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# Effect of NB-UVB on levels of MCP-1 and CCR6 mRNA in patients with psoriasis vulgaris

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**ABSTRACT.** The aim of this study is to explore the effect of narrow band ultraviolet B (NB-UVB) on the chemokine receptor CCR6 mRNA levels in patients with psoriasis. Psoriasis area and severity index (PASI) values were recorded before and after the treatment with NB-UVB phototherapy of 30 psoriasis vulgaris patients. The reverse transcription-polymerase chain reaction method was used to detect the expression level of CCR6 mRNA in peripheral blood mononuclear cells, and compared with 30 healthy subjects. The PASI value of the 30 psoriasis vulgaris patients decreased significantly after 15 iterations of phototherapy treatment ($P < 0.01$). The expression level of CCR6 mRNA in psoriasis patients was significantly higher than in the healthy controls ($P < 0.01$), while the expression level of CCR6 mRNA decreased significantly after phototherapy ($P < 0.01$). Reduction of CCR6 level may be one of the mechanisms through which NB-UVB can treat psoriasis.

**Key words:** Psoriasis; Narrow-band ultraviolet B; Chemokine receptors

 

## INTRODUCTION

Psoriasis is a type of chronic inflammatory proliferative disease, which has a serious impact on the quality of life of patients. Its pathogenesis is complex, and research currently focuses on the role of T cells and keratinocytes. A key question concerns the method by which T cells and inflammatory cells escape blood vessels and interact with keratinocytes leading to the clinical and pathological features of psoriasis. Recent studies suggest that chemokines and their receptors play an important role. Inflammatory cells need an appropriate local cytokine concentration to be able to migrate, and at the same time, the corresponding target cells will migrate (Peters et al., 2013). The role of cytokines and their receptors is thought to be important to the pathogenesis of the disease. The clinical curative effect of narrow band ultraviolet B (NB-UVB) phototherapy in the treatment of psoriasis has been widely recognized, but the mechanism is not completely clear. This study used the reverse transcription polymerase chain reaction (RT-PCR) method to determine the effect of NB-UVB on the expression of CCR6 mRNA in psoriasis patients, so as to observe the possible role of MCP-1 and CCR6 in the NB-UVB treatment of psoriasis (Chen et al., 2012).

## MATERIAL AND METHODS

### Sample collection

This study included 30 patients [17 males, 13 females; median age, 35.5 years (range, 8-50 years)] who were clinically and pathologically diagnosed with active stage psoriasis vulgaris at the Department of Dermatology in the Affiliated Hospital of Binzhou Medical College. Among the patients, 10 had guttate psoriasis and 20 had plaque psoriasis, and the average psoriasis area and severity index (PASI) score was17.969 ± 0.963. All subjects had neither used vitamin A, retinoic acid, corticosteroids, nor other immunosuppressive agents in the last 3 months. None had used any drugs for the treatment of psoriasis in the last month, and also had no dysfunction of heart, brain, kidney, or other important organs. As a normal control group 30 healthy subjects from the hospital were used. This group was comprised of 15 males and 15 females aged between 11 and 62 years with a median age of 32.1 years. The two groups had no statistically significant differences in age and gender composition ($P > 0.05$).

### Treatment methods

#### *NB-UVB treatment*

Thirty patients treated with NB-UVB phototherapy wore special black eye protection, and men had to wear underwear to protect genitals (Krzysiek et al., 2000). After determination of minimal erythema dose in patients with NB-UVB treatment, the patients were given 50% minimal arythema dose during the first treatment, then according to the individual differences, the doses increased 10-20% each time. A total of 15 exposures was one course of treatment. When the patients developed erythema, blisters, or perceived pain, the treatment was stopped for 2-3 exposures. The maximum cumulative dose should not exceed 2.0 J/cm$^2$. The PASI score of all of the patients was evaluated before and after the phototherapy treatment, and the score improvement rate (%) = (PASI total score before phototherapy - PASI score after pho-

totherapy) / PASI total score before phototherapy x 100%. A score improvement ratio ≥90% was considered a clinical cure; 70-89% was considered a markedly improved; 25-69% was considered improved; <25% was considered ineffective. The total effective rate = clinical cure rate + markedly improved rate.

### *Blood sampling*

Samples of 8 mL peripheral blood were collected from each subject before and after therapy. To this was added 300 µL heparin for anticoagulation. Samples were stored in a -70°C freezer.

### *Isolation mononuclear cells from peripheral blood*

Heparin anticoagulant venous blood (8 mL) was 2X diluted with calcium and magnesium free Hanks' solution. After mixing, the samples were left at 37°C for 40 min, and the supernatant was slowly added along down the side wall of a test tube containing demixing liquid at a 3:2 ratio. The samples were then centrifuged at 2000 rpm for 20 min. After centrifugation, the cell layer was washed with Hanks' solution five times to obtain mononuclear cells. These were then counted under a microscope. A sample of 8 x $10^6$ cells was reserved.

### *Use of TRNzol-A$^+$ to isolate total RNA*

To 8 x $10^6$ mononuclear cells isolated from peripheral blood was added 1 mL TRNzol-A$^+$. Following sufficient agitation and standing at 15° to 30°C for 5 min, the nucleic acid protein complexes separated completely. The samples were then centrifuged at 12,000 rpm for 10 min a 4°C. The supernatant was transferred to a fresh RNase-free Eppendorf tube, and 150 µL chloroform was added. This was fiercely agitated for 15 s, stored at room temperature for 3 min, and then centrifuged at 12,000 rpm for 10-15 min at 4°C. A 500-µL portion of the aqueous phase was transferred to a new Eppendorf tube, and the same volume of isopropanol was added. This was mixed at room temperature for 20 to 30 min, centrifuged at 1000 rpm for 10 min at 4°C, and the supernatant was discarded. To wash the precipitate, 1 mL 75% ethanol for washing the precipitation was added. After centrifugation at 5000 rpm for 3 min at 4°C, the liquid was discarded and the remaining material dried at room temperature. Into this was added 40 µL RNase-free dd$H_2O$, and complete dissolution was achieved following repeated pipetting and mixing. This completed RNA extraction.

### *One-step RT-PCR*

In an ice bath, 50 µL reaction solution was prepared. This comprised 5 µL 10X RT-PCR buffer, 2 µL dNTP mixture, 10 µL 5X RT-PCR enhancer, 0.5 µL RNasin, 2.5 µL HotMaster Taq polymerase, 0.5 µL Quant RTase, and 5 µL RNA template L. This was made up to 50 µL with RNase-free dd$H_2O$. Amplification conditions were: reverse transcription reaction at 50°C for 30 min, PCR initial degeneration at 94°C for 2 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 65°C for 2 min, for a total of 35 cycles, then finally extension at 65°C for 10 min. The primer sequence for CCR6 mRNA amplification fragment was 316 bp, the upstream primer: 5'-TGGCTGTTGGTTTGTGGA-3', downstream primer: 5'-AGGAGTAGGCAATCGGTA-3'. The β-actin-amplified fragment was

180 bp, the upstream primer: 5'-ATTGCCGACAGGATGCAGAA-3', the downstream primer: 5'-GCTGATCCACATCTGCTGGA-3'. Semi-quantitative RT-PCR used β-actin as an internal reference, which was amplified with the target gene fragments. The absorbance value was determined to calculate the relative ratio of CCR6 and β-actin, which represented the relative level of CCR6 (Brembilla et al., 2013).

Using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), the MCP-1 values in peripheral blood were detected before and after treatment of patients with psoriasis, as shown in Table 1 (MCP-1 expression level before and after 1 NB-UVB therapy). The method was in strict accordance with the instruction manual. The series of concentration standard samples and serum samples were added to the corresponding ELISA plate well coated with monoclonal antibody, followed by the addition of enzyme-linked complexes, which were incubated at room temperature for 1 h. The plate was rinsed with PBS five times and to each well was added in turn substrate I and substrate II. This was then left to react at room temperature for 15 min. The stop buffer was then added to terminate the reaction. The OD absorbance values were read at 450 nm (Homey et al., 2000).

With the OD value as the vertical ordinate, and the standard sample concentration as the horizontal ordinate, the standard curve was generated using the Logit-Log linear regression model. According to OD values, the concentrations of the serum samples were identified using the standard curve.

The main experimental instruments and reagents were: NB-UVB treatment instrument (Waldmann Co., Germany), Hema 480 gene amplification instrument (HEMA Medical Instrument Co. Ltd., Zhuhai, China), XL-70 high speed centrifuge (Beckman, USA), TFX-20M UV detector (Vilberlourmat, France), CS9000 dual-wavelength flying spot scanner (SHIMADZU, Japan), DYY-III 8B regulator for the steady flow timing electrophoresis apparatus (Beijing Six-One Instrument Factory), Nikon F301 camera (Nikon, Japan), Nikon 52 mm, 1:2.8 close-up lens, Nikon filter, WH-851-type vortex oscillator (Beijing East Instrument Limited Company of Science and Technology), Trizol (Gibco/BRL), and Super Script™ First-Strand Synthesis (Shanghai Huamei Biological Engineering Company).

**Table 1.** Expression level of MCP-1 NB-UVB before and after treatment (means ± SD).

| Group | MCP-1 (pg/mL) |
|---|---|
| Normal control | 96.26 ± 1.30 |
| Psoriasis patients | |
| Before treatment | 200.52 ± 2.23* |
| After treatment | 101.88 ± 1.90** |

*P < 0.05; **P < 0.01.

## Statistical methods

Data are reported as means ± SD, and the SPSS 12.0 statistical analysis software was used for analysis. The normal control group and the patient group were compared using the two sample *t*-test, comparisons before and after the treatment were done using paired *t*-test data analysis. Correlation analysis used the Pearson correlation. Results were considered significant if $P < 0.05$.

## RESULTS

### Clinical curative effect

Over the 30 patients, the PASI average score before treatment was 17.969 ± 0.963, after treatment it was 4.352 ± 0.481, and the difference was statistically significant ($t = 76.291$, $P < 0.01$). Overall, 14 cases were clinically cured, 13 cases were markedly improved, two cases improved, and in one case the treatment was ineffective. The total efficiency was 90%.

### Reaction products of RT-PCR electrophoresis

The levels of CCR6 mRNA in the peripheral blood mononuclear cells of psoriasis vulgaris patients before treatment were much higher than those of healthy people, and this difference was statistically significant ($P < 0.01$; Figure 1). The expression level of CCR6 mRNA decreased significantly after phototherapy treatment, and the difference was statistically significant ($P < 0.01$; Table 2).

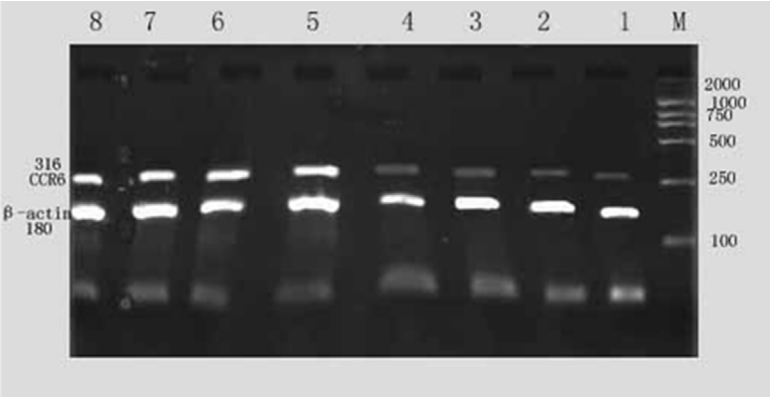


**Figure 1.** CCR6 mRNA reverse transcriptase-polymerase chain reaction product electrophoresis. *Lane M*: DNA marker; *lanes 1* and *2*: normal control; *lanes 3* and *4*: psoriasis patients after treatment: *lanes 5* and *8*: psoriasis patients before treatment.

**Table 2.** Expression level of CCR6 mRNA in peripheral blood mononuclear cells before and after NB-UVB treatment (mean ± SD).

| Group (N) | *t* | P |
|---|---|---|
| Normal control (30) | 0.519 ± 0.056 | |
| Patients before treatment (30) | 0.911 ± 0.027 | 34.889*0.000 |
| Patients after treatment (30) | 0.623 ± 0.061 | 28.590**0.000 |

*Comparison between the patients before the treatment and the normal control group, **comparison of the patients before and after the treatment.

### Correlation analysis

The correlation coefficient (r) of CCR6 difference value before and after UVB treatment with PASI difference value before and after UVB treatment was -0.223, which showed a weak-negative correlation.

## DISCUSSION

Psoriasis is an exciting topic within the field of dermatology; however, its pathogenic mechanism is not clear (Teraki et al., 2004). Currently, it is considered that T cell-mediated immunity plays an important role. At the same time, chemokines, adhesion molecules, growth factors, and other active molecules interact closely with T cells, neutrophils and other cells, and are jointly involved in pathogenic mechanisms in psoriasis (Hedrick et al., 2009). Chemokines are a type of cytokine secreted by various types of cells, which can cause the chemotactic properties of the leukocytes (Springer, 1994). This is in addition to chemotaxis and leukocyte activation, which has variety of biological activities such as stimulating cell proliferation, promoting the formation of new blood vessels, and also plays an important role in the pathogenesis of inflammatory skin disease (Van Coillie et al., 1999). The biological effects of chemokines are realized via combination with the chemokine receptor on the surface of the target cell.

CCR6 is the only receptor of MIP-3a (recently named CCL20) (Nakamura et al., 1995), which is expressed in memory T cells, regulatory T cells, immature dendritic cells, and B cells. MIP-3a/CCR6 interaction plays a key role in the control of B cell differentiation and development, and mediates non-dendritic cells and memory/mature T cells migrating to inflammatory sites. Vestergaard et al. (2004) discovered that CCR6 and MIP-3a had higher expression in psoriatic lesions. Homey et al. (2000) and Chen et al. (2013a) also found that CCR6 and MIP-3a had high expression in psoriatic lesions, and the skin homing CLA+ memory T cells also had a high level of CCR6, and that CCR6 also increased significantly in peripheral blood mononuclear cells in psoriasis patients, suggesting that the CCR6 might play an important role in the migration of T cells to the epidermis of psoriasis. Teraki et al. (2004) and Hedrick et al. (2009) also had similar views.

This study found that the CCR6 mRNA levels in the peripheral blood mononuclear cells of psoriatic patients were significantly higher than in the control group; this was similar to earlier findings. Presumably more CCR6 might induce a chemotactic effect by binding with its ligand MIP-3a and gathering memory/effector T cells and immature dendritic cells at the epidermis, resulting in the occurrence and development of psoriasis.

After the application of NB-UVB phototherapy to treat psoriasis, we found that CCR6 mRNA levels decreased in patients. Presumably with the decreased expression of CCR6 mRNA, its combination with MIP-3a might reduce, and chemotaxis of T cells and other inflammatory cells to the epidermis might reduce. This would thereby block the vicious cycle that leads to psoriasis. However, the difference value of CCR6 before and after phototherapy treatment and the PASI difference value were negatively correlated, so it cannot be ruled out that the expression level of CCR6 in peripheral blood might also be a concomitant phenomenon or be a process related to disease development (Chen et al., 2013b).

MCP was the first CC chemotactic factor to be discovered. This family has at least four members (MCP-1, -2, -3, and -4). MCP-1 is mainly responsible for chemotaxis of mononuclear cells, memory T cells, dendritic cells, and natural killer cells. Using transgenic mouse model studies, Krzysiek et al. (2000) found that external stimulation can increase the expression of MCP-1 in epidermal cells. Langerhans cells and macrophage cells chemotaxis to the epidermis, and are involved in the inflammatory immune response (Siebert et al., 2000). In psoriasis patients, there is excessive proliferation of keratinocytes and a variety of inflammatory cell infiltrate, suggesting that the pathogenesis of psoriasis may be related to the high expression of MCP-1 in epidermal keratinocytes (Dzenko et al., 2001). It has also been found

that serum MCP-1 levels in active-stage psoriasis patients were not only significantly higher than in the control group but also higher than in the resting-stage patients. This suggests that high expression of MCP-1 in peripheral serum was not only involved in psoriasis, but also related to the condition changes and development of the patients. By using Goeckerman therapy in the treatment of psoriasis, Pohl et al. (2009) found that peripheral serum levels of MCP-1 and RANTES were significantly decreased, therefore indicating that the therapy might achieve the aim of treating psoriasis by affecting chemokine levels in the peripheral serum of patients (Ge et al., 2013). Our experiments found that peripheral blood MCP-1 levels in psoriatic patients were significantly higher than those in the normal control group, and the difference was statistically significant ($P < 0.01$). This was a similar result to previous experiments (Salcedo et al., 2000). After 15 sessions of NB-UVB phototherapy treatment, the MCP-1 level in the peripheral blood of patients decreased significantly, suggesting that NB-UVB might play a role in the treatment of psoriasis by reducing MCP-1 expression in peripheral blood.

Because of the complex etiology of psoriasis, various kinds of cytokines and inflammatory cells, mediators of inflammation have complicated roles in the pathogenesis of psoriasis and also interact with each other resulting in a complex interplay of effects (Viedt et al., 2002). CCR6 and its ligand are no exception, and this study was limited to measuring the level of the gene, and this result was not verified by also measuring the protein level, which is the next stage in our study. Therefore, the influence mechanism between CCR6 and psoriasis and NB-UVB on CCR6 level of psoriasis patients needs to be studied further (Nascimento et al., 2013).

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