Effect of brucea javanica oil injection combined with neoadjuvant chemotherapy on malignant molecule expression and antitumor immune response in patients with gastric cancer

Shuang-Xiu Zhou¹, Ying Xu², Chao Zhou³

¹ Internal Medicine Department, Wuhan Xinzhou District Hospital of Traditional Chinese Medicine in Hubei Province, Wuhan City, Hubei Province, 430400
² Digestive System Department, Wuhan Hankou Hospital in Hubei Province, Wuhan City, Hubei Province, 430132
³ Department of Blood Donor Services, Wuhan Blood Center in Hubei Province, Wuhan City, Hubei Province, 430030

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ABSTRACT

Objective: To study the effect of brucea javanica oil injection combined with neoadjuvant chemotherapy on malignant molecule expression and antitumor immune response in patients with gastric cancer. Methods: A total of 78 patients with gastric cancer undergoing preoperative neoadjuvant chemotherapy in our hospital between May 2013 and July 2016 were selected and randomly divided into two groups, intervention group received brucea javanica oil injection combined with neoadjuvant chemotherapy, and the control group accepted neoadjuvant chemotherapy. Serum tumor marker levels and peripheral blood regulatory molecule expression were determined before and after treatment, and the malignant molecule expression levels in gastric cancer lesions were determined after the operation. Results: 2 cycles and 4 cycles after treatment, serum CEA, DKK1, exosc2 and ANXA2 levels of both groups of patients were significantly lower than those before treatment, PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of control group were significantly higher than those before treatment, PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of intervention group were significantly lower than those before treatment, serum CEA, DKK1, exosc2 and ANXA2 levels as well as PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of intervention group were significantly lower than those of control group, and the GKN1 and GKN2 mRNA expression in gastric cancer lesions were significantly higher than those of control group while GOLPH3 and PTP1B mRNA expression were significantly lower than those of control group. Conclusion: Brucea javanica oil injection combined with neoadjuvant chemotherapy can more effectively kill the gastric cancer cells and improve the antitumor immune response.

1. Introduction

Gastric cancer is one of the gastrointestinal malignancies with high incidence in China, its incidence is increasing year by year, the prognosis is poor, and the five-year survival rate is low. At present, surgery-based comprehensive therapy is the main means for clinical treatment of gastric cancer, preoperative neoadjuvant chemotherapy can effectively kill the cancer cells and narrow the scope of cancer cell infiltration, and it is conducive to eradicating tumor lesions by surgery and improving the prognosis[1,2]. However, when some patients with gastric cancer accept preoperative neoadjuvant chemotherapy, more significant adverse reactions will occur, which not only suppress the antitumor immune response, but also affect the body’s tolerance to surgical resection. Brucea javanica oil injection is the antitumor active ingredient extracted from brucea javanica fruit, which can not only inhibit the topoisomerase activity and interfere with DNA replication and cell proliferation, but can also enhance the body's specific and nonspecific immune function. Brucea javanica oil injection in combination with intravenous

Footnotes:
Corresponding author: Shuang-Xiu Zhou, Internal Medicine Department, Wuhan Xinzhou District Hospital of Traditional Chinese Medicine in Hubei Province, Wuhan City, Hubei Province, 430400.
Tel: 15927659007
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chemotherapy has achieved positive curative effect in the treatment of patients with advanced gastric cancer\[3,4\], but the effect of the preparation remains uncertain for preoperative neoadjuvant chemotherapy in patients with gastric cancer. In the following research, the effect of brucea javanica oil injection combined with neoadjuvant chemotherapy on malignant molecule expression and antitumor immune response in patients with gastric cancer was analyzed.

2. Case information and research methods

2.1 General case information

A total of 78 patients with gastric cancer undergoing preoperative neoadjuvant chemotherapy in our hospital between May 2013 and July 2016 were selected as the research subjects, all the patients were in accordance with the pathological diagnosis of gastric cancer and the indications for surgical resection, and they intended to receive preoperative neoadjuvant chemotherapy. Patients who are allergic to chemotherapy drugs and those who are associated with other chemotherapy contraindications were ruled out. The included patients were randomly assigned to the intervention group and the control group, 39 cases in each group. Intervention group received brucea javanica oil injection combined with neoadjuvant chemotherapy, including 26 male cases and 13 female cases that were 52-68 years old; control group control group accepted neoadjuvant chemotherapy, including 28 male cases and 11 female cases that were 51-65 years old. There was no significant difference in general information between the two groups of patients (\(P>0.05\)).

2.2 Therapy

The control group received preoperative neoadjuvant chemotherapy according to SOX solution, and the method was as follows: Tegafur Gimeracil Oteracil Potassium Capsule 120 mg/d, oral administration, on the 1-14 d; Oxaliplatin Injection 130 mg/m\(^2\), intravenous drip, on the first day; three weeks as a course of treatment, 4 courses in a row, and performing radical operation for gastric cancer at least four weeks after the end of last chemotherapy. Intervention group, on the basis of SOX solution neoadjuvant chemotherapy, received auxiliary brucea javanica oil injection therapy as follows: brucea javanica oil injection 30 mL in saline 250 mL, intravenous drip, 1 time/d, on the 1-14 d of each cycle of chemotherapy.

2.3 Serum index detection methods

Before treatment as well as 2 cycles and 4 cycles after treatment, 3-5 mL of peripheral blood was collected, let stand for coagulation and centrifuged to separate serum, and enzyme-linked immunosorbent assay kits were used to detect CEA, DKK1, exosc2 and ANXA2 content.

2.4 Gene expression detection methods

After radical operation for gastric cancer, gastric cancer lesions were taken to extract the RNA in tissue, and then the fluorescence quantitative PCR kits were used to determine the GKN1, GKN2, GOLPH3 and PTP1B mRNA expression in tissue; before treatment as well as 2 cycles and 4 cycles after treatment, 3-5 mL of peripheral blood was collected, added in Ficoll separating medium and centrifuged to separate peripheral blood mononuclear cells, the RNA in the cells was extracted, and then the fluorescence quantitative PCR kits were used to determine PD-1, TIM-3 and Foxp3 mRNA expression.

2.5 Statistical processing

SPSS 18.0 software was used to input and analyze serum indexes, gene expression and other data, measurement data analysis between two groups was by t test and \(P<0.05\) indicated statistical significance in differences.

3. Results

3.1 Serum tumor marker levels

Before treatment as well as 2 cycles and 4 cycles after treatment, analysis of serum tumor markers CEA (μg/L), DKK1, exosc2 (ng/L) and ANXA2 (μg/L) between two groups of patients was as follows: before treatment, serum CEA, DKK1, exosc2 and ANXA2 levels were not significantly different between two groups of patients (\(P>0.05\)); 2 cycles and 4 cycles after treatment, serum CEA, DKK1, exosc2 and ANXA2 levels of both groups of patients were significantly lower than those before treatment (\(P<0.05\)), and serum CEA, DKK1, exosc2 and ANXA2 levels of intervention group were significantly lower than those of control group (\(P<0.05\)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>CEA</th>
<th>DKK1</th>
<th>Exosc2</th>
<th>ANXA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>39</td>
<td>Before treatment</td>
<td>27.38±4.52</td>
<td>79.67±49.35</td>
<td>752.11±93.52</td>
<td>12.85±1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 weeks after treatment</td>
<td>12.11±1.52(^a)</td>
<td>46.31±7.52(^a)</td>
<td>524.52±78.54(^a)</td>
<td>5.84±0.93(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>9.58±1.15(^a)</td>
<td>32.12±4.75(^a)</td>
<td>389.52±52.14(^a)</td>
<td>4.12±0.52(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>27.93±4.92</td>
<td>80.11±10.12</td>
<td>756.09±89.86</td>
<td>12.91±1.88</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>2 weeks after treatment</td>
<td>18.64±2.25(^c)</td>
<td>61.28±7.82(^c)</td>
<td>631.12±89.87(^c)</td>
<td>8.26±0.94(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>12.88±1.95(^c)</td>
<td>43.58±7.12(^c)</td>
<td>522.32±72.91(^c)</td>
<td>6.21±0.88(^c)</td>
</tr>
</tbody>
</table>

\(^{1}\): comparison within group before and after treatment, \(P<0.05\); \(^{2}\): comparison between groups after treatment, \(P<0.05\); \(^{3}\): comparison between groups after treatment, \(P<0.05\).
3.2 Malignant molecule expression in gastric cancer lesions

After treatment, analysis of malignant molecules GKN1, GKN2, GOLPH3 and PTP1B mRNA expression in gastric cancer lesions between two groups of patients was as follows: GKN1 and GKN2 mRNA expression in gastric cancer lesions of intervention group were significantly higher than those of control group while GOLPH3 and PTP1B mRNA expression were significantly lower than those of control group. Differences were statistically significant in GKN1, GKN2, GOLPH3 and PTP1B mRNA expression in gastric cancer lesions between two groups of patients ($P<0.05$).

3.3 Peripheral blood immune regulatory molecule expression

Before treatment as well as 2 cycles and 4 cycles after treatment, analysis of immune regulatory molecules PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells between two groups of patients was as follows: before treatment, PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells were not significantly different between two groups of patients ($P>0.05$); 2 cycles and 4 cycles after treatment, PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of control group were significantly higher than those before treatment ($P<0.05$), PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of intervention group were significantly lower than those before treatment ($P<0.05$), and PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of intervention group were significantly lower than those of control group ($P<0.05$).

4. Discussion

The value of preoperative neoadjuvant chemotherapy is to kill cancer cells and reduce tumor load, which is helpful for complete removal of tumor lesions by surgical treatment. Brueca javanica oil injection is an adjuvant chemotherapy drug used in the treatment of advanced malignant tumors in recent years, and it has been proven that its combination with intravenous chemotherapy has achieved positive value in advanced gastric cancer treatment. In the study, adjuvant brueca javanica oil injection chemotherapy was used for preoperative neoadjuvant chemotherapy. In order to define the killing effect of brueca javanica oil injection combined with neoadjuvant chemotherapy on gastric cancer cells, the changes in serum tumor marker levels were analyzed in the study, and the results showed that serum CEA, DKK1, exosc2 and ANXA2 levels of both groups of patients after treatment were significantly lower than those before treatment, and serum CEA, DKK1, exosc2 and ANXA2 levels of intervention group after treatment were significantly lower than those of control group. CEA is a marker for screening for malignant tumors in the digestive tract, the sensitivity and specificity are not strong, but it can reflect the malignancy of the tumor; DKK1 is a member of DKKs secretory protein family, it can compete with Wnt molecules to be combined with Lrp5/6 and cause β-catenin degradation, and abnormal secretion of DKK1 is closely related to the progression of gastric cancer, colon cancer and other malignant tumors[5,6]; exosome is a small vesicle containing a variety of cell membrane-related proteins and molecules, it has negative immunomodulatory effect, and exosc2 is an important subunit of exosome and associated with the immune escape and the growth of cancer cells[7,8]; ANXA2 is a member of ANXA family, which is involved in the regulation of cancer cell proliferation and differentiation[9]. The results show that preoperative neoadjuvant chemotherapy can effectively kill gastric cancer cells, and the combination with brueca javanica oil injection can enhance the killing effect on gastric cancer cells.

The growth of cancer cells in the gastric cancer lesion is regulated by a variety of proliferation-related genes. GKN1 and GKN2 of the GKNs family are the molecules that have significant inhibitory effects on the proliferation of gastric cancer cells. GKN1 can inhibit the proliferation of gastric cancer cells and induce the apoptosis of gastric cancer cells by FAS/FASL and p16/Rb pathways[10,11]; GKB2 is able to inhibit CyclinD1 and CyclinE1 to make the proliferation of gastric cancer cells arrest in the G1/S phase so as to inhibit cell proliferation[12,13]. GOLPH3 is a kind of Golgi matrix protein that can regulate cell proliferation after connected with carbohydrate.

### Table 2.
Malignant molecule expression in gastric cancer lesions of two groups of patients after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>GKN1</th>
<th>GKN2</th>
<th>GOLPH3</th>
<th>PTP1B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>39</td>
<td>2.33±0.42</td>
<td>1.98±0.25</td>
<td>0.38±0.05</td>
<td>0.41±0.07</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>1.03±0.16</td>
<td>1.06±0.14</td>
<td>0.98±0.11</td>
<td>1.02±0.18</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>
<td></td>
</tr>
</tbody>
</table>

### Table 3.
Peripheral blood immune regulatory molecule expression of two groups of patients before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>PD-1</th>
<th>TIM-3</th>
<th>Foxp3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>39</td>
<td>Before treatment</td>
<td>1.03±0.18</td>
<td>1.07±0.13</td>
<td>0.99±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 weeks after treatment</td>
<td>0.89±0.13*</td>
<td>0.83±0.12*</td>
<td>0.80±0.11*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>0.73±0.09**</td>
<td>0.69±0.08**</td>
<td>0.52±0.08**</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>Before treatment</td>
<td>1.06±0.20</td>
<td>0.97±0.14</td>
<td>1.04±0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 weeks after treatment</td>
<td>1.32±0.18*</td>
<td>1.29±0.18*</td>
<td>1.36±0.14*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks after treatment</td>
<td>1.63±0.20*</td>
<td>1.58±0.20*</td>
<td>1.69±0.25*</td>
</tr>
</tbody>
</table>

*: comparison within group before and after treatment, $P<0.05$; #: comparison between groups after treatment, $P<0.05$. 

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acyl transferase, and can regulate cell migration after connected with myosin and actin; knocking down the expression of GOLPH3 has significant inhibitory effect on the proliferation and migration of gastric cancer cells[14]. PTP1B is a member of the PTPs family with characteristics similar to proto-oncoge, and multiple signaling pathways such as ErbB2, Akt/P13K and Ras are regulated by PTP1B in cell proliferation[15]. In order to further clarify the killing effect of brucea javanica oil injection combined with neoadjuvant chemotherapy on gastric cancer cells, the proliferation-related gene expression in gastric cancer lesions were analyzed in the study, and the results showed that GKN1 and GKN2 mRNA expression in gastric cancer lesions of intervention group were significantly higher than those of control group while GOLPH3 and PTP1B mRNA expression were significantly lower than those of control group. This means that preoperative brucea javanica oil injection combined with neoadjuvant chemotherapy can more effectively inhibit the proliferation of gastric cancer cells and induce the apoptosis of gastric cancer cells.

Immune escape is an important pathological link that causes abnormal proliferation of cancer cells. T lymphocyte is the key cell mass for the body to complete antitumor immune response, and different T lymphocyte subsets can synthesize and secrete a variety of cytokines to kill cancer cells. Treg is a T lymphocyte subset with immunosuppressive effect, which can not only secrete TGF-β, IL-10 and other inhibitory cytokines to suppress the antitumor immune response, but can also directly inhibit the differentiation and maturation of a variety of immune cells through the surface marker molecule Foxp3[16]. PD-1 and TIM-3 are the costimulatory molecules that play negative regulation role in the maturation of T cells and the differentiation of different subgroups, and high expression of PD-1 and TIM-3 can suppress T cell maturation and cause immunosuppression[17,18]. In the peripheral blood of patients with stomach cancer, the negative costimulatory molecules pd-1 and TIM-3 as well as the inhibitory Treg cell surface marker Foxp3 are all highly expressive. In the study, the analysis of changes in above immune regulatory molecule expression in peripheral blood before and after treatment showed that PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of control group after treatment were significantly higher than those before treatment while PD-1, TIM-3 and Foxp3 mRNA expression in peripheral blood mononuclear cells of intervention group after treatment were significantly lower than those before treatment. This suggests that preoperative neoadjuvant chemotherapy can cause immune damage and suppress immune response; the combind use of brucea javanica oil injection has protective effect on the immune damage caused by neoadjuvant chemotherapy and can improve the anti-tumor immune response.

Based on above discussion, it is believed that brucea javanica oil injection combined with neoadjuvant chemotherapy has better value for gastric cancer treatment than neoadjuvant chemotherapy alone, and the therapy can significantly kill gastric cancer cells, inhibit gastric cancer cell proliferation, induce gastric cancer apoptosis and also improve the antitumor immune response.

References