Effect of rhPDGF gel on wound healing as well as inflammation and angiogenesis in the wound of rats with diabetes ulcer

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Objective: To study the effect of recombinant human platelet-derived growth factor (rhPDGF) gel on wound healing as well as inflammation and angiogenesis in the wound of rats with diabetes ulcer. Methods: Clean male Wistar rats were selected as the research objects, made into diabetes ulcer models and then divided into model group, low dose group and high dose group who received blank gel, 30 μg/g rhPDGF gel and 100 μg/g rhPDGF gel respectively for intervention. 4, 6, 8 and 10 d after intervention, the area of ulcer wound was observed; 6 and 10 d after intervention, wound tissue was collected to detect the expression of inflammation and angiogenesis molecules. Results: 4, 6, 8 and 10 d after intervention, ulcer wound area of low dose group and high dose group were significantly less than those of model group (P<0.05), and ulcer wound area of high dose group were significantly less than those of low dose group (P<0.05); 6 and 10 d after intervention, TNF-α and IL-2 levels in ulcer wounds of low dose group and high dose group were significantly lower than those of model group (P<0.05) while VEGF, eNOS and NO levels were significantly higher than those of model group (P<0.05); TNF-α and IL-2 levels in ulcer wounds of high dose group were significantly lower than those of low dose group (P<0.05) while VEGF, eNOS and NO levels were significantly higher than those of low dose group (P<0.05). Conclusions: rhPDGF gel has promoting effect on the wound healing in rats with diabetes ulcer, and can also inhibit inflammation and promote angiogenesis.

1. Introduction

Type 2 diabetes mellitus is the most common endocrine system disease in China, its incidence is rising year by year, and in the development and change of type 2 diabetes mellitus, there is a higher onset risk of diabetic foot in addition to a variety of macrovascular and microvascular complications[1,2]. Difficult ulcer wound healing is the prominent characteristic of diabetic foot and also the main reason that increases the amputation rate of patients with diabetic foot. At present, the common means for clinical treatment of diabetic ulcers wound include debridement, anti-infection and improving microcirculation, the wound of some patients can effectively heal, but the wound healing is still poor in some patients, it will further develop into gangrene and amputation will be required[3,4]. Platelet-derived growth factor (PDGF) is one of the main cytokines to promote the wound repair and healing, and recombinant human platelet-derived growth factor (rhPDGF) gel has been increasingly used in the dressing therapy for a variety of wounds[5,6]. However, there is no related research on the effect of rhPDGF gel for treatment of diabetic foot ulcer wound. In the following study, the effect of rhPDGF gel on wound healing as well as inflammation and angiogenesis in the wound of rats with diabetes ulcer was analyzed.

2. Materials and methods

2.1. Experimental materials

Experimental animals were 36 clean male Wistar rats, they were provided by the laboratory animal center of Sichuan University, and the qualified animal number was SCXK (Sichuan) 2014-083; streptozotocin was purchased in the Sigma Company; 100 and 30 μg/g rhPDGF gel and blank gel were bought from the Tasly...
Pharmaceutical Group Co., LTD.; enzyme-linked immunosorbent assay kits were purchased from Guangzhou Runkwon Biological Technology Co., LTD.

2.2. Experimental methods

2.2.1. Diabetes ulcer rat model establishment methods
Male Wistar rats were adaptively raised for 2 weeks and then used for diabetes model establishment, and the method was as follows: the rats received high-fat diet and conventional water for 12 weeks and then received intraperitoneal injection of 35 μg/kg streptozotocin, blood was collected by caudal vein after 2 weeks to detect blood glucose, and the 30 rats with random blood glucose level > 16.7 mmol/L were selected as the diabetes model rats and used for the establishment of ulcer wound; ulcer wound establishment method was as follows: 30 rats received intraperitoneal chloral hydrate anesthesia, the back was shaved to expose skin, puncher with 2 cm in diameter was used to punch a hole in the left-side skin of spine deep down to the fascia layer, the hold was covered with sterile gauze, and the ulcer wound was formed after 3 d.

2.2.2. Experimental animal grouping and intervention methods
A total of 30 diabetes ulcer model rats were randomly divided into model group, low dose group and high dose group, model group received blank gel for wound daubing, low dose group received 30 μg/g rhPDGF gel for wound daubing, and high dose group received 100 μg/g rhPDGF gel for wound daubing. The gel was applied once a day, the quality of applied gel was 100 mg/cm² each time, and the gel was applied for 10 d in a row.

2.2.3. Wound healing and molecule expression evaluation methods
4, 6, 8 and 10 d after intervention, the wounds were photographed, recorded and input in the computer respectively, and image analysis software was used to calculate the area of the wounds; 6 and 10 d after intervention, five rats were randomly selected from every group and executed, wound tissue samples were collected, cleaned with saline, frozen with liquid nitrogen, added in protein lysis buffer and homogenized, the obtained homogenate suspension was centrifuged for 20 min in 4 °C centrifuge at 12 000 r/min to separate supernatant, protein was determined, and then TNF-α, IL-2, VEGF, eNOS and NO levels, BCA kits were used to determine total protein content, and then TNF-α, IL-2, VEGF, eNOS and NO levels per each mg total protein were calculated.

2.3. Statistical analysis

Table 2
Comparison of inflammatory factors TNF-α and IL-2 in the wounds among three groups of rats (μg/mg total protein, n=5, x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α 6 d after intervention</th>
<th>IL-2 6 d after intervention</th>
<th>TNF-α 10 d after intervention</th>
<th>IL-2 10 d after intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>22.32±3.12</td>
<td>17.76±2.03</td>
<td>20.14±2.39</td>
<td>15.92±1.72</td>
</tr>
<tr>
<td>Low dose group</td>
<td>15.23±1.93</td>
<td>11.34±1.57</td>
<td>12.74±1.48</td>
<td>9.03±1.06</td>
</tr>
<tr>
<td>High dose group</td>
<td>10.32±1.47^a</td>
<td>6.76±0.78^a</td>
<td>7.86±0.92^a</td>
<td>4.42±0.57^a</td>
</tr>
<tr>
<td>F</td>
<td>10.958</td>
<td>14.182</td>
<td>18.855</td>
<td>22.685</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*: compared with model group, P<0.05; #: compared with low dose group, P<0.05.

3. Results

3.1. Ulcer wound area change
4, 6, 8 and 10 d after intervention, analysis of ulcer wound area among three groups of rats is as follows: ulcer wound area of low dose group and high dose group were significantly less than those of model group (P<0.05), and ulcer wound area of high dose group were significantly less than those of low dose group (P<0.05). Differences in pair-wise comparison of ulcer wound area were statistically significant among three groups of rats 4, 6, 8 and 10 d after intervention (P<0.05). The specific data are shown in Table 1.

Table 1
Comparison of ulcer wound area among three groups of rats (cm², n=5, x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>4 d</th>
<th>6 d</th>
<th>8 d</th>
<th>10 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>3.88±0.47</td>
<td>3.24±0.38</td>
<td>2.92±0.34</td>
<td>2.67±0.31</td>
</tr>
<tr>
<td>Low dose group</td>
<td>3.11±0.39^*</td>
<td>2.61±0.32^*</td>
<td>2.24±0.28^*</td>
<td>1.73±0.23^*</td>
</tr>
<tr>
<td>High dose group</td>
<td>2.78±0.34^a</td>
<td>2.69±0.25^a</td>
<td>1.67±0.20^a</td>
<td>0.92±0.11^a</td>
</tr>
<tr>
<td>F</td>
<td>7.183</td>
<td>7.876</td>
<td>7.336</td>
<td>8.768</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*: compared with model group, P<0.05; #: compared with low dose group, P<0.05.

3.2. Inflammatory factors TNF-α and IL-2 expression in the wounds
6 and 10 d after intervention, analysis of inflammatory factors TNF-α and IL-2 expression in the wounds among three groups of rats is as follows: TNF-α and IL-2 levels in ulcer wounds of low dose group and high dose group were significantly lower than those of model group (P<0.05), and TNF-α and IL-2 levels in ulcer wounds of high dose group were significantly less than those of low dose group (P<0.05). Differences in pair-wise comparison of TNF-α and IL-2 levels in ulcer wounds were statistically significant among three groups of rats 6 and 10 d after intervention (P<0.05). The specific data are shown in Table 2.
3.3. Angiogenesis molecules VEGF, eNOS and NO expression in the wounds

6 and 10 d after intervention, analysis of angiogenesis molecules VEGF, eNOS and NO expression in the wounds among three groups of rats is as follows: VEGF, eNOS and NO levels in ulcer wounds of low dose group and high dose group were significantly higher than those of model group (P<0.05), and VEGF, eNOS and NO levels in ulcer wounds of high dose group were significantly higher than those of low dose group (P<0.05). Differences in pair-wise comparison of VEGF, eNOS and NO levels in ulcer wounds were statistically significant among three groups of rats 6 d and 10 d after intervention (P<0.05). The specific data are shown in Table 3.

4. Discussion

Diabetic foot is a rather severe complication in diabetic patients, which specifically refers to the lower limb infection and ulceration caused by diabetes combined with neuropathy and peripheral vascular lesions. The formation of diabetes ulcer wound is closely related to neuropathy and microvascular lesion, the local pathological factors are complex, and therefore, the healing of diabetic foot is poor, and the risk of amputation greatly increases[9,10]. In the study, analysis of the expression of both inflammatory factors in the local wound, causes epithelial cell and endothelial cells damage in the wound, and affects wound healing process[9,10]. IL-2 and TNF-α are the cytokines closely related to the inflammation in diabetic wound. IL-2 is produced by the activated lymphocytes, and it can induce the activation and proliferation of a variety of inflammatory cells and immune cells, and thus mediate the inflammatory reactions in wounds[11]; TNF-α is produced by the activated mononuclear macrophages, and it can promote the neutrophil degranulation, increase the production of inflammatory media and free radicals, and thus cause damage to the wounds[12].

In the study, analysis of the expression of both inflammatory factors in diabetes ulcer wounds showed that 6 and 10 d after intervention, TNF-α and IL-2 levels in ulcer wounds of low dose group and high dose group were significantly lower than those of model group (P<0.05), and TNF-α and IL-2 levels in ulcer wounds of high dose group were significantly lower than those of low dose group (P<0.05). This means that rhPDGF gel can inhibit inflammation in diabetic ulcer wound and high-dose rhPDGF gel has better anti-inflammatory effect than low-dose rhPDGF gel.

In the process of ulcer wound healing, the capillary structure formed by the endothelial cell proliferation is involved in the formation of granulation tissue, and the process is regulated by VEGF, NO and many other angiogenesis molecules[13,14]. In the protracted diabetic ulcer wound, angiogenesis molecule expression is significantly abnormal. VEGF is the mitogen with strongest pro-angiogenesis effect in the body, has promoting effect on endothelial cell differentiation, proliferation and migration, and can promote the formation of new blood vessels[15,16]; the NO catalytically produced from endothelial NOS is an important vasodilator factor in local wound, has protective effect on endothelial cells, and can also promote angiogenesis[17,18]. In the study, analysis of the expression of above three angiogenesis molecules in diabetic ulcer wounds showed that 6 and 10 d after intervention, VEGF, eNOS and NO levels in ulcer wounds of low dose group and high dose group were significantly higher than those of model group (P<0.05), and VEGF, eNOS and NO levels in ulcer wounds of high dose group

Table 3

Comparison of angiogenesis molecules VEGF, eNOS and NO in the wounds among three groups of rats (μg/mg total protein, n=5, x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>6 d after intervention</th>
<th>10 d after intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEGF</td>
<td>eNOS</td>
</tr>
<tr>
<td>Model group</td>
<td>5.24±0.74</td>
<td>2.75±0.33</td>
</tr>
<tr>
<td>Low dose group</td>
<td>7.76±0.92*</td>
<td>3.89±0.41*</td>
</tr>
<tr>
<td>High dose group</td>
<td>10.35±1.85*</td>
<td>5.68±0.72*</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*: compared with model group, P<0.05; &: compared with low dose group, P<0.05.

Infection is an important factor that affects diabetes ulcer wound healing, and under the action of infection factors, the inflammatory response is significantly enhanced, then secretes and releases various inflammatory factors in the local wound, causes epithelial cell and endothelial cells damage in the wound, and affects wound healing process[9,10]. IL-2 and TNF-α are the cytokines closely related to the inflammation in diabetic wound. IL-2 is produced by the activated lymphocytes, and it can induce the activation and proliferation of a variety of inflammatory cells and immune cells, and thus mediate the inflammatory reactions in wounds[11]; TNF-α is produced by the activated mononuclear macrophages, and it can promote the neutrophil degranulation, increase the production of inflammatory media and free radicals, and thus cause damage to the wounds[12].
were significantly higher than those of low dose group \((P<0.05)\). This means that rhPDGF gel has promoting effect on angiogenesis in diabetes ulcer wounds and high-dose rhPDGF gel has pro-angiogenesis effect than low-dose rhPDGF gel.

To sum up, rhPDGF gel has promoting effect on the wound healing in rats with diabetes ulcer, it can also inhibit inflammation and promote angiogenesis in the wounds, and the above effect of high-dose rhPDGF gel are better than those of low-dose rhPDGF gel.

References


