Effect of NB-UVB on cytokines and endoplasmic reticulum stress in psoriasis vulgaris lesions

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Objective: To study the effect of NB-UVB on cytokines and endoplasmic reticulum stress in psoriasis vulgaris lesions. Methods: Patients with psoriasis vulgaris who received NB-UVB therapy in People’s Hospital of Beijing Daxing District between May 2014 and January 2017 were selected, proper amount of skin lesion tissue was collected before treatment as well as 4 weeks and 8 weeks after treatment respectively to extract the protein in it, and the protein expression levels of inflammatory cytokines, transcription factors and endoplasmic reticulum stress molecules in tissue protein were determined. Results: 4 weeks and 8 weeks after treatment, IFN-γ, IL-2, IL-17, Runx3 and RORγt expression in lesions were significantly lower than those before treatment while IL-4, IL-10, Foxp3, IRE-1, XBP1, ATF6, CHOP and GADD34 expression were significantly higher than those before treatment; 8 weeks after treatment, IFN-γ, IL-2, IL-17, Runx3 and RORγt expression in lesions were significantly lower than those 4 weeks after treatment while IL-4, IL-10, Foxp3, IRE-1, XBP1, ATF6, CHOP and GADD34 expression were significantly higher than those 4 weeks after treatment. Conclusion: NB-UVB can regulate the differentiation and maturation of CD4+ T cell subsets Th1/Th2 and Th17/Treg as well as the apoptosis mediated by endoplasmic reticulum stress in psoriasis vulgaris lesions.

1. Introduction

Psoriasis is a common chronic inflammatory skin disease, psoriasis vulgaris is the most common in clinical practice, and scale erythema is a prominent clinical manifestation. Abnormal keratinocyte proliferation and apoptosis, immune response and inflammatory reaction disorders are the important pathological features of local psoriasis vulgaris lesions, keratinocyte proliferation and apoptosis is closely related to abnormal endoplasmic reticulum stress pathway, and the occurrence of immune responses and inflammation disorders are closely related to the pro-inflammatory factor and anti-inflammatory factor secretion imbalance[1,2]. Narrow band ultraviolet B (NB-UVB) is the ultraviolet ray with around 311 nm wavelength, it has the characteristics of strong penetrability, weak erythema effect and small DNA damage, and it is widely used in clinical treatment of psoriasis vulgaris[3,4]. But the mechanism of NB-UVB to exert therapeutic effect is not fully elucidated. In order to define the mechanism of NB-UVB for psoriasis vulgaris treatment, the effect of NB-UVB on cytokines and endoplasmic reticulum stress in psoriasis vulgaris lesions was specifically analyzed.

2. Case information and research methods

2.1 Case information

Patients with psoriasis vulgaris who received NB-UVB therapy in People’s Hospital of Beijing Daxing District between May 2014 and January 2017 were selected as the research subjects, all the patients were in line with the diagnostic criteria for psoriasis vulgaris, lesion...
area was > 10% and PASI score was > 90 when they saw a doctor, they never received local drug therapy or systemic immune therapy, and those allergic to NB-UVB were excluded. A total of 46 cases of patients with psoriasis vulgaris were included, 24 cases were male and 22 cases were female, and they were 23-55 years old.

2.2 NB–UVB therapy

SS–09 ultraviolet phototherapy instrument from Shanghai sigma company was used for treatment, wavelength was 310-313 nm, and irradiation distance was 20 cm; Before irradiation, sunlight ultraviolet simulator was used to determine the minimum erythema dose of lesion NB-UVB, namely MED, the lesion area was fully exposed during irradiation, the eyes, external genitals and other non-lesion areas were covered, the initial irradiation dose was set as 50% of the MED, 0.1 J/cm² was added each irradiation, the exposure dose was maintained when there was light erythema, and 0.1 J/cm² continued to be added if there is no light erythema. Irradiation was conducted three times a week, for a total of eight weeks.

2.3 Detection of molecule expression in lesions

Before treatment as well as 4 weeks and 8 weeks after treatment, the right amount of skin tissue was taken respectively, added in PBS buffer and fully split, the split tissue suspension was put in the centrifuge and centrifuged at 4 °C and 12 000 r/min for 10 min to separate the upper clear protein suspension, and enzyme-linked immunosorbent assay kits were used to detect IFN-γ, IL-4, IL-10, Runx3, RORγt, Foxp3, IRE-1α, XBP1, ATF6, CHOP and GADD34 protein expression.

2.4 Statistical processing methods

SPSS 20.0 software was used to input and analyze data, analysis among different points in time before and after treatment was by repeated measures analysis of variance and SPSS 20.0 software was used to input and analyze data, analysis of statistical significance in differences.

3. Results

3.1 Cytokine expression in lesions

Before treatment as well as 4 weeks and 8 weeks after treatment, the analysis of pro-inflammatory factors IFN-γ (ng/L), IL-2 (ng/L) and IL-17 (ng/L) as well as anti-inflammatory factors IL-4 (ng/L) and IL-10 (ng/L) expression in lesions was as follows: 4 weeks and 8 weeks after treatment, IFN-γ, IL-2 and IL-17 expression in lesions were significantly lower than those before treatment while IL-4 and IL-10 expression were significantly higher than those before treatment; 8 weeks after treatment, IFN-γ, IL-2 and IL-17 expression in lesions were significantly lower than those 4 weeks after treatment while IL-4 and IL-10 expression were significantly higher than those 4 weeks after treatment. Differences in pair-wise comparison of IFN-γ, IL-2, IL-17, IL-4 and IL-10 expression in lesions were statistically significant among different points in time before and after treatment (P<0.05), shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>IFN-γ (ng/L)</th>
<th>IL-2 (ng/L)</th>
<th>IL-4 (ng/L)</th>
<th>IL-10 (ng/L)</th>
<th>IL-17 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>46</td>
<td>3.41±0.55</td>
<td>1.98±0.22</td>
<td>77.52±9.41</td>
<td>102.48±15.62</td>
<td>92.11±10.23</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>46</td>
<td>2.12±0.36</td>
<td>1.24±0.16</td>
<td>121.24±16.63</td>
<td>178.49±22.13</td>
<td>56.73±7.62</td>
</tr>
<tr>
<td>8 weeks after treatment</td>
<td>46</td>
<td>1.52±0.29</td>
<td>0.80±0.10</td>
<td>180.53±22.32</td>
<td>288.54±31.48</td>
<td>34.18±5.28</td>
</tr>
</tbody>
</table>

*: comparison between 4 weeks as well as 8 weeks after treatment and before treatment, P<0.05; †: comparison between 4 weeks after treatment and 8 weeks after treatment, P<0.05.

3.2 Transcription factor expression in lesions

Before treatment as well as 4 weeks and 8 weeks after treatment, the analysis of transcription factors Runx3 (μg/L), RORγt (ng/L) and Foxp3 (ng/L) expression in lesions was as follows: 4 weeks and 8 weeks after treatment, Runx3 and RORγt expression in lesions were significantly lower than those before treatment while Foxp3 expression were significantly higher than before treatment; 8 weeks after treatment, Runx3 and RORγt expression in lesions were significantly lower than those 4 weeks after treatment while Foxp3 expression were significantly higher than that after treatment; 8 weeks after treatment, Runx3 and RORγt expression in lesions were significantly lower than those 4 weeks after treatment while Foxp3 expression was significantly higher than that 4 weeks after treatment. Differences in pair-wise comparison of Runx3, RORγt and Foxp3 expression in lesions were statistically significant among different points in time before and after treatment (P<0.05), shown in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>Runx3 (μg/L)</th>
<th>RORγt (ng/L)</th>
<th>Foxp3 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>46</td>
<td>2.31±0.39</td>
<td>114.65±17.96</td>
<td></td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>46</td>
<td>1.25±0.17</td>
<td>194.58±26.74</td>
<td></td>
</tr>
<tr>
<td>8 weeks after treatment</td>
<td>46</td>
<td>0.72±0.10</td>
<td>325.69±41.48</td>
<td></td>
</tr>
</tbody>
</table>

*: comparison between 4 weeks as well as 8 weeks after treatment and before treatment, P<0.05; †: comparison between 4 weeks after treatment and 8 weeks after treatment, P<0.05.

3.3 Endoplasmic reticulum stress molecule expression in lesions

Before treatment as well as 4 weeks and 8 weeks after treatment, the analysis of endoplasmic reticulum stress molecules IRE-1α (ng/L), XBP1 (μg/L), ATF6 (μg/L), CHOP (ng/L) and GADD34 (μg/L) expression in lesions was as follows: 4 weeks and 8 weeks after treatment, IRE-1α, XBP1, ATF6, CHOP and GADD34 expression were significantly lower than those before treatment; 8 weeks after treatment, IRE-1α, XBP1, ATF6, CHOP and GADD34 expression were significantly higher than that before treatment; 8 weeks after treatment, IRE-1α, XBP1, ATF6, CHOP and GADD34 expression were significantly lower than those 4 weeks after treatment while IRE-1α, XBP1, ATF6, CHOP and GADD34 expression were significantly higher than that 4 weeks after treatment. Differences in pair-wise comparison of IRE-1α, XBP1, ATF6, CHOP and GADD34 expression in lesions were statistically significant among different points in time before and after treatment (P<0.05), shown in Table 3.
in lesions were significantly higher than those before treatment; 8 weeks after treatment, IRE-1, XBP1, ATF6, CHOP and GADD34 expression in lesions were significantly higher than those 4 weeks after treatment. Differences in pair-wise comparison of IRE-1 α, XBP1, ATF6, CHOP and GADD34 expression in lesions were statistically significant among different points in time before and after treatment (P<0.05), shown in Table 3.

### Table 3
Endoplasmic reticulum stress molecule expression in lesions before and after treatment.

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>IRE-1</th>
<th>XBP1</th>
<th>ATF6</th>
<th>CHOP</th>
<th>GADD34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>46</td>
<td>93.41±11.23</td>
<td>2.83±0.35</td>
<td>1.21±0.18</td>
<td>131.35±17.68</td>
<td>0.92±0.12</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>46</td>
<td>176.52±20.32</td>
<td>5.51±0.89</td>
<td>3.28±0.55</td>
<td>289.64±42.18</td>
<td>1.84±0.25</td>
</tr>
<tr>
<td>8 weeks after treatment</td>
<td>46</td>
<td>302.59±52.18</td>
<td>7.35±0.94</td>
<td>4.52±0.67</td>
<td>425.64±66.21</td>
<td>3.52±0.66</td>
</tr>
</tbody>
</table>

*: comparison between 4 weeks as well as 8 weeks after treatment and before treatment, P<0.05; *: comparison between 4 weeks after treatment and 8 weeks after treatment, P<0.05.

### 4. Discussion

NB-UVB irradiation is the common method for clinical treatment of psoriasis vulgaris, NB-UVB has the characteristics of strong penetrability, weak erythema effect and small DNA damage, and its effect is ideal on treatment of psoriasis vulgaris[5,6]. However, it is still not clear about the molecular mechanism of NB-UVB for treatment of psoriasis vulgaris. The inflammatory cell infiltration as well as inflammatory response and immune response unbalance in local lesion are the important pathological features of psoriasis vulgaris, and the abnormal secretion of a variety of pro-inflammatory factors and anti-inflammatory factors are the important factors causing abnormal keratinocyte proliferation in lesions[7,8]. IFN-γ, IL-2 and IL-17 are the pro-inflammatory cytokines that can not only make inflammatory cell and mediate cascade amplification of inflammatory reaction in lesion, but can also significantly promote the keratinocyte proliferation[9,10]; IL-4 and IL-10 are the anti-inflammatory cytokines that have inhibitory effect on both activation of inflammatory cells and cascade amplification of inflammatory reaction[11]. In order to define the effect of NB-UVB on inflammatory response and immune response in psoriatic lesions, the levels of above pro-inflammatory factors and anti-inflammatory factors were analyzed in the study, and the results showed that IFN-γ, IL-2 and IL-17 expression in lesions gradually decreased while IL-4 and IL-10 expression gradually increased after treatment. This means that NB-UVB can adjust the balance of inflammatory and immune response in psoriatic lesions, inhibit the expression of pro-inflammatory factors and increase the expression of anti-inflammatory factors.

The expression and secretion of pro-inflammatory factor and anti-inflammatory factors in psoriatic lesions mainly come from the different CD4+T lymphocyte subsets. Th1 subsets mainly secrete IFN-γ, IL-2 and other pro-inflammatory factors, Th2 subsets mainly secrete IL-4, IL-5 and other anti-inflammatory factors, and the two kinds of cell subsets interact with and inhibit each other; Treg subsets mainly secrete IL-10, TGF-β1 and other anti-inflammatory factors, Th17 mainly secrete IL-17, IL-22, IL-23 and other pro-inflammatory factors, and the two kinds of cell subsets can not only interact with each other, but can also affect the Th1/Th2 balance[12,13]. Runx3, ROR γ t, Foxp3 and other transcription factors have played a key regulating role in CD4+ T lymphocyte subset differentiation and maturation process. Runx3 is the Runx transcription factor family member involved in regulating Th1/Th2 balance, and the high expression of Runx3 can promote the Th1 subset differentiation and maturation, and inhibit Th2 differentiation and maturation; ROR γ t can guide CD4+T cell differentiation to Th17 subset, and Foxp3 is marker molecule on Treg subset surface and participates in the subset differentiation and maturation[14,15]. In the study, analysis of the expression of above transcription factors in psoriatic lesions showed that Runx3 and ROR γ t expression gradually decreased while Foxp3 expression gradually increased after treatment. It proves that NB-UVB can adjust the expression of transcription factors in psoriatic lesions to influence the differentiation and maturation of CD4+T cell subsets Th1/Th2 and Th17/Treg, and then influence the expression and secretion of corresponding cytokines.

The abnormal proliferation of keratinocytes in psoriatic lesions is the important factor causing the formation of skin lesions, keratinocyte proliferation is not only affected by pro-inflammatory factors and anti-inflammatory factors, but also affected by multiple signaling pathways related to cell proliferation and apoptosis, and the endoplasmic reticulum stress is one of the important molecular pathways that affect cell proliferation[16,17]. Endoplasmic reticulum stress downstream IRE-1 α, ATF6 and other transmembrane proteins can directly participate in the process of cell apoptosis. After forming homodimer, IRE-1 α can undergo autophosphorylation, thus cut XBP1 into the form with transcription activity and mediate apoptosis; ATF6 is activated and enters the nucleus under the action of SIP1, SP2 and other proteases, it is combined with reaction elements CRE and ERSE-1 to start the CHOP and other gene expression, and it can further induce the expression of GADD34, increase endoplasmic reticulum overload and induce cell apoptosis. The study, analysis of the degree of endoplasmic reticulum stress in psoriatic lesions showed that IRE-1 α, XBP1, ATF6, CHOP...
and GADD34 expression in lesions gradually increased after treatment. This means that NB-UVB can activate the endoplasmic reticulum stress in psoriatic lesions and then induce cell apoptosis by endoplasmic reticulum stress downstream IRE-1α, ATF6 and other transmembrane proteins.

To sum up, it is believed that the molecular mechanisms of NB-UVB to treat psoriasis vulgaris include regulating the differentiation and maturation of CD4+T cell subsets Th1/Th2 and Th17/Treg in lesions, affect the expression and secretion of corresponding cytokines and inhibit the apoptosis mediated by endoplasmic reticulum stress.

References