Immune function and illness molecule expression in focus tissue after ALA–PDT combined with CO₂ laser treatment of condyloma acuminatum

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ABSTRACT

Objective: To study the immune function and illness molecule expression in focus tissue after ALA-PDT combined with CO₂ laser treatment of condyloma acuminatum. Methods: A total of 62 cases of patients with condyloma acuminatum were selected for study and divided into combined treatment group (n=29) and laser treatment group (n=33) according to different treatment methods. The changes of condyloma acuminatum focus tissue were observed, and immune indexes in peripheral blood as well as the expression levels of apoptosis-related genes and proliferation-related genes in focus tissue were detected. Results: Condyloma acuminatum lesion area of combined treatment group was significantly smaller than that of laser treatment group, and the lesion healing was more ideal; CD3⁺CD4⁺T lymphocyte proportion as well as TLR1, TLR3 and TLR9 expression levels on T cell surface in peripheral blood of combined treatment group were higher than those of laser treatment group while CD3⁺CD8⁺T lymphocyte and CD4⁺CD25⁺Foxp3⁻Treg cell proportion was lower than that of laser treatment group; p16INK4, CyclinD1, CDK4, CDK6, pRb and E2F as well as Livin and XIAP contents in focus tissue of combined treatment group were significantly lower than those of laser treatment group while Fas, FasL, SHP-1, PDCD4, TRAIL and Caspase-3 contents were significantly higher than those of laser treatment group. Conclusions: ALA-PDT combined with CO₂ laser treatment can more effectively clear condyloma acuminatum focus, improve cellular immune function as well as inhibit cell proliferation and promote cell apoptosis in focus tissue.

1. Introduction

Condyloma acuminatum (CA) is a clinical common sexually transmitted disease caused by human papilloma virus (HPV) infection, and the cure is difficult[1]. Traditional treatments include surgery treatment, drug therapy, the CO₂ laser treatment, etc., but the effect is not very ideal, and the recurrence rate is higher[2]. ALA photodynamic therapy (ALA-PDT) is a newly developed tissue ablation technique that uses the characteristic of high affinity between lesions and photosensitizer for treatment, and makes the photosensitizer gathered within the lesions and kill diseased cells[3]. In the following research, the immune function and illness molecule expression in focus tissue after ALA-PDT combined with CO₂ laser treatment of condyloma acuminatum were analyzed.

2. Materials and methods

2.1. Subjects

A total of 62 cases of patients diagnosed with condyloma acuminatum in our hospital from June 2014 to September 2015 were selected for study and divided into combined treatment group (n=29) and laser treatment group (n=33) according to different treatment methods. Combined treatment group received ALA-PDT combined with CO₂ laser treatment, including 18 male cases
and 11 female cases who were (37±4) years old; laser treatment group received CO₂ laser treatment, including 20 male cases and 13 female cases who were (35±3) years old. Comparison of general information between two groups showed no differences.

2.2. Treatment methods

Skin lesions were confirmed by Acetowhite test and then treatment was conducted. Laser group received CO₂ laser treatment, which was as follows: CO₂ laser was used for vaporization of skin lesion tissue, the depth reached the dermis, and the range was 2-5 mm around skin lesion tissue; combined treatment group received ALA-PDT combined with CO₂ laser treatment, which was as follows: at first, CO₂ laser was used for vaporization of focus tissue epidermis, and then 5-aminolevulinic acid was daubed on the pads, the skin lesions were covered by the pads, packed with plastic film for 4 h and irradiated with 635 nm wavelength red therapeutic apparatus, the energy density set to 200 mW/cm² and the irradiation time for 30 min.

2.3. Efficacy evaluation

Before treatment and 2 weeks after treatment, the conditions of condyloma acuminatum focus tissue of two groups were observed, and skin lesions were photographed and recorded.

2.4. Immune function indexes in peripheral blood

Two weeks after treatment, 5 mL peripheral blood of both groups was collected and separated to get mononuclear cells, different fluorescently-labeled CD3, CD4, CD8, CD25, Foxp3, TLR1, TLR3 and TLR9 monoclonal antibodies were incubated respectively, and then flow cytometer was used to analyze the contents of T cell subsets and the expression levels of TLRs.

2.5 Expression levels of apoptosis-related molecules in focus tissue

Two weeks after treatment, focus tissue was collected and homogenized, and then ELISA method was used to detect p16INK4, CyclinD1, CDK4, CDK6, pRb, E2F, Livin, XIAP, Fas, FasL, SHP-1, PDCD4, TRAIL and Caspase-3 contents.

2.6. Statistical methods

SPSS 14.0 statistical software was used for data analysis, analysis of measurement data between two groups was by t test and differences were considered to be statistically significant at the level of P<0.05.

3. Results

3.1. Skin lesion conditions before and after treatment

Before treatment, condyloma acuminatum lesion tissue of two groups were shown in Figure 1, the skin lesion area and shape were basically the same between two groups; after treatment, the pictures of condyloma acuminatum lesion tissue of two groups were shown in Figure 2, condyloma acuminatum lesion area of combined treatment group was significantly smaller than that of laser treatment group, and the lesion healing was more ideal.

Figure 1. Condyloma acuminatum lesion tissue of two groups before treatment.
Left: combined treatment group; right: laser treatment group.

Figure 2. Condyloma acuminatum lesion tissue of two groups after treatment.
Left: combined treatment group; right: laser treatment group.

3.2. Immune function indexes in peripheral blood

CD3⁺CD4⁺T lymphocyte proportion in peripheral blood of combined treatment group was higher than that of laser treatment group while CD3⁺CD8⁺T lymphocyte and CD4⁺CD25⁺Foxp3⁺Treg cell proportion was lower than that of laser treatment group; TLR1, TLR3 and TLR9 expression levels on T cell surface in peripheral blood of combined treatment group were higher than those of laser treatment group (Table 1).

3.3. Proliferation–related genes

p16INK4, CyclinD1, CDK4, CDK6, pRb and E2F contents in focus tissue of combined treatment group were significantly lower than those of laser treatment group; Livin and XIAP contents in focus tissue of combined treatment group were significantly lower than those of laser treatment group (Table 2).

3.4. Apoptosis–related genes
Fas, FasL, SHP-1, PDCD4, TRAIL and Caspase-3 contents in focus tissue of combined treatment group were significantly higher than those of laser treatment group (Table 3).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>CD3^+CD4^+ (%)</th>
<th>CD3^+CD8^+ (%)</th>
<th>CD4^+CD25^+Foxp3^+ (%)</th>
<th>TLR1 (%)</th>
<th>TLR3 (%)</th>
<th>TLR9 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment</td>
<td>39.25±4.13</td>
<td>26.42±3.16</td>
<td>6.49±0.81</td>
<td>14.56±1.68</td>
<td>7.15±0.88</td>
<td>7.36±0.84</td>
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<tr>
<td>Laser treatment</td>
<td>32.45±3.88</td>
<td>31.62±3.87</td>
<td>9.32±1.05</td>
<td>8.45±0.94</td>
<td>4.71±0.55</td>
<td>4.41±0.54</td>
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<tr>
<td>p</td>
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<td>&lt; 0.05</td>
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### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>p16INK4 (ng/L)</th>
<th>CyclinD1 (ng/L)</th>
<th>CDK4 (ng/L)</th>
<th>CDK6 (ng/L)</th>
<th>pRb (pg/L)</th>
<th>E2F (pg/L)</th>
<th>Livin (ng/L)</th>
<th>XIAP (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment</td>
<td>2.96±0.33</td>
<td>1.74±0.22</td>
<td>5.2e±0.61</td>
<td>3.7e±0.45</td>
<td>68.4±7.8</td>
<td>92.6±10.3</td>
<td>7.97±0.93</td>
<td>9.16±1.07</td>
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<tr>
<td>Laser treatment</td>
<td>5.14±0.64</td>
<td>3.08±0.38</td>
<td>13.35e±1.64</td>
<td>8.14±0.95</td>
<td>142.5±16.7</td>
<td>147.8±16.2</td>
<td>13.87±1.62</td>
<td>15.42±1.78</td>
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<tr>
<td>p</td>
<td>&lt; 0.05</td>
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### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Fas (ng/L)</th>
<th>FasL (ng/L)</th>
<th>SHP-1 (pg/L)</th>
<th>PDCD4 (pg/L)</th>
<th>TRAIL (ng/L)</th>
<th>Caspase-3 (ng/L)</th>
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<tbody>
<tr>
<td>Combined treatment</td>
<td>15.92±1.85</td>
<td>25.59±3.05</td>
<td>142.25±17.62</td>
<td>226.53±26.59</td>
<td>53.43±6.13</td>
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<tr>
<td>Laser treatment</td>
<td>8.14±0.99</td>
<td>11.35±1.27</td>
<td>88.45±9.69</td>
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<td>29.24±3.62</td>
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<tr>
<td>p</td>
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### 4. Discussion

The principle of CO₂ laser treatment of condyloma acuminatum is the carbonization and vaporization of condyloma acuminatum tissue by high temperature and the clearance of focus tissue and locally infected HPV. However, at the same time of clearing condyloma acuminatum tissue, CO₂ laser would have a splash on the surrounding normal tissues and make the adjacent normal tissues infected by HPV, thereby causing the recurrence of condyloma acuminatum[4]. ALA photodynamic therapy (ALA-PDT) is a newly developed tissue ablation technology, rapidly proliferated cells in focal tissue can absorb exogenous photosensitizer and convert into protoporphyrin IX in cells, and normal tissue won't absorb the photosensitizer. When the tissue is exposed to a particular wavelength of laser, protoporphyrin IX will form the singlet oxygen and killer cells[5,6]. Based on CO₂ laser treatment, ALA-PDT was used for treatment in the research, and observation of the changes of focus tissue showed that condyloma acuminatum lesion area of combined treatment group was significantly smaller than that of laser treatment group, and the lesion healing was more ideal. It indicated that ALA-PDT combined with CO₂ laser treatment could more effectively clear condyloma acuminatum focus.

Cellular immune dysfunction is associated with persistent infection of HPV and the pathogenesis of condyloma acuminatum. T lymphocyte is the key cell group to perform cellular immune response, and the abnormality of its content and function will affect the completion of cellular immune response[7]. CD3^+CD4^+ T lymphocytes and CD3^+CD8^+ T lymphocytes are T cell subsets with immune adjuvant effect and immunosuppressive effect respectively, CD4^+CD25^+Foxp3^+Treg cells are regulatory T cells with inhibiting effect[8,9]. After treatment, CD3^+CD4^+ T lymphocyte proportion in peripheral blood of combined treatment group was higher than that of laser treatment group while CD3^+CD8^+ T lymphocyte and CD4^+CD25^+Foxp3^+Treg cell proportion was lower than that of laser treatment group. It indicated that ALA-PDT combined with CO₂ laser treatment could more effectively clear condyloma acuminatum focus.
expression of a variety of cell cycle-related genes and anti-apoptotic genes abnormally increases. Cell cycle-related genes include Cyclins, and cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitor (CKI), and the cell cycle pathway closely related to the cell proliferation within the condyloma acuminatum lesions is p16INK4-CyclinD1-CDK4/6-pRb-E2F[10]. Livin and XIAP are two types of anti-apoptotic molecules closely related to the onset of condyloma acuminatum, and can enhance the anti-apoptotic ability of cells and promote cell proliferation[11,12]. In the research, analysis of the expression levels of above molecules in focus tissue showed that p16INK4, CyclinD1, CDK4, CDK6, pRb and E2F as well as Livin and XIAP contents in focus tissue of combined treatment group were significantly lower than those of laser treatment group. It indicated that ALA-PDT combined with CO2 laser treatment could more effectively inhibit cell proliferation in focus tissue.

In the development process of condyloma acuminatum, there is not only abnormal proliferation-related genes expression, but also abnormal expression of apoptosis-related genes. Insufficient expression of pro-apoptotic genes such as Fas/FasL, SHP-1, PDCD4, TRAIL and Caspase-3 is closely related to condyloma acuminatum. Combination of Fas and FasL can induce apoptosis through death receptor pathway[13]; SHP-1 can be combined with ITIM on growth factor receptors to change the space conformation of receptors, terminate the transduction of growth signals and induce apoptosis; PDCD4-encoded proteins are mainly located in the nucleus, and can restrain the function of a variety of cell cycle molecules and cause cell apoptosis[14]; TRAIL is a member of the tumor necrosis factor superfamily, and the combination with corresponding receptor can induce apoptosis through two pathways Caspase-3 and NF-kB[15]. In the research, analysis of the expression levels of above molecules in focus tissue showed that Fas, FasL, SHP-1, PDCD4, TRAIL and Caspase-3 contents in focus tissue of combined treatment group were significantly higher than those of laser treatment group. It indicated that ALA-PDT combined with CO2 laser treatment could more effectively induce cell apoptosis in focus tissue.

Based on above discussion, it can be concluded that ALA-PDT combined with CO2 laser treatment can more effectively clear condyloma acuminatum focus, improve cellular immune function as well as inhibit cell proliferation and promote cell apoptosis in focus tissue.

References


