Correlation of substance P expression in diabetic foot ulcer tissue with oxidative stress and apoptosis
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ABSTRACT

Objective: To study the correlation of substance P (SP) expression in diabetic foot ulcer tissue with oxidative stress and apoptosis. Methods: Patients with diabetic foot ulcer who were treated in the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology between January 2016 and March 2017 were selected as the diabetic foot ulcer (DFU) group of the research, the diabetic foot ulcer tissue samples were collected, patients who received debridement surgery due to trauma in the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology during the same period were selected as the control group of the research, and normal wound tissue samples were collected. The contents of SP, oxidative stress molecules and apoptosis molecules in the wound specimens were detected. Results: SP, Prdx6, GSHPX and SOD contents in diabetic foot ulcer wound specimens of DFU group were significantly lower than those in normal wound specimens of control group while MDA, AOPP, Cyto-C, Caspase-9, Fas, Fasl, Caspase-8 and Caspase-3 contents were significantly higher than those in normal wound specimens of control group; Prdx6, GSHPX and SOD contents in wound of patients with high SP were significantly higher than those of patients with low SP while MDA, AOPP, Cyto-C, Caspase-9, Fas, Fasl, Caspase-8 and Caspase-3 contents were significantly lower than those of patients with low SP. Conclusion: The low expression of SP in diabetic foot ulcer tissue can activate oxidative stress response and apoptosis to participate in the formation of ulcer wound.

1. Introduction

Diabetes is a metabolic disease with increasing morbidity in recent years. Long-term blood glucose increase can increase the risk of multiple complications, including microvascular complications and macrovascular complications. Diabetic foot is one of the serious complications of diabetic patients. It is the result of the joint action of peripheral vascular lesion and neuropathy, the wound ulcer is difficult to heal and the amputation rate is high[1,2]. Excessive activation of oxidative stress and excessive apoptosis in diabetic ulcer wound are important pathological features, but the specific regulation mechanism is not completely clear. Substance P (SP) is a new nerve regulation substance discovered in recent years, which can promote the recruitment of epidermal stem cells in the wounds and granulation tissue in the process of wound healing, and is conducive to wound healing[3-4]. In the following study, in order to define the role of SP in the development and changes in diabetic foot, we analyzed the correlation of substance P expression in diabetic foot ulcer tissue with oxidative stress and apoptosis.

2. Research subjects and methods

2.1 General information of research subjects

Patients with diabetic foot ulcer who were treated in the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology between January 2016 and March 2017 were selected as the diabetic foot ulcer (DFU) group of the research, all patients were admitted to hospital due to diabetic foot
chronic ulcer, and there were a total of 40 cases, including 23 men and 17 women that were 41-59 years old. Patients who received debridement surgery due to trauma in the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology during the same period were selected as the control group of the research, all patients were without history of diabetes or wound infection, and there were a total of 48 cases, including 28 men and 20 women that were 37-55 years old. There was no statistically significant difference in general information between the two groups ($P>0.05$).

2.2 Experimental methods of the research

2.2.1 Wound sample collecting
Proper amount of diabetic foot ulcer tissue was collected from DFU group during debridement and wound dressing, and right amount of normal wound tissue was collected from control group during debridement and suturing. The specimens were washed with saline, then frozen in liquid nitrogen for 20-30 min, and finally stored in the -70 °C refrigerator.

2.2.2 Wound molecule testing
The right amount of wound sample was collected and added in RIPA lysate to extract total protein, enzyme-linked immunosorbent assay kit was used to determine the contents of SP, Prdx6, Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3, and radioimmunoprecipitation kit was used to determine the contents of GSHPX, SOD, MDA and AOPP.

2.3 Statistical methods
SPSS 17.0 software was used to input and analyze data, and the median of SP contents in DFU group was calculated and used to divide them into patients with high SP content and those with low SP content. Analysis of measurement data between two groups were by t test, and $P<0.05$ indicated statistical significance in differences in test results ($P<0.05$).

3. Results

3.1 SP content in wound
SP content in diabetic foot ulcer wound specimens of DFU group was (1.85±0.23) pg/mL, and SP content in normal wound specimens of control group was (5.49±0.77) pg/mL. After t test analysis, SP content in diabetic foot ulcer wound specimens of DFU group was significantly lower than that in normal wound specimens of control group, and differences in SP contents in wound were statistically significant between the two groups ($P<0.05$).

3.2 Oxidative stress molecule contents in wound
Analysis of oxidative stress molecules Prdx6 (ng/L), GSHPX (U/L), SOD (U/L), MDA (μmol/L) and AOPP (μmol/L) contents in wound between two groups of patients was as follows: Prdx6, GSHPX and SOD contents in diabetic foot ulcer wound specimens of DFU group were significantly lower than those in normal wound specimens of control group while MDA and AOPP contents were significantly higher than those in normal wound specimens of control group. Differences in Prdx6, GSHPX, SOD, MDA and AOPP contents in wound were statistically significant between the two groups of patients ($P<0.05$).

Analysis of oxidative stress molecules Prdx6, GSHPX, SOD, MDA and AOPP contents in wound between DFU group of patients with different SP contents was as follows: Prdx6, GSHPX and SOD contents in diabetic foot ulcer wound specimens of DFU group were significantly lower than those with low SP while MDA and AOPP contents were significantly lower than those of patients with low SP. Differences in Prdx6, GSHPX, SOD, MDA and AOPP contents in wound were statistically significant between the two groups of patients with different SP contents ($P<0.05$).

Table 1.
Comparison of oxidative stress molecules Prdx6, GSHPX, SOD, MDA and AOPP contents in wound.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Prdx6</th>
<th>GSHPX</th>
<th>SOD</th>
<th>MDA</th>
<th>AOPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFU group</td>
<td>40</td>
<td>23.15±2.56</td>
<td>68.91±8.92</td>
<td>89.31±10.37</td>
<td>7.69±0.84</td>
<td>10.31±1.67</td>
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<tr>
<td>Control group</td>
<td>48</td>
<td>57.74±7.52</td>
<td>176.31±20.36</td>
<td>203.56±26.71</td>
<td>2.31±0.39</td>
<td>3.87±0.47</td>
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<tr>
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<td></td>
<td>&lt;0.05</td>
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<td>P</td>
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</tr>
</tbody>
</table>

Table 2.
Comparison of oxidative stress molecules in wound between DFU group of patients with different SP contents.

<table>
<thead>
<tr>
<th>SP content</th>
<th>n</th>
<th>Prdx6</th>
<th>GSHPX</th>
<th>SOD</th>
<th>MDA</th>
<th>AOPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High SP</td>
<td>20</td>
<td>69.33±8.25</td>
<td>233.67±29.62</td>
<td>257.61±32.68</td>
<td>5.21±0.77</td>
<td>6.28±0.78</td>
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<tr>
<td>Low SP</td>
<td>20</td>
<td>42.62±5.69</td>
<td>110.52±13.28</td>
<td>151.24±17.79</td>
<td>9.95±1.06</td>
<td>14.29±2.03</td>
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<tr>
<td>P</td>
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</tr>
</tbody>
</table>
Comparison of apoptosis molecules in wound between DFU group of patients with different SP contents.

Table 4.

Comparison of apoptosis molecules in wound between DFU group of patients with different SP contents.

<table>
<thead>
<tr>
<th>SP content</th>
<th>n</th>
<th>Cyto-C (ng/L)</th>
<th>Caspase-9 (ng/L)</th>
<th>Fas (pg/L)</th>
<th>FasL (pg/L)</th>
<th>Caspase-8 (pg/L)</th>
<th>Caspase-3 (pg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High SP</td>
<td>20</td>
<td>1.95±0.22</td>
<td>1.21±0.14</td>
<td>168.7±20.3</td>
<td>203.5±26.7</td>
<td>124.5±14.6</td>
<td>2.21±0.29</td>
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<tr>
<td>Low SP</td>
<td>20</td>
<td>3.96±0.47</td>
<td>2.13±0.28</td>
<td>347.8±42.8</td>
<td>421.7±52.6</td>
<td>231.2±30.8</td>
<td>4.78±0.57</td>
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<td>T</td>
<td></td>
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<td></td>
<td>11.039</td>
<td>8.184</td>
<td>12.327</td>
<td>12.184</td>
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<tr>
<td>P</td>
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<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

3.3 Apoptosis molecule contents in wound

Analysis of apoptosis molecules Cyto-C (ng/L), Caspase-9 (ng/L), Fas (pg/L), FasL (pg/L), Caspase-8 (pg/L) and Caspase-3 (ng/L) contents in wound between two groups of patients was as follows: Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in diabetic foot ulcer wound specimens of DFU group were significantly higher than those in normal wound specimens of control group. Differences in Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in wound were statistically significant between the two groups of patients (P<0.05).

Analysis of apoptosis molecules Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in wound between DFU group of patients with different SP contents was as follows: Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in wound of patients with high SP were significantly lower than those of patients with low SP. Differences in Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in wound were statistically significant between DFU group of patients with different SP contents (P<0.05).

4. Discussion

Diabetic foot is one of the serious complications of diabetic patients, and the peripheral vascular and nervous disease caused by long-term poor blood glucose control is the foundation of diabetic foot[5]. In the repair process of diabetic foot wound, the abnormality of inflammatory response, cell proliferation, granulation formation and other processes in the wound can affect the wound repair and cause the formation of chronic ulcer wound[6,7]. At present, the formation mechanism of diabetic foot ulcer is not completely elucidated, and the key molecules that regulate wound healing are not clear. SP is the important neuropeptide in the body, which promotes proliferation and differentiation in the process of wound healing, and can promote epidermal cell differentiation and recruitment, accelerate granulation tissue formation and influence apoptosis and oxidative stress[8,9]. In order to define the role of SP in diabetic foot ulceration, the differences of SP contents in diabetic foot ulcer wound and normal wound were analyzed in the study, and the results showed that SP content in diabetic foot ulcer wound specimens of DFU group was significantly lower than that in normal wound specimens of control group. This means that the lower SP expression within the wound is closely related to diabetic foot ulceration, and affecting the oxidative stress and apoptosis may be the molecular pathways of low SP expression to be involved in diabetic foot ulceration.

The excessive activation of oxidative stress is the pathological link closely related to a variety of complications from diabetes, and the constant generation of advanced glycation end-products will increase the synthesis and release of oxygen free radicals, which causes oxidative stress reaction activation[10,11]. Prdx6, GSHPX and SOD are the antioxidants that scavenge oxygen free radicals in the process of oxidative stress, Prdx6 can restrain the peroxidation of oxygen free radicals to lipid, and GSHPX and SOD can catalyze reduction reaction to remove oxygen free radicals[12]. The oxygen free radicals massively generated in the diabetic foot wound can continuously deplete the antioxidants, and can also react with lipid and protein to produce MDA and AOPP. In the study, analysis of the differences of oxidative stress molecule contents in diabetic foot ulcer wound and normal wound showed that Prdx6, GSHPX and SOD contents in diabetic foot ulcer wound specimens of DFU group were significantly lower than those in normal wound specimens of control group while MDA and AOPP contents were significantly higher than those in normal wound specimens of control group. This indicates that the excessive activation of oxidative stress induced by excessive generation of oxygen free radicals and massive consumption of antioxidants is closely related to the formation of diabetic foot ulcer wound. Further analysis of the influence of SP in diabetic foot ulcer on oxidative stress showed that Prdx6, GSHPX and SOD contents in wound of patients with high SP were significantly higher than
those of patients with low SP while MDA and AOPP contents were significantly lower than those of patients with low SP. It confirms that the lower expression of SP in diabetic foot ulcer wound can increase the production of oxygen free radicals and the consumption of antioxidants, and then participate in the formation of diabetic foot chronic ulcer wound through the oxidative stress reaction activation. Massive oxygen free radical generation within the wound will not only directly cause tissue damage, but can also activate apoptosis, and the excessive apoptosis is not conducive to the formation of granulation tissue and the proliferation of epithelial cells in wound[13]. Mitochondrial apoptosis pathway and death receptor apoptosis pathway are common mechanisms for regulating apoptosis. The activation of mitochondrial apoptosis depends on the release of Cyto-C in the mitochondria, and the Cyto-C in the cytoplasm can activate the cascade activation response of apoptosis by caspase-9[14,15]; the activation of death receptor apoptosis depends on the binding of Fas and FasL, which will activate the cascade activation response of apoptosis through caspase-8. The cascade activation reactions mediated by Caspase-9 and Caspase-8 will eventually lead to the activation of Caspase-3, which then mediates apoptosis[16]. In the study, analysis of the differences of apoptosis molecule contents in diabetic foot ulcer wound and normal wound showed that Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in diabetic foot ulcer wound specimens of DFU group were significantly higher than those in normal wound specimens of control group. This shows that the excessive activation of mitochondrial apoptosis and death receptor apoptosis is closely related to the formation of diabetic foot ulcer wound. Further analysis of the influence of SP in diabetic foot ulcer on apoptosis showed that Cyto-C, Caspase-9, Fas, FasL, Caspase-8 and Caspase-3 contents in wound of patients with high SP were significantly lower than those of patients with low SP. It confirms that the lower expression of SP in diabetic foot ulcer wound can promote the activation of mitochondrial apoptosis pathway and death receptor apoptosis pathway, which causes excessive apoptosis to participate in the formation of diabetic foot chronic ulcer wound. To sum up, it is concluded that the expression of SP significantly decreases in diabetic foot ulcer tissue; the lowly expressed SP can activate the oxidative stress response and apoptosis to participate in the formation of ulcer wounds.

References


