Effect of vacuum sealing drainage combined with biological dressings on the angiogenesis and inflammatory response after diabetic foot ulcer wound grafting

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ABSTRACT

Objective: To study the effect of vacuum sealing drainage combined with biological dressings on the angiogenesis and inflammatory response after diabetic foot ulcer wound grafting.

Methods: Patients with diabetic foot who were treated in Sichuan Provincial People’s Hospital between May 2014 and February 2017 were selected and randomly divided into vacuum drainage group and normal control group who received vacuum sealing drainage combined with biological dressings as well as conventional debridement and dressing change to deal with the wound respectively. Before treatment as well as 1 d, 3 d and 5 d after treatment, the wound tissue was collected to determine the expression of angiogenesis molecules, angiogenesis signaling pathway molecules and inflammatory response molecules.

Results: 1 d, 3 d and 5 d after treatment, VEGF, VEGFR, CD105, MMP9, PI3K, AKT, cyclinD1, p38MAPK and NF-kB protein expression in wound tissue of vacuum drainage group were significantly higher than those of normal control group while COX-2, iNOS, TNF-α and IL-6 mRNA expression were significantly lower than those of normal control group.

Conclusion: Vacuum sealing drainage combined with biological dressings promotes the angiogenesis and inhibits the inflammatory response after diabetic foot ulcer wound grafting.

1. Introduction

Type 2 diabetes mellitus is a common clinical endocrine metabolism disease, which can cause vessel and nerve injury during the disease course progression and lead to many complications. Diabetic foot is a serious complication of diabetes, which is closely related to the lower limb vasculopathy and neuropathy, difficult to handle in clinic and with high amputation rate[1,2]. At present, the main clinical therapies for diabetic foot are debridement, anti-infection, local dressing change and improving circulation, but the treatment effect is not satisfactory, and the ulcer wound healing is not ideal. Vacuum sealing drainage is a new means of wound treatment developed in recent years, and the combination of biological dressing can achieve extensive wound drainage and make the wound in a clean and moist local environment, which is advantageous to the local angiogenesis and inflammatory mediator removal, and thus promotes the healing of wounds[3]. It is reported that the vacuum sealing drainage combined with biological dressings can improve the wound healing of the fracture combined with soft tissue damage[4]. In the following studies, we analyzed the effects of vacuum sealing drainage combined with biological dressings on the angiogenesis and inflammatory response after diabetic foot ulcer wound grafting.

2. Case information and research methods

2.1 General case information

Patients with diabetic foot who were treated in our hospital between May 2014 and February 2017 were selected, and all patients were in accordance with the diagnostic criteria for diabetic foot, conformed to the skin grafting indications and refused amputation; the patients with diabetic foot of the Wngner IV-V grade, the patients with poor overall condition and unable to tolerate the operation and multiple dressing change, and the patients with arterial occlusion confirmed by lower extremity vessel ultrasonography were excluded. A total of 56 cases were enrolled and divided into two groups by random number table, each with 23...
cases. Vacuum drainage group included 14 men and 9 women that were 42-59 years old; control group included 13 men and 10 women that were 41-60 years old. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Wound treatment

Both groups of patients received intensive insulin treatment after admission, fasting blood glucose was controlled below 7 mmol/L, then debridement and skin grafting were conducted, the area with small defect was moderately released and covered with adjacent soft tissue, and the area with big defect received split-thickness skin grafting. After skin grafting, vacuum drainage group received the vacuum sealing drainage combined with biological dressings for wound treatment, and the method was as follows: the wound surface was referred to cut VSD, the cut dressing was placed on wound surface, the drainage tube was inserted inside the foam, then tegaderm transparent dressing was used to close the wound, vacuum drainage was connected, and the pressure was about 10.0 kPa. Control group were treated with conventional wound dressing.

2.3 Gene protein expression detection

Before treatment as well as 1 d, 3 d and 5 d after treatment, appropriate wound tissue was collected during dressing change, washed with PBS to remove the secreta adhered on the wound surface and then added in RIPA lysis buffer to separate the total protein in the tissue, and then enzyme-linked immunosorbent assay kit was used to determine VEGF, VEGFR, CD105, MMP9, PI3K, AKT, cyclinD1, p38MAPK and NF-kB protein expression.

2.4 Gene mRNA expression detection

The wound tissue before treatment as well as 1 d, 3 d and 5 d after treatment were taken and added in RNAiso lysis buffer from Takara to isolate the RNA in the tissue, the reverse transcription kit from Takara was used to synthesize the RNA into cDNA by reverse transcription, finally the fluorescent quantitative PCR kit from Takara company was used to amplify COX-2, iNOS, TNF-α and IL-6, and the mRNA expression was calculated.

2.5 Statistical methods

SPSS 20.0 was used to input and analyze data, measurement data analysis between two groups was by t test and P<0.05 indicated statistical significance in differences in test results.

3. Results

3.1 Angiogenesis molecule expression in wound tissue

Before treatment and at different time points after treatment, analysis of angiogenesis molecules VEGF (ng/mL), VEGFR (ng/mL), CD105 (pg/mL) and MMP9 (pg/mL) expression in wound tissue between vacuum drainage group and normal control group was as follows: before treatment, VEGF, VEGFR, CD105 and MMP9 protein expression in wound tissue were not significantly different between two groups of patients; 1 d, 3 d and 5 d after treatment, VEGF, VEGFR, CD105 and MMP9 protein expression in wound tissue of vacuum drainage group were significantly higher than those of normal control group (P<0.05).

3.2 Angiogenesis pathway signal molecule expression in wound tissue

Before treatment and at different time points after treatment, analysis of angiogenesis pathway signal molecules PI3K (ng/mL), AKT (ng/mL), cyclinD1 (ng/mL), p38MAPK (pg/mL) and NF-kB (pg/mL) expression in wound tissue between vacuum drainage group and normal control group was as follows: before treatment,
Inflammatory response molecule mRNA expression in wound tissue of two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>COX-2</th>
<th>INOS</th>
<th>TNF-α</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum drainage</td>
<td>29</td>
<td>Before treatment</td>
<td>1.02±0.15</td>
<td>1.05±0.15</td>
<td>0.98±0.14</td>
<td>1.01±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 d after treatment</td>
<td>0.73±0.09*</td>
<td>0.69±0.09*</td>
<td>0.64±0.07*</td>
<td>0.71±0.09*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>0.56±0.08*</td>
<td>0.52±0.07*</td>
<td>0.49±0.06*</td>
<td>0.44±0.08*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d after treatment</td>
<td>0.32±0.06*</td>
<td>0.38±0.04*</td>
<td>0.31±0.04*</td>
<td>0.24±0.05*</td>
</tr>
<tr>
<td>Control</td>
<td>29</td>
<td>Before treatment</td>
<td>1.04±0.13</td>
<td>0.99±0.11</td>
<td>1.01±0.13</td>
<td>0.97±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 d after treatment</td>
<td>0.87±0.11*</td>
<td>0.81±0.08*</td>
<td>0.84±0.10*</td>
<td>0.85±0.12*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 d after treatment</td>
<td>0.64±0.08*</td>
<td>0.72±0.08*</td>
<td>0.69±0.08*</td>
<td>0.62±0.08*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d after treatment</td>
<td>0.50±0.07*</td>
<td>0.55±0.06*</td>
<td>0.46±0.07*</td>
<td>0.54±0.07*</td>
</tr>
</tbody>
</table>

*: comparison between vacuum drainage group and normal control group, P<0.05; *: comparison between before and after treatment, P<0.05.

3.3 Inflammatory response molecule expression in wound tissue

Before treatment and at different time points after treatment, analysis of inflammatory response molecules COX-2, iNOS, TNF-α and IL-6 expression in wound tissue between vacuum drainage group and normal control group was as follows: before treatment, COX-2, iNOS, TNF-α and IL-6 mRNA expression in wound tissue were not significantly different between two groups of patients; 1 d, 3 d and 5 d after treatment, PI3K, AKT, cyclinD1, p38MAPK and NF-kB protein expression in wound tissue of vacuum drainage group were significantly higher than those of normal control group (P<0.05).

4. Discussion

Diabetic foot is one of the most serious complications of diabetic patients, which mainly involves lower segment of leg, ankle, planta and toe, and is with great clinical treatment difficulty and high amputation rate[5]. The occurrence of diabetic foot is closely related to lower limb neuropathy and vasculopathy, neuropathy will cause sensory hypofunction of corresponding parts and increase the chance of injury, and vasculopathy can cause luminal stenosis and poor blood supply and make rupture area difficult to heal. After the occurrence of diabetic foot, local rupture wound is easily infected, and the effect of conventional debridement and dressing change is less effective[6]. Vacuum sealing drainage is a new complex wound treatment developed in recent years, which can promote wound healing through the combined action of multiple aspects[7,8]: (1) biological dressing cover can keep local wound in a slightly acidic and humid environment, which is conducive to the granulation and angiogenesis; (2) the vacuum sealing drainage can avoid the wound infection from external pathogen and also drain the exudation of local wound in time, which is conducive to reducing the degree of local tissue edema and the degree of inflammation. Vacuum sealing drainage combined with biological dressing has achieved positive value for the treatment of fracture combined with soft tissue injury wound[9], and the treatment was used in the study for wound after diabetic foot skin grafting.

The angiogenesis in local wound is an important biological activity in the granulation and wound healing. VEGF is a cytokine with powerful pro-angiogenesis effect, which can induce endothelial cell proliferation and form vascular structures after by binding to the corresponding membrane receptor VEGFR[10,11]. CD105 is a marker molecule highly expressed in proliferative vascular endothelial cells, which mediates the signal transduction between endothelial cells and intercellular substance and participates in the process of angiogenesis. MMP9 is a protease that can hydrolyze the collagen, laminin and other compositions in basement membrane and extracellular matrix, which can promote vascular basement membrane and endothelial extracellular matrix in the process of angiogenesis, is advantageous to the migration and proliferation of endothelial cells in the local wounds, and thus promotes the angiogenesis[12]. In order to define the angiogenesis in wounds after diabetic foot skin grafting, the changes in angiogenesis molecule expression in wound tissue were analyzed before and after the treatment, and the results showed that VEGF, VEGFR, CD105 and MMP9 protein expression in wound tissue of both groups increased significantly after treatment, and VEGF; VEGFR, CD105 and MMP9 protein expression in wound tissue of vacuum drainage group were significantly higher than those of normal control group (P<0.05). This indicates that the angiogenesis in the wound is gradually increasing after skin grafting, which is beneficial to the healing of the wound; vacuum sealing drainage combined with biological dressings can increase the expression of angiogenesis molecules and promote the process of angiogenesis so as to create more favorable conditions for wound healing.

The process of angiogenesis involves complex signal transduction, and the regulation of VEGF, MMP9 and others on angiogenesis processes is also affected by upstream and downstream signaling pathways. PI3K/AKT is an intracellular signaling pathway that promotes proliferation, and the endothelial cell proliferation effects mediated by VEGF depend on the signaling pathway; increased VEGF generation can start PI3K and AKT activation through VEGF receptors, and the activated AKT transfers into the nucleus and then start the expression of cyclinD1, accelerate the process of cell cycle and promote cell proliferation[13]. p38MAPK is an upstream signaling molecule regulating a variety of cytokines in the cells, the activated p38MAPK caused by external stimuli can start downstream NF-kB transposition into the nucleus, and the NF-kB in nucleus can identify and be combined with VEGF gene promoter regions to increase the expression of VEGF[14]. In order to further clarify the effect of vacuum sealing drainage combined with biological dressing on angiogenesis process within the wound, the angiogenesis pathway signal molecule expression in the wound were analyzed in the study, and the results showed that PI3K, AKT,
cell infiltration in local area and mediate inflammatory response are pro-inflammatory cytokines that can promote inflammatory wound tissue of both groups of patients decreased significantly after and suppress the inflammatory response within the wound. Above all, it is believed that the vacuum sealing drainage combined with biological dressings can promote the activation of PI3K/AKT and p38MAPK pathway, increase the angiogenesis and promote wound healing.

Continued exposure of diabetic foot wound can increase the risk of infection, the vacuum sealing drainage can not only avoid the chance of wound to contact with the external pathogen, but also make wound secretion drained in time, and it can greatly reduce the risk of infection. Inflammatory response activation is an important pathological feature of wound infection, which is mediated by the secretion of multiple inflammatory mediators. COX-2 is the rate-limiting enzyme that catalyzes prostaglandin E2 generation, and the catalyse has pro-inflammatory effect of activating inflammation[15]; iNOS is induced enzyme that mediates NO generation, iNOS is massively induced in the process of inflammatory reaction and increases the synthesis of NO, and high levels of NO has cytotoxic effects and can cause inflammatory reactions[16]. TNF-α and IL-6 are pro-inflammatory cytokines that can promote inflammatory cell infiltration in local area and mediate inflammatory response cascade activation[17]. In the study, analysis of inflammatory response molecule expression in wounds before and after treatment indicated that COX-2, iNOS, TNF-α and IL-6 mRNA expression in wound tissue of both groups of patients decreased significantly after treatment, and COX-2, iNOS, TNF-α and IL-6 mRNA expression in wound tissue of vacuum drainage group were significantly higher than those of normal control group. This indicates that the angiogenesis process mediated by PI3K/AKT and p38MAPK pathway within the wounds after skin grafting is in activated state, and vacuum sealing drainage combined with biological dressings can promote the activation of PI3K/AKT and p38MAPK pathway, increase the angiogenesis and promote wound healing.

References