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Original Research Article

Effects of retinoic acid on immunity and inflammation in patients with *Psoriasis vulgaris*

Yi Liu[1], Min Li[2], Zhangjun Ding[3]*
[1]Department of Dermatological, Nanxishan Hospital of Guangxi Zhuang Autonomous Region, Guilin City, Guangxi, [2]Department of Dermatology, Taihe Hospital, Hubei University of Medicine, Hubei, [3]Department of Dermatological, Dongtai People's Hospital, Dongtai, China

For correspondence:* ***Email:* *gbjid@qq.com;* ***Tel:*** *+86-018962096677*

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Abstract

Purpose: *To investigate the effect of retinoic acid on immune and inflammatory factors in patients with psoriasis vulgaris, and its underlying mechanism of action.*
Methods: *A total of 20 P. vulgaris (PV) patients from Dongtai People's Hospital were divided into two groups, with 10 patients receiving retinoic acid and 10 receiving calcipotriol. The patients' clinical parameters, psoriasis area and severity index were evaluated after one month of treatment. Enzyme-linked immunosorbent assay (ELISA) was used to measure the concentrations of interleukin-10 and interleukin-17 in the patients' peripheral blood, while the proportion of T helper immune cells that expressed IL-17a and regulatory T immune cells were determined via flow sorting. Expressions of Notch1 in psoriatic lesions and CD4+T cells were determined by quantitative real time-polymerase chain reaction (qRT-PCR).*
Results: *Retinoic acid significantly reduced psoriasis area and severity index. Furthermore, retinoic acid decreased the concentration of IL-17, increased the concentration of IL-10, lowered the percentage of Th17 cells, but elevated the percentage of Treg immune cells ($p < 0.05$). Expression of Notch1 in skin lesions and CD4+T cells was lower in the retinoic acid group than in the calcipotriol group ($p < 0.05$).*
Conclusion: *Expression of Notch1 is decreased in skin tissue lesions of PV patients receiving oral retinoic acid treatment. Thus, retinoic acid influences Th17/Treg immune balance by regulating Notch1 signaling pathway in PV patients, and therefore can potentially be developed for the treatment of psoriasis.*

Keywords: *Psoriasis, Notch1 pathway, Psoriasis area, Severity index, Symptom score reduction index (SSRI)*

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INTRODUCTION

Psoriasis, also clinically called pustular psoriasis, is an autoimmune skin disease mediated by cluster of differentiation 4 (CD4) $^{+}$T cells, and it manifests as chronic inflammatory responses. Besides, the abnormal proliferation and differentiation of keratinocytes can be observed under the microscope, and more infiltrating immune cells appear in the epidermis and dermis [1]. These pathological changes lead to the development of psoriasis.

Patients with psoriasis experience inflammatory symptoms on their skin, characterized by the presence of red scales and erythema. These symptoms are often accompanied by itching and discomfort [2], and this disease affects 2 - 3 % of the world's population [3]. Although the etiology of psoriasis has not been determined yet, changes in the immune system caused by T cell activation exacerbates the onset and sustenance of the disease.

The Notch signaling pathway plays a crucial role in cell differentiation, proliferation, and the determination of fate in various organisms and tissues. It is involved in regulating early T cell development and the differentiation of peripheral T cells within the thymus [4-6]. The Notch system exerts a crucial effect in the growth and differentiation of epidermal cells [7,8]. It consists of four transmembrane receptors (Notch1-4), five transmembrane ligands and three regulatory proteins that modulate ligand-receptor-induced signals [9]. Previous studies have shown that the Notch1 may participate in psoriasis [10,11].

Local treatment is the basic treatment strategy for mild-to-moderate psoriasis as well as the initial and auxiliary treatment for severe psoriasis. Corticosteroids, vitamin D3 analogues and retinoids are clinically used for the local treatment of psoriasis. Retinoic acid is a natural compound related to vitamin A, and promotes the function and growth of skin cells. There are reports that retinoic acid regulates changes in the Notch1 pathway and inhibits epithelial-mesenchymal transition and cancer cell migration in breast cancer patients [12]. However, whether retinoic acid can relieve psoriasis dermatitis has not been determined. The aim of this study was to investigate the effects of retinoic acid on inflammation and immune activities in psoriasis, as well as its mechanisms of action.

METHODS

Materials

The materials used include Ficoll-Hypaque density gradient centrifugation (Amersham Biosciences, Chicago, IL, USA); antibodies against CD4, CD25 and forkhead box p3 (Foxp3) (Abcam, Cambridge, MA, USA); a PrimeScript™ Real-Time (RT) reagent kit (Toyobo, Osaka, Japan), enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) and retinoic acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China).

Patients

A total of 20 patients with psoriasis vulgaris (PV) patients were treated with retinoic acid twice a day (RA group) or with calcipotriol once a day (PV group). Samples were collected from the peripheral blood and psoriatic skin lesions at one week after drug administration.

Drug intervention

This study was approved by the Ethics Committee of Dongtai People's Hospital and followed the guidelines of Helsinki Declaration. Signed written informed consent was obtained from all participants before the study. The 20 PV patients admitted to the hospital from April 2017 to May 2019 were randomly divided into two groups. In the RA group, the patients were randomly treated with retinoic acid at a ratio of 1:1. They were aged 18 - 65 years (male or female) and had been diagnosed with PV at a stable stage. Besides, their lesions accounted for at least 10 % of the body surface area, and the physician global assessment (PGA) was at least 2 points. Patients who were allergic to the drugs used in the research or diagnosed with other types of psoriasis were not included in the study. After one month of treatment, the diseased tissues and blood samples were collected and cryopreserved at -80 °C. Before cryopreservation, the blood samples were centrifuged at 25 °C and 2500 rpm for 5 min.

Quantitative real time-polymerase chain reaction (qRT-PCR)

Total ribonucleic acids (RNAs) were separated from the sera of the two groups of PV patients using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and the quality of RNA samples was evaluated based on the ratio of absorbance at 260 nm to that at 280 nm (ranging from 1.9 to 2.0). Then, the PrimeScript™ RT kit was used for complementary deoxyribose nucleic acid (cDNA) synthesis, and SYBR Premix ExTaq™ II was utilized for quantifying the relative expression level of Notch messenger RNA (mRNA). The sequences of primers used are shown in Table 1.

Skin inflammation assessment

Skin inflammation was evaluated using three indicators, namely, erythema, thickness and area. These indicators were assessed independently using a scoring system (0 point: none, 1 point: mild, 2 points: moderate, 3 points: marked and 4 points: severe). Psoriasis area and severity index (PASI) scores were the sum of the scores in the three indicators.

Table 1: Primer sequence

Gene		Primer sequence
Notch1	Forward	5'-GTCAACGCCGTAGATGACC-3'
	Reverse:	5'-TTGTTAGCCCCGTTCTTCAG-3'
Interleukin-17 (IL-17)	Forward	5'-TGTCCACCATGTGGCCTAAGAG-3'
	Reverse:	5'-GTCCGAAATGAGGCTGTCTTTGA-3'
IL-10	Forward	5'-GGACTTTAAGGGTTACCTGGGTTGCC-3'
	Reverse:	5'-GCCTTGATGTCTGGGTCTTGGTTCTC-3'
β-Actin	Forward	5'-AGTTGCGTTACACCCTTTCTTG-3'
	Reverse:	5'-TCACCTTCACCGTTCCAGTTT-3'

Evaluation of symptom score reduction index (SSRI)

The effectiveness of retinoic acid in treating PV can be determined by the level of SSRI. An SSRI value of 90 % or higher indicated a complete cure of PV, while an SSRI value between 60 % and 90 % suggests that retinoic acid is effective in treating PV. When the SSRI value is between 20 % and 60 %, it indicates an improvement in the symptoms of PV. Finally, an SSRI value below 20 % signifies the aforementioned condition.

Determination of serum inflammatory cytokines

ELISA was conducted to determine the concentrations of IL-22 and tumor necrosis factor-alpha (TNF-α), which were expressed as mean ± standard deviation. Three replicate wells were set for each serum sample.

Flow sorting of T helper immune cells

Ficoll-Hypaque density gradient centrifugation was performed to separate peripheral blood mononuclear cells (PBMCs), and unmodified $CD4^+$T cells were obtained by immunomagnetic beads. Thereafter, $CD4^+$T cells were incubated with phorbol 12-myristate 13-acetate (PMA) 5 ng/mL and 1 μg/mL ionomycin for 5 h, followed by staining with CD4 antibody for 30 min and with IL-17 antibody for 30 min. Thereafter, Th17 cells were sorted using a flow sorter. Subsequently, the same procedures were carried out to determine the number of Treg cells. Specifically, $CD4^+$T cells were incubated with antibodies against CD4, CD25 and Foxp3 for staining, and finally the target cells were sorted with the sorter.

Statistical analysis

Statistical analysis of the data was performed using Statistic Package for Social Science software (SPSS 17.0, SPSS Inc, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). The t-test was used for analyzing measurement data while differences between two groups were determined by Student's t-test. Comparison among multiple groups was done using one-way ANOVA followed by post hoc test (least significant difference). $P < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics of PV patients

A total of 40 PV patients were collected for research and analysis. Through the statistical analysis, it was found that there were no significant differences in age, gender, weight, number of smokers, smoking history and proportion of hypertension population between the two groups of patients. Moreover, significant differences were also not found in high-sensitivity C-reactive protein (hs-CRP), PASI and PV history in a biochemical examination (Table 2), indicating that the baseline characteristics of the two groups of patients were comparable.

Table 2: Biographical data of PV patients

Variable	PV group (n =	RA group (n =
Age	42	47
Male [n (%)]	9 (45)	8 (40)
Weight (kg)	70.2	68.9
BMI (kg/m^2)	29	28.4
Number of	8	10
Hs-CRP	11.1	9.3
PASI	30.2	26.4

Retinoic acid alleviated the clinical symptoms of PV

The changes in skin scales, erythema and epidermal thickness in the two groups of PV patients were observed to evaluate the effect of retinoic acid on psoriatic skin inflammation. The results revealed that skin inflammation in patients in RA group was significantly lower than that in PV group patients, while PASI score in RA group decreased significantly on the 6th day ($p < 0.01$;

Figure 1 A). The SSRI value in RA group (77.11 %) was significantly higher than that in PV group (59.43 %, $p < 0.05$; Figure 1 B), which suggests that retinoic acid is effective against psoriasis.

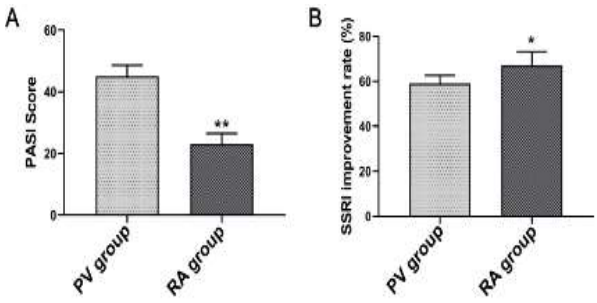

Figure 1: Changes in the clinical symptoms of PV patients treated with retinoic acid. (A) PASI value of each group of patients. The PASI value in RA group was significantly lower than that in PV group ($p < 0.01$). (B) compared with those in PV group, patients in RA group had an increased SSRI percentage ($p < 0.05$)

Effect of retinoic acid on the expressions of serum inflammatory mediators in PV patients

The treated patients in RA group exhibited significantly lower concentrations of IL-17 and higher concentrations of IL-10 in the peripheral blood than in PV group. It can be seen that retinoic acid play an anti-inflammatory role, promotes the secretion of anti-inflammatory factors and reduces the secretion of pro-inflammatory factors ($p < 0.01$; Figure 2).

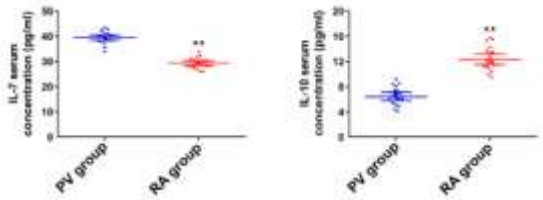

Figure 2: IL-17 and IL-10 concentrations in the peripheral blood of patients with PV. The concentration of IL-17 in the peripheral blood of patients in RA group was reduced, whereas that of IL-10 was raised ($p < 0.01$)

Effect of retinoic acid on the levels of Th17 and Treg cells in CD4$^+$T cells in peripheral blood

The results of flow sorting demonstrated that the treated patients in the RA group had a higher percentage of Th17 immune cells ($p < 0.01$), but lower percentage of Treg immune cells ($p < 0.05$) than in PV group; thus, Th17/Treg decreased ($p < 0.01$), which indicates that retinoic acid is able to facilitate the recovery of Th17/Treg and improve the immune function of patients (Figure 3).

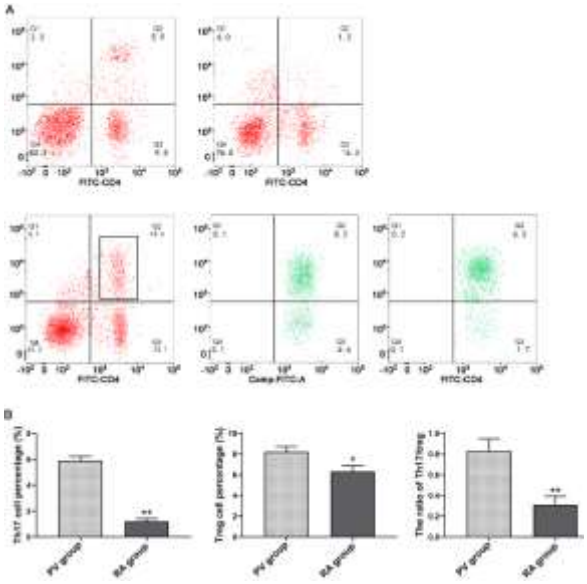

Figure 3: Effects of retinoic acid on changes in Th17 and Treg immune cells in PA (A) Flow capture of Th17 and Treg immune cells; (B) In comparison to the PV group, the RA group exhibited a decrease in the number of Th17 cells, while there was an increase in the number of Treg cells. Additionally, the ratio of Th17/Treg cells decreased significantly ($p < 0.05$) in the RA group

Retinoic acid suppressed Notch1 signaling pathway in psoriatic skin lesions

It was discovered from qRT-PCR analysis that the Notch1 mRNA level in psoriatic lesions in RA group was decreased by about 1.5 times compared with that in PV group ($p < 0.05$). Besides, the relative expression level of Notch1 mRNA in CD4$^+$T cells in RA group was significantly lower than that in PV group ($p < 0.01$), in consistency with the results in skin lesions (Figure 4).

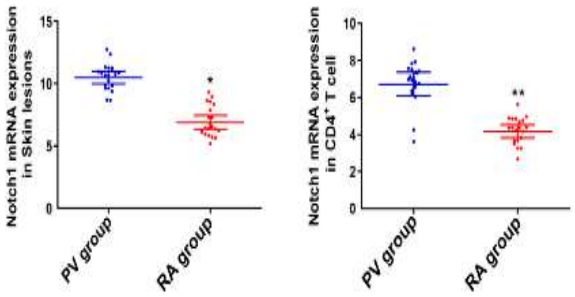

Figure 4: Changes in the expression of Notch1 in skin lesions and CD4$^+$T cells of PV patients. Compared with PV group, the relative expression of Notch1 in skin tissue lesions and CD4$^+$T cells in the patients in RA group were significantly reduced ($p < 0.05$)

DISCUSSION

Psoriasis is a skin disease driven by T cells [12]. Strategies targeting T cells and related cytokines are effective for the treatment of psoriasis and are widely used in patients with psoriasis in clinical practice [13,14]. In this study, it was demonstrated that retinoic acid inhibited PV. Retinoic acid intervention reduces clinical symptoms and skin inflammation such as psoriatic skin scales, erythema and epidermal thickness in PA patients. According to further research results, it was found that retinoic acid regulated the immune and inflammatory responses of the body through the Notch1 signaling pathway.

Retinoic acid contains 0.025 % all-trans tretinoin, and exerts various pharmacological activities. It efficaciously modulates keratinocyte proliferation and alleviates the inflammatory symptoms of psoriasis. This study demonstrated that retinoic acid significantly improved skin condition, erythema area and epidermal thickness and reduced the PASI score in the treatment of PV patients, indicating that the clinical symptoms of PV were relieved. When SSRI ≥ 60 %, the treatment method was effective. Changes in the levels of IL-17 (the main pro-inflammatory factor) and IL-10 (an anti-inflammatory factor) affect the inflammatory activities in the body. The levels of the inflammatory mediators IL-17 and IL-10 in the peripheral blood were notably lower in the RA group compared to the PV group. This indicates that retinoic acid possesses anti-inflammatory effects by enhancing the release of anti-inflammatory factors while diminishing the release of pro-inflammatory factors. Hence, retinoic acid effectively suppresses inflammation and ameliorates the inflammatory symptoms of PA.

Immune cells are the key "executors" in maintaining the body's immune balance. Th17 immune cells facilitate the development of autoimmune and inflammatory diseases, but Treg cells exert opposite effects. The Treg cells maintain self-tolerance through contact with effective immune cells and prevent autoimmune and inflammatory diseases. On the other side, they release inhibitory cytokines, IL-10 and TGF-β, and suppress immunity to T cells [15-17]. Th17/Treg immune imbalance is the pathogenesis of multiple autoimmune and inflammatory diseases. In this study, flow sorting was carried out for $CD4^+$ immune cells in psoriatic skin tissue lesions. The results presented indicate that retinoic acid may decrease Th17 immune cells, increase Treg immune cells, and reduce the Th17/Treg ratio. These findings suggest that retinoic acid has the potential to be used as a treatment for PV.

The Notch signaling cascade pathway is involved in the differentiation, proliferation, and functioning of CD4+ T cells, as well as other activities [4,6,18]. The Notch signaling pathway restores Th17/Treg immune imbalance in some autoimmune and inflammatory diseases [19-21]. It has been reported that Notch1 expression is up-regulated in psoriatic lesions and mediates the abnormal differentiation of epidermal keratinocytes, indicating that Notch1 pathway participates in the pathological changes of psoriasis [22,23].

CONCLUSION

Notch1 level is elevated in skin lesions, indicating the involvement of Notch1 signals in the development and progression of PV. Furthermore, the expression of Notch1 is lowered in skin tissue lesions of PV patients undergoing oral retinoic acid treatment. As a result, retinoic acid affects the balance between Th17 and Treg immune cells by modulating Notch1 signaling pathway in PV patients. Retinoic acid influences the quantity of Th17 and Treg cells by regulating Notch1 expression in psoriatic skin tissue lesions, thereby restoring Th17/Treg immune balance and effectively improving the clinical symptoms of psoriatic skin inflammation. This discovery may present a new approach to treating PV, suggesting that retinoic acid holds potentials as a drug for PV treatment.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Yi Liu and Min Li contributed equally to this work.

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