can treat severe plaque psoriasis effectively.<sup>18</sup> The PASI score decreased significantly after treatment and was positively correlated with the decrease in mitotic index. The current study indicates that oxymatrine not only improved clinical symptoms, but also inhibited mitosis of epidermal cells and the abnormal proliferation of keratinocytes. It also showed that oxymatrine promoted the recovery of mitosis and improved the pathology of excessive proliferation in psoriasis. We found that the efficacy of oxymatrine on lesion symptoms was comparable with that of acitretin. The two treatments also had no significant difference in the inhibition of epidermal cell mitosis and keratinocytes after treatment.

PCNA and Ki-67 are two widely used markers to evaluate tissue or tumour proliferative status.<sup>28</sup> PCNA is a DNA clamp that acts as a processivity-promoting factor for DNA polymerase  $\delta$  in eukaryotic cells and is essential for replication. The increase of PCNA can be induced by growth factors or as a response to damaged DNA even after the cell is no longer active in the cell cycle. PCNA is involved in the repair

of abnormal nucleotides and is thus also expressed in non-proliferating cells undergoing DNA repair.<sup>29,30</sup> All of the points mentioned above could explain the increase in PCNA cell immunoreactivity compared with other proliferation markers, such as Ki-67, in many studies.<sup>31,32</sup> Ki-67 is a large (395 kDa) nuclear protein that is present during all active phases of the cell cycle except for the G<sub>0</sub> phase.<sup>33</sup> Because Ki-67 degrades rapidly after mitosis,<sup>34</sup> Ki-67 is a more sensitive and specific proliferation marker than PCNA to determine the growth fraction of cells.<sup>35</sup> The expression levels of these two markers are usually low in normal skin tissue with weak immunoreactivity present at the basal and spinous layers. We observed higher expression of PCNA and Ki-67 at these layers in the skin lesions of patients with psoriasis than in normal skin, which is consistent with previous studies.<sup>36,37</sup> The result suggests that epidermal keratinocytes of psoriatic skin are actively proliferating. After oxymatrine or acitretin treatment, the expression of PCNA and Ki-67 declined significantly. The proliferation index was positively associated with PASI, and in all layers of the skin there was no statistical difference between the two groups after treatment. Thus, oxymatrine reduced the abnormal proliferation of the epidermis in psoriasis. Consistent with our results, a previous study by Pileri *et al.* reported that oxymatrine markedly reduced the levels of PCNA and Ki-67 in primary mediastinal B-cell lymphoma.<sup>38</sup>

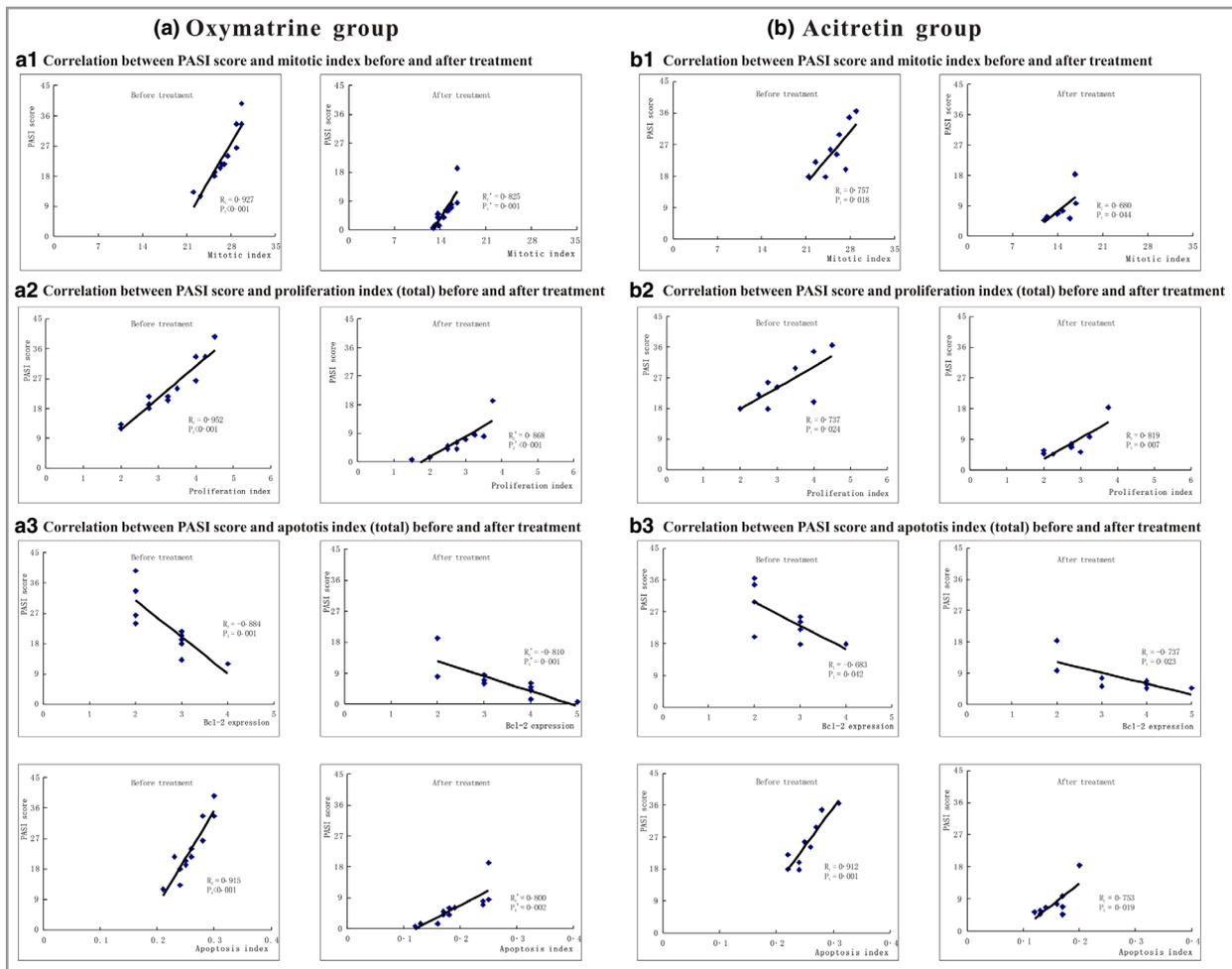


Fig 6. Correlation between Psoriasis Area and Severity Index (PASI) score and proliferation (total), apoptosis index (total).

Bcl-2 is an oxidative stress-sensitive protein and a key regulator of cell proliferation and apoptosis.<sup>39</sup> There is high-to-moderate expression of Bcl-2 at the basal layer in normal skin, but the expression in psoriasis was significantly lower.<sup>25,40</sup> We observed that there was low expression of Bcl-2 in psoriatic skin lesions before treatment compared with normal, healthy controls. Eight weeks of oxymatrine or acitretin treatment significantly increased the expression of Bcl-2 and restored apoptosis to normal levels. The PASI score was negatively correlated with Bcl-2 before and after treatment. Therefore, oxymatrine can reverse the abnormal biological behaviour of epidermal cells in psoriasis and partially restore the balance between cell division, differentiation and apoptosis. It has been reported that oxymatrine induced apoptosis in human pancreatic cancer PANC-1 cells via the regulation of Bcl-2 and IAP family expression, and the release of cytochrome c.<sup>26</sup> TUNEL-positive keratinocytes in the normal epidermis are distributed only in the upper granular layer. In contrast, in all layers of the psoriatic epidermis, most keratinocytes are TUNEL-positive.<sup>41,42</sup> Apoptosis is an autonomous and orderly process of cell death controlled by genes and is an important mechanism for eliminating redundant or

potentially harmful cells and maintaining internal microenvironments.<sup>43</sup> Increased apoptosis in psoriatic lesions may be a compensatory response of the body.<sup>44</sup> In our study, measurement of apoptotic cells by the TUNEL assay showed that there was elevated apoptosis of epidermis cells in the psoriatic skin. Although apoptotic keratinocytes are still detected in some cases after treatment in both groups, this increase in cellular apoptosis was inhibited by oxymatrine or acitretin treatment, but remained lower relative to normal skin, thus contributing to the recovery of the normal cell cycle. Similarly, Song *et al.* reported that oxymatrine inhibited the proliferation and induced apoptosis of human hepatoma cells,<sup>45</sup> further confirming that oxymatrine treatment was effective. Compared with pretreatment levels, the reports of apoptotic keratinocytes in psoriasis lesions detected by TUNEL assay after treatment are inconsistent,<sup>42,46</sup> which might be attributed to differences in the treatment plan and sampling time after treatment in patients with psoriasis. Further studies are needed to clarify these inconsistencies in the future.

In addition, we analysed the relationship between PASI scores and the expression of PCNA, Ki-67, Bcl-2, mitotic index and AI. We found that a higher PASI score suggested a

more serious clinical condition reflecting a higher abnormal proliferative and mitotic state. Abnormal proliferation of epidermal cells is a pathological feature of psoriasis.<sup>47</sup> Keratinocytes are the main cells of skin epidermis, with a key role in proliferation and differentiation, contributing to skin renewal, protection and immunity. Overproliferation and prosoplasia of keratinocytes occur in psoriasis,<sup>48</sup> manifesting as hypertrophic and papulosquamous lesions. After 8 weeks of treatment, the oxymatrine group and acitretin group PASI scores decreased significantly, and proliferation indices such as PCNA, Ki-67 and AI decreased at different degrees in all cases and the apoptotic index of Bcl-2 increased. All indices tended to return to normal, indicating that oxymatrine not only changed psoriasis lesions, but also regulated abnormal proliferation, differentiation and apoptosis of epidermis cells.

In summary, oxymatrine regulated mitosis, and inhibited the excessive expression of PCNA and Ki-67 in the skin lesions and promoted the restoration of apoptotic Bcl-2 expression. With the recovery of the balance between cell proliferation, differentiation and apoptosis in the skin, oxymatrine improved psoriasis skin lesions and is likely to be a promising agent for the treatment of psoriasis.

## Acknowledgments

The authors would like to thank the patients who were involved in this study. This study is supported by the Natural Science Foundation of Ningxia (NZ17154).

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