Effect of ozone water rinse on wound healing in rats with \textit{Pseudomonas aeruginosa} infection

Ju-Hua Ye*, Jun-Wu Huang, Hong-Yun Shi

Operation Room, Huanggang Central Hospital, Huanggang City, Hubei Province, 438000, China

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\textbf{Objective:} To study the promoting effect of ozone water rinse on wound healing in rats with \textit{Pseudomonas aeruginosa} infection. \textbf{Methods:} Wistar male rats were selected as experimental animals and randomly divided into control group, chlorhexidine group and ozone water group, \textit{Pseudomonas aeruginosa}-infected wounds were made and cleaned with normal saline, chlorhexidine and ozone water respectively; wound healing of three groups was observed, wound tissue was collected and contents of inflammatory factors, apoptosis molecules and autophagy markers were detected. \textbf{Results:} Wound healing rates of chlorhexidine group and ozone water group were higher than that of control group and wound healing time was shorter than that of control group, wound healing rate of ozone water group was higher than that of chlorhexidine group and wound healing time was shorter than that of chlorhexidine group; TNF-$\alpha$, IL-1, IL-2, Fas, FasL and Beclin-1 contents and LC3 II/LC3 I ratios in wound tissue of chlorhexidine group and ozone water group were lower than those of control group, and TNF-$\alpha$, IL-1, IL-2, Fas, FasL and Beclin-1 contents and LC3 II/LC3 I ratios in wound tissue of ozone water group were lower than those of chlorhexidine group. \textbf{Conclusions:} Compared with normal saline and chlorhexidine, ozone water rinse helps to promote wound healing, improve wound healing rate and shorten wound healing time in rats with \textit{Pseudomonas aeruginosa} infection, and meanwhile it can inhibit cell apoptosis and autophagy in the wounds.

1. Introduction

Wound healing is the most basic and most common problem in surgery, and the process involves complicated pathophysiologic changes and requires the participation of a variety of molecules. In clinical practice, wound infection is the most common reason that causes delay of wound healing or difficulty to heal[1]. Long-term exposure of wounds and weakened body resistance can cause large growth and reproduction of some conditioned pathogens in local area, thus affecting wound healing process through the synthesis and release of protease, oxygen free radicals, inflammatory factors, and so on[2,3]. Therefore, how to effectively deal with infected wounds and promote wound healing is an important clinical topic. In recent years, ozone water has displayed positive value in promoting wound healing and anti-infection. In the following research, the promoting effect of ozone water rinse on wound healing in rats with \textit{Pseudomonas aeruginosa} infection was analyzed, hereby reported as follows.

2. Materials and methods

2.1. Experimental materials

A total of 30 Wistar male rats were provided by the university animal center, \textit{Pseudomonas aeruginosa} was provided by Microbiology Laboratory and ozone water preparation device was from German Haas Company.
2.2. Animal experiment methods

A total of 30 Wistar rats were randomly divided into control group, chlorhexidine group and ozone water group, the following methods were followed to build Pseudomonas aeruginosa-infected wound models of three groups, and details were as follows: hair on the backs of rats was removed by depilatory, skin was revealed and disinfected, then skin defect of 3 cm × 2 cm was prepared, wound depth reached muscular layer, Pseudomonas aeruginosa suspension was applied to the wounds after hemostasis by compression, wounds were covered with dressing, wounds were observed after 48h, and rats with formed wounds were used in subsequent studies. Wounds of control group were cleaned with normal saline every day, wounds of chlorhexidine group were rinsed with chlorhexidine every day and wounds of ozone water group were rinsed with ozone water every day; after rinse, wounds were covered with gauze.

2.3. Evaluating methods of wound healing

Before wound cleaning and 3 d, 7 d, 14 d and 21 d after wound cleaning, wounds were photographed and recorded, photos were input into computer to calculate wound area, and wound healing rate = (wound area before wound cleaning - wound area after wound cleaning) / wound area before wound cleaning.

2.4. Detection of molecule contents in wound tissue

Wound tissue was collected, frozen with liquid nitrogen and then used for detection. When mRNA contents were detected, appropriate amount of wound tissue was taken and grinded to extract RNA and reverse-transcribe it into cDNA for PCR amplification, and mRNA contents of TNF-α, IL-1, IL-2, Beclin-1, LC3 I and LC3 II were detected; when protein contents were detected, appropriate amount of wound tissue was taken and grinded to get protein suspension, ELISA kits were used to detect TNF-α, IL-1 and IL-2 contents, BCA kits were used to detect total protein content, and contents of cytokines in each mg of total protein specimen were calculated.

2.5. Statistical methods

SPSS21.0 software was used to input and statistically process data, comparison among three groups was performed by variance analysis, pair wise comparison was performed by LSD-t test and differences were considered to be statistically significant at a level of P < 0.05.

3. Results

3.1. Wound healing

Analysis of wound healing rate showed that within 21 d after treatment, wound healing rates of three groups showed increasing trend, wound healing rates at different points in time of three groups were different, wound healing rates of chlorhexidine group and ozone water group were higher than that of control group, and wound healing rate of ozone water group was higher than that of chlorhexidine group; analysis of wound healing time showed that wound healing time of chlorhexidine group and ozone water group was shorter than that of control group, and wound healing time of ozone water group was shorter than that of chlorhexidine group (Table 1).

3.2. Inflammatory factor contents in wounds

Comparison results of inflammatory factor contents in wound tissue of three groups by variance analysis were as follows: mRNA contents and protein contents of TNF-α, IL-1 and IL-2 in wound tissue of three groups were different; pair wise comparison among groups by LSD method showed that mRNA contents and protein contents of TNF-α, IL-1 and IL-2 in wound tissue of chlorhexidine group and ozone water group were lower than those of control group, and mRNA contents and protein contents of TNF-α, IL-1 and IL-2 in wound tissue of ozone water group were lower than those of chlorhexidine group (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wound healing rate (%)</th>
<th>Wound healing rate (%)</th>
<th>Wound healing time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3d after treatment</td>
<td>7d after treatment</td>
<td>14d after treatment</td>
</tr>
<tr>
<td>Ozone water group</td>
<td>7.25±0.87</td>
<td>32.06±4.23</td>
<td>68.12±6.98</td>
</tr>
<tr>
<td>Chlorhexidine group</td>
<td>4.31±0.52</td>
<td>19.02±2.14</td>
<td>45.97±4.89</td>
</tr>
<tr>
<td>Control group</td>
<td>3.13±0.34</td>
<td>9.34±0.89</td>
<td>32.68±3.87</td>
</tr>
<tr>
<td>F</td>
<td>7.948</td>
<td>10.494</td>
<td>17.844</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
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<table>
<thead>
<tr>
<th>Groups</th>
<th>mRNA content (ratio with β-actin)</th>
<th>Protein content (ng/mg total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF-α</td>
<td>IL-1</td>
</tr>
<tr>
<td>Ozone water group</td>
<td>31.24±3.58</td>
<td>37.54±4.02</td>
</tr>
<tr>
<td>Chlorhexidine group</td>
<td>73.85±7.69</td>
<td>70.29±7.72</td>
</tr>
<tr>
<td>Control group</td>
<td>100±12.91</td>
<td>100±10.58</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
3.3. Apoptosis molecule expression levels in wounds

Comparison results of apoptosis molecule expression levels in wound tissue of three groups by variance analysis were as follows: mRNA contents of Fas and FasL in wound tissue of three groups were different; pair wise comparison among groups by LSD method showed that mRNA contents of Fas and FasL in wound tissue of chlorhexidine group and ozone water group were lower than those of control group, and mRNA contents of Fas and FasL in wound tissue of ozone water group were lower than those of chlorhexidine group (Figure 1).

![Figure 1. Apoptosis molecule expression levels in wounds of three groups.](image)

3.4. Autophagy marker expression levels in wounds

Comparison results of autophagy marker expression levels in wound tissue of three groups by variance analysis were as follows: mRNA contents of Beclin-1 and LC3 II/LC3 I ratios in wound tissue of three groups were different; pair wise comparison among groups by LSD method showed that mRNA contents of Beclin-1 and LC3 II/LC3 I ratios in wound tissue of chlorhexidine group and ozone water group were lower than those of control group, and mRNA content of Beclin-1 and LC3 II/LC3 I ratio in wound tissue of ozone water group were lower than those of chlorhexidine group (Figure 2).

![Figure 2. Autophagy marker expression levels in wounds of three groups.](image)

4. Discussion

Wound infection is the most common reason that causes the difficulty for wounds to heal, and infiltration of large amounts of inflammatory cells in infected lesions will release large amounts of proteases, oxygen free radicals, inflammatory factors, and so on, thus causing local tissue damage and collagen degradation rate exceeding synthesis rate, and resulting in delayed wound healing and repair barriers[4,5]. Pseudomonas aeruginosa is the most common pathogen that causes wound infection, the bacteria is parasitic in both skin surface and the intestines, decreased body resistance or immunity as well as the slow healing of local wounds will cause infection, and it is within the scope of conditioned pathogen[6-8]. Common method of clinical treatment of wounds is cleaning the wounds with chlorhexidine, although it can clear the wound exudates, chlorhexidine itself doesn’t have the sterilization effect, and therefore its effect on dealing with infected wounds is not ideal.

Ozone is a new material used for the treatment of skin wound in recent years, and studies of foreign and domestic scholars show that medial ozone has good repairing effect on wounds such as diabetic foot and skin ulcers[9,10]. Ozone dissolves in aqueous solution and then forms ozone water, the solution has strong sterilization function as well as decomposition and catalyst effect, and on the one hand, it directly inhibits the growth and reproduction of bacteria and reduces the synthesis of toxic byproducts; on the other hand, it has decomposing and catalyzing effect on oxygen free radicals, inflammatory factors, proteases, etc, and reduces the accumulation of local toxic byproducts[11,12]. Based on the effect of above two aspects, it was speculated that ozone water could promote the healing of infected wounds. In the research, Pseudomonas aeruginosa-infected wound models in rats were built, and comparison of the effect of chlorhexidine and ozone water on wound healing showed that wound healing rate of ozone water group was higher than that of chlorhexidine group and wound healing time was shorter than that of
chlorhexidine group, which indicated that ozone water could more effectively promote the healing of infected wounds.

After *Pseudomonas aeruginosa* infects wounds, the most direct pathophysiologic changes are the activation of inflammatory response and the release of large amounts of inflammatory mediators. In local wounds, inflammatory mediators within a certain range of concentration can help to kill infectious pathogens and limit further growth of pathogens and expansion of infected lesions. Therefore, inflammatory response is also regarded as body’s defense reaction and self compensatory mechanism. However, in the development process of infected wounds, if pathogens cannot be effectively removed and controlled, they will increase local inflammation and lead to large release of inflammatory mediators, thus imposing an adverse influence on wound healing[13]. TNF-α, IL-1 and IL-2 are three types of inflammatory mediators that play an important role in the process of wound infection, and TNF-α has the effect of starting inflammatory response and inflammatory cascade amplification reaction; IL-1 and IL-2 can directly mediate inflammatory response and exert bacteria-killing effect[14]. Analysis of inflammatory mediator contents in wounds showed that mRNA contents and protein contents of TNF-α, IL-1 and IL-2 in wounds of ozone water group were significantly lower than those of chlorhexidine group.

Studies about pathophysiologic changes in healing process of infected wounds in recent years believe that abnormal cell apoptosis pathway is related to delayed wound healing. Fas/FasL is a classic apoptosis signal transduction pathway. Fas is a member of tumor necrosis factor receptor superfamily, and combination of its ligand FasL with it can start cell apoptosis process and induce cell apoptosis. In infected wounds, stimulated by pathogen infection and large release of inflammatory mediators, Fas and FasL are largely expressed and cause increased cell apoptosis and delayed wound healing. In addition to the involvement of FasL/Fas in the regulation of cell apoptosis and wound healing, autophagy process is also closely related to the regulation of cell apoptosis, and it is also known as “Type II programmed cell death”[15]. LC3 and Beclin-1 are marker proteins of autophagy process, elevated LC3 II/LC3 I and increased Beclin-1 mark enhanced autophagy process and increased cell apoptosis[16]. After wounds were cleaned with chlorhexidine and ozone water, cell apoptosis was detected, and results showed that mRNA contents of Fasl, Fas and Beclin-1 as well as LC3 II/LC3 I in wounds of ozone water group was significantly lower than those of chlorhexidine group, which indicated that ozone water could inhibit cell apoptosis in infected wounds.

Based on above discussion, it can be concluded that compared with normal saline and chlorhexidine, ozone water rinse helps to promote wound healing, improve wound healing rate and shorten wound healing time in rats with *Pseudomonas aeruginosa* infection, and meanwhile it can inhibit cell apoptosis and autophagy in the wounds.

**References**


