Effect of local ozone treatment on inflammatory cytokine, growth cytokine and apoptosis molecule expression in anal fistula wound

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ABSTRACT

Objective: To study the effect of local ozone therapy on inflammatory cytokine, growth cytokine and apoptosis molecule expression in anal fistula wound. Methods: Patients with low simple anal fistula who received anal fistulectomy in Eighth Hospital of Wuhan between June 2014 and December 2016 were selected and randomly divided into ozone group and control group who received ozone combined with routine treatment and routine treatment respectively. 5 d, 10 d and 15 d after treatment, proper amount of wound granulation tissue was clamped to determine the expression of inflammatory cytokines IFN-γ, TNF-α, IL-1, IL-8 and IL-12, growth cytokines VEGF, FGF-1, FGF-2 and EGF as well as apoptosis molecules Bax, P53 and caspase-3. Results: 5 d, 10 d and 15 d after treatment, IFN-γ, TNF-α, IL-1, IL-8, IL-12, VEGF, FGF-1, FGF-2 and EGF protein expression in anal fistula wound of ozone group were significantly higher than those of control group while Bax, P53 and caspase-3 protein expression were significantly lower than those of control group. Conclusion: Local ozone treatment can increase the expression of inflammatory cytokines and growth cytokines and inhibit the expression of apoptosis molecules in anal fistula wound, and it is conducive to wound healing.

1. Introduction

Anal fistula is a clinical common rectum and anus disease, the main cause is that the rectal bacteria invade the anal sinuses and cause infection, and surgical treatment is needed. The wound after anal fistula surgery is special, local wound pollution is serious because of the special physiological function of daily defeation of anus, the healing process is accompanied by repair and pollution, and it’s easy to cause delay in wound repair and healing[1,2]. Ozone is the means for complex wound treatment in recent years, which has strong oxidizing property, can not only kill pathogens and induce fibroblast proliferation, but can also adjust the function of endothelial cells and macrophages, and plays a significant role in promoting repair of wounds[3,4]. The wound healing process involves inflammatory response, cell growth and tissue reconstruction, and various cytokines and apoptotic molecules are involved in regulating the process. In the following study, in order to define the value of ozone for the treatment of anal fistula wound, the effect of local ozone therapy on inflammatory cytokine, growth cytokine and apoptosis molecule expression in anal fistula wound was analyzed in detail.

2. Information of clinical patients and methods of clinical research

2.1 General information of clinical patients

Patients with low simple anal fistula who received anal fistulectomy in Eighth Hospital of Wuhan between June 2014 and December 2016 were selected as the research subjects, and all the patients were in line with the diagnostic criteria for anal fistula, accepted fistulectomy and opened wounds. Patients with tuberculous anal fistula and anal fistula combined with perianal eczema were

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ruled out. A total of 68 cases were enrolled and randomly divided into two groups, each with 34 cases. Ozone group included 22 men and 12 women that were 32-55 years old; control group included 20 men and 14 women that were 33-53 years old. There was no significant difference in general information between the two groups of patients ($P>0.05$).

2.2 Wound treatment

Control group received routine dressing change, iodine disinfection and then vaseline gauze placement, once a day; ozone group, on the basis of routine dressing change, received local ozone treatment, and the method was as follows: ozone apparatus from Wuhan Sinotech Medical Technology Co., Ltd., was used for ozone treatment, ozone concentration was set to 10 mg/L, ozone water flushing mode and aerosol fumigation mode were used for treatment, 3 min each time, and routine dressing change was conducted after treatment.

2.3 Detection of molecule expression in wound

5 d, 10 d and 15 d after dressing change, a little wound granulation tissue was clamped, added in protein lysis buffer and fully homogenized, the total protein in tissue was separated, and enzyme-linked immunosorbent assay kit was used to detect the protein expression of IFN-γ, TNF-α, IL-1, IL-8, IL-12, VEGF, FGF-1, FGF-2, EGF, Bax, P53 and caspase-3.

2.4 Statistical method

SPSS 18.0 software was used to input and analyze data, protein expression data between two groups were by t test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Inflammatory cytokine expression in anal fistula wound

5 d, 10 d and 15 d after dressing change, analysis of inflammatory cytokines IFN-γ, TNF-α, IL-1, IL-8 and IL-12 expression in anal fistula wound between two groups of patients was as follows: IFN-γ, TNF-α, IL-1, IL-8 and IL-12 protein expression in anal fistula wound of ozone group were significantly higher than those of control group. Differences in IFN-γ, TNF-α, IL-1, IL-8 and IL-12 expression in anal fistula wound were statistically significant between two groups of patients 5 d, 10 d and 15 d after dressing change ($P<0.05$).

3.2 Growth cytokine expression in anal fistula wound

5 d, 10 d and 15 d after dressing change, analysis of growth cytokines VEGF (ng/L), FGF-1 (pg/L), FGF-2 (ng/L) and EGF (ng/L) expression in anal fistula wound between two groups of patients was as follows: VEGF, FGF-1, FGF-2 and EGF protein expression in anal fistula wound of ozone group were significantly higher than those of control group. Differences in VEGF, FGF-1, FGF-2 and EGF expression in anal fistula wound were statistically significant between two groups of patients 5 d, 10 d and 15 d after dressing change ($P<0.05$).

3.3 Apoptosis molecule expression in anal fistula wound

5 d, 10 d and 15 d after dressing change, analysis of apoptosis molecules Bax (ng/L), P53 (pg/L) and caspase-3 (ng/L) expression

<table>
<thead>
<tr>
<th>Table 1. Inflammatory cytokine expression in anal fistula wound after dressing change (ng/L).</th>
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<tbody>
<tr>
<td><strong>Groups</strong></td>
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<tr>
<td>Ozone group</td>
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<td>Control group</td>
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$^*$: comparison between ozone group and control group, $P<0.05$.

<table>
<thead>
<tr>
<th>Table 2. Growth cytokine expression in anal fistula wound after dressing change.</th>
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<td><strong>Groups</strong></td>
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<tr>
<td>Ozone group</td>
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<td>Control group</td>
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$^*$: comparison between ozone group and control group, $P<0.05$. 
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<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time (d)</th>
<th>Bax</th>
<th>P53</th>
<th>Caspase-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone group</td>
<td>34</td>
<td>5</td>
<td>1.87±0.24*</td>
<td>86.53±10.24*</td>
<td>4.29±0.62*</td>
</tr>
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<td></td>
<td>10</td>
<td>1.32±0.17</td>
<td>67.51±8.24*</td>
<td>3.04±0.52*</td>
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<tr>
<td></td>
<td>15</td>
<td>1.05±0.15</td>
<td>48.64±7.25*</td>
<td>2.41±0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.94±0.42</td>
<td>131.25±16.74</td>
<td>7.58±0.94</td>
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</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>10</td>
<td>2.13±0.26</td>
<td>112.42±14.52</td>
<td>5.61±0.66</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.88±0.25</td>
<td>104.46±12.83</td>
<td>4.24±0.55</td>
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</tbody>
</table>

*: comparison between ozone group and control group, P<0.05.

4. Discussion

Postoperative anal fistula wound is quite special and accompanied by both pollution and repair, the tissue repair process involves inflammation, cell growth and tissue reconstruction, and the pollution caused by waste discharge will affect the repair process[5,6]. Therefore, effective local dressing change and treatment have positive value for the healing of anal fistula wound. The chronic inflammatory response is an important pathologic change in the process of wound healing, and moderate inflammation helps the wound repair and healing[7]. Macrophages are the cells that mediate the phagocytosis and antigen presenting during inflammatory response. In the microenvironment of wound healing, macrophages can be activated into M1 macrophages, synthesize IL-1, IL-8, IL-12 and other pro-inflammatory factors under the induction of IFN-γ, TNF-α and other cytokines, and then participate in the destruction of pathogenic microorganisms. IL-1 and IL-12 have strong pro-inflammatory activity, which can enhance the phagocytosis of neutrophils and macrophages, and can also mediate the cascade activation of the inflammatory response[8]; IL-8 is a cytokine with chemotactic activity, which can promote the aggregation and infiltration of lymphocytes, neutrophils and other inflammatory cells to local wound, and then kill local pathogen via inflammatory response[9]. In order to define the effect of local ozone treatment on the inflammatory response in anal fistula wound healing, the inflammatory cytokine expression were analyzed in the study, and the results showed that IFN-γ, TNF-α, IL-1, IL-8 and IL-12 protein expression in anal fistula wound of ozone group were significantly higher than those of control group. This shows that the local ozone treatment can activate the inflammatory response in the anal fistula wound to a certain extent, and it is beneficial to the healing of the wound.

The proliferation of fibroblasts, endothelial cells, epithelial cells and other cells is an important biological process in the repair and healing of wound. Growth cytokines such as VEGF, FGF-1, FGF-2 and EGF are the important endogenous active substances mediating the proliferation and growth of cells, and play an important role in the process of wound repair. VEGF is the family including VEGF-A, -B, -C, -D, -E and other members, which can promote new capillary generation and enrich the granulation tissue in the process of wound repair, and can also improve the microcirculation in local wound and provide the nutrients needed for repairing[10]. FGF-1 and FGF-2 are the members of the FGF family involved in wound repair, have promoting effect on the proliferation of fibroblasts, endothelial cells and keratinocyte, and can accelerate tissue repair and angiogenesis in local tissue[11]. EGF is the cytokine that can specifically promote epidermal cell proliferation, and it can accelerate the epithelial process of wound, and also promote the connective tissue formation and contraction within wound[12]. In order to define the effect of local ozone treatment on the secretion of growth cytokines in the process of anal fistula wound healing, the growth cytokine expression were analyzed in the study, and the results showed that VEGF, FGF-1, FGF-2 and EGF protein expression in anal fistula wound of ozone group were significantly higher than those of control group. This shows that the local ozone treatment can promote the secretion of various growth cytokines in the wound to a certain extent and thus promote the healing of the wound.

Ozone, as a kind of strong oxidizer, it can effectively kill the pathogenic bacteria in wound through its own strong oxidizing property, and meanwhile, ozone is automatically decomposed into oxygen and water after killing pathogenic bacteria, which doesn’t leave behind pollution, and can adjust the secretion of cytokines, cell proliferation and apoptosis, and other biological processes in wound[13–15]. The proliferation activity of fibroblasts, endothelial cells, epithelial cells and others in wound are not only associated with VEGF, FGF-1, FGF-2, EGF and other growth cytokines, but also closely related to the cell apoptosis process regulated by apoptosis molecules such as Bax, P53, caspase-3. Bax is a molecule that exerts pro-apoptosis effect through the mitochondrial pathway, it is located in mitochondrial membrane and can form dimers, and Bax-Bax homodimer is the channel for cytochrome C from mitochondrial membrane into the cytoplasm; Bax promotes cellular mitochondrial pathway apoptosis by promoting cytochrome C into cytoplasm[16]. P53 is the regulator of multiple apoptotic pathways in the body, which can inhibit cell proliferation and induce cell apoptosis by blocking cell cycle[17]. Caspase-3 is the executive molecule of apoptosis, and different apoptotic pathways can all activate caspase-3 and cause apoptosis. In the study, analysis of the apoptosis molecule expression in wound healing process showed...
that Bax, P53 and caspase-3 protein expression in anal fistula wound of ozone group were significantly lower than those of control group. This means that local ozone treatment can inhibit the cell apoptosis in anal fistula wound to a certain extent, is conducive to the cell proliferation in the wound, and then promotes the healing of wounds. Local ozone treatment has promoting effect on anal fistula wound healing, and increasing the expression of inflammatory cytokines and growth cytokines and inhibiting the expression of apoptosis molecules are the molecular mechanisms for local ozone treatment to promote wound healing.

References


