Effect of Aidi injection combined with FOLFOX4 chemotherapy on tumor stem cell characteristics and antitumor immune response in patients with advanced colon cancer

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1. Introduction

Colon cancer is one of the common malignant tumors of digestive system, and its incidence is rising year by year. Although the early diagnostic rate and resection rate of colon cancer has been improved significantly in recent years, intravenous chemotherapy is still the important means in the clinical treatment of colon cancer, and the patients with advanced colon cancer and recurrent patients after radical operation for colon cancer both need to receive intravenous chemotherapy. FOLFOX4 is a common chemotherapy regimen for the malignant digestive tract tumor that has significant killing effect on cancer cells and can effectively inhibit the tumor lesion growth and prolong patients’ survival time[1,2]. However, the side reaction of FOLFOX4 chemotherapy is significant, it will not only cause liver and kidney function damage, but can also affect the body’s antitumor immune response, and the overall effect is not ideal. Aidi injection is an antitumor Chinese medicine preparation, its main compositions include cantharidin, ginsenoside, ginseng polysaccharides and astragalus polysaccharides, and it has broad-spectrum antitumor activity and can improve immune response. During intravenous chemotherapy, Aidi injection application can not only help the chemotherapeutics to kill cancer cells, but can also improve immune response and reduce the inhibiting effect of chemotherapeutics on the immune response[3,4]. In the following study, the effect of Aidi injection combined with FOLFOX4 chemotherapy on tumor stem cell characteristics and antitumor immune response in patients with advanced colon cancer was analyzed.

ARTICLE INFO

ABSTRACT

Objective: To study the effect of Aidi injection combined with FOLFOX4 chemotherapy on tumor stem cell characteristics and antitumor immune response in patients with advanced colon cancer. Methods: Patients with advanced colon cancer who received chemotherapy in our hospital between May 2013 and June 2016 were selected and randomly divided into the combined chemotherapy group who received Aidi injection combined with FOLFOX4 chemotherapy and the FOLFOX4 group who received FOLFOX4 chemotherapy alone. After chemotherapy, the serum was collected to determine the levels of tumor markers, tumor lesions were collected to determine the expression of tumor stem cell markers and immune cell markers, and peripheral blood mononuclear cells were collected to determine the expression of immune cell markers. Results: After 2 and 4 cycles of treatment, serum CEA, CA199, CCSA-3 and CCSA-4 levels of combined chemotherapy group were significantly lower than those of FOLFOX4 group, and the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 in peripheral blood mononuclear cells were significantly higher than those of FOLFOX4 group; after four cycles of chemotherapy, CD133, Musashi-1, Piwil2, Nanog and Sox-2 protein content in tumor lesions were significantly lower than those of FOLFOX4 group, and the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 were significantly higher than those of FOLFOX4 group. Conclusion: Aidi injection combined with FOLFOX4 chemotherapy treatment of advanced colon cancer will help reduce the tumor load, inhibit tumor stem cell characteristics and enhance antitumor immune response.
2. Subjects and methods

2.1 Research subjects

114 patients with advanced colon cancer who received chemotherapy in our hospital between May 2013 and June 2016 were selected as the research subjects of this study, all patients were diagnosed with colon cancer by pathology biopsy under colonoscopy, imageological examination confirmed that they were at TNM IIIc-IV stage, and intravenous chemotherapy was proposed after patients signed informed consent. Random number table was used to divide the 114 patients with advanced colon cancer into the combined chemotherapy group and FOLFOX4 group, 57 cases in each group. Combined chemotherapy group received Aidi injection combined with FOLFOX4 chemotherapy, they included 39 male cases and 18 female cases, they were 52-67 years old, 33 cases were at TNM IIIc stage and 24 cases were at TN IV stage; FOLFOX4 group received FOLFOX4 chemotherapy alone, they included 41 male cases and 16 female cases, they were 51-69 years old, 35 cases were at TNM IIIc stage and 22 cases were at TNM IV stage. The two groups of patients were not significantly different in general data (P>0.05).

2.2 Chemotherapy methods

FOLFOX4 group received FOLFOX4 chemotherapy, which was as follows: oxaliplatin 85 mg/m², intravenous drip, 2 h and d 1, leucovorin calcium 200 mg/m², intravenous drip, 2 h and d 1-2, 5-fluorouracil 400 mg/m², intravenous drip, 2 h and d 1-2, 5-fluorouracil 600 mg/m², continuous intravenous drip, 22 h and d 1-2; treatment was repeated once every 2 weeks and as one cycle. Combined chemotherapy group received Aidi injection combined with FOLFOX4 chemotherapy, which as follows: the same FOLFOX4 chemotherapy as that of FOLFOX4 group, aidi injection during chemotherapy, intravenous drip and d 1-5; treatment was repeated once every 2 weeks and as one cycle.

2.3 Detection methods of serum tumor marker levels

After 2 and 4 cycles of treatment, peripheral venous blood was collected from two groups of patients and centrifuged to separate peripheral blood mononuclear cells and incubate the fluorescent monoclonal antibodies of CD3, CD4, CD8, CD16 and CD56, and the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 were determined at flow cytometer. Serum CEA, CA199, CCSA-3 and CCSA-4 levels were statistically significant between combined chemotherapy group and FOLFOX4 group. Differences in serum CEA, CA199, CCSA-3 and CCSA-4 levels were statistically significant between combined chemotherapy group and FOLFOX4 group (P<0.05).

2.4 Detection methods of stem cell marker levels in tumor lesions

After 4 cycles of treatment, proper amount of tumor lesion tissue was collected from two groups of patients, added in PBS buffer for 2-3 times, then added in RIPA lysis buffer and ground to get tissue suspension, enzyme-linked immunosorbent assay kits were used to determine CD133, Musashi-1, Nanog and Sox-2 levels. BCA protein quantification kits were used to detect total protein content, and the CD133, Musashi-1, Nanog, 0.05 and Sox-2 levels per unit mass total protein were calculated.

2.5 Detection methods of immune cell marker molecule expression in peripheral blood and tumor lesions

After 2 and 4 cycles of treatment, peripheral venous blood was collected from two groups of patients, added in lymphocyte separation medium and centrifuged to separate peripheral blood mononuclear cells and incubate the fluorescent monoclonal antibodies of CD3, CD4, CD8, CD16 and CD56, and the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 were determined at flow cytometer; tumor lesions after 4 cycles of treatment were collected and ground to get tissue suspension and incubate the fluorescent monoclonal antibodies of CD3, CD4, CD8, CD16 and CD56, and the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 were determined at flow cytometer.

2.6 Statistical processing methods

SPSS 17.0 software was used to input and analyze experimental data, differences in measurement data between two groups was analyzed by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum tumor marker levels of two groups of patients

After 2 and 4 cycles of treatment, analysis of serum tumor markers CEA (ng/mL), CA199 (U/mL), CCSA-3 (ng/mL) and CCSA-4 (ng/mL) were statistically significant between two groups of patients was as follows: serum CEA, CA199, CCSA-3 and CCSA-4 levels of combined chemotherapy group were significantly lower than those of FOLFOX4 group. Differences in serum CEA, CA199, CCSA-3 and CCSA-4 levels were statistically significant between combined chemotherapy group and FOLFOX4 group (P<0.05).

Table 1.

Comparison of serum tumor marker levels between two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Chemotherapy time</th>
<th>CEA</th>
<th>CA199</th>
<th>CCSA-3</th>
<th>CCSA-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined chemotherapy group</td>
<td>57</td>
<td>2 cycles</td>
<td>7.76±0.93</td>
<td>18.76±2.24</td>
<td>5.63±0.71</td>
<td>1.42±0.17</td>
</tr>
<tr>
<td>FOLFOX4 group</td>
<td>57</td>
<td>2 cycles</td>
<td>4.41±0.56</td>
<td>13.24±1.65</td>
<td>3.46±0.44</td>
<td>1.03±0.12</td>
</tr>
</tbody>
</table>

* comparison between combined chemotherapy group and FOLFOX4 group at the time point of chemotherapy, differences were statistically significant, P<0.05.
3.2 Stem cell marker expression in tumor lesions of two groups of patients

After 4 cycles of treatment, analysis of stem cell markers CD133, Musashi-1, Piwi12, Nanog and Sox-2 expression in tumor lesions between two groups of patients was as follows: CD133, Musashi-1, Piwi12, Nanog and Sox-2 protein content in tumor lesions of combined chemotherapy group were significantly lower than those of FOLFOX4 group. Differences in CD133, Musashi-1, Piwi12, Nanog and Sox-2 protein content in tumor lesions were statistically significant between combined chemotherapy group and FOLFOX4 group (P<0.05).

3.3 Immune cell marker molecule expression in peripheral blood and tumor lesions of two groups of patients

After 2 and 4 cycles of treatment, analysis of immune cell marker molecules CD3, CD4, CD8, CD16 and CD56 expression in peripheral blood between two groups of patients was as follows: the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 in peripheral blood mononuclear cells of combined chemotherapy group were significantly higher than those of FOLFOX4 group; after four cycles of chemotherapy, analysis of immune cell marker molecules CD3, CD4, CD8, CD16 and CD56 expression in tumor lesions between two groups of patients was as follows: the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 in tumor lesions of combined chemotherapy group were significantly higher than those of FOLFOX4 group. Differences in CD3, CD4, CD8, CD16 and CD56 expression in peripheral blood and tumor lesions were statistically significant between two groups of patients after treatment (P<0.05).

4. Discussion

Aidi injection is the common traditional Chinese medicine preparation for auxiliary treatment of advanced malignant tumors, and the active ingredients of the preparation include cantharidin, ginsenoside, ginseng polysaccharides and astragalus polysaccharide [5]. Modern pharmacology study proves that cantharidin, ginsenoside and ginseng polysaccharide in Aidi injection have killing effects of a variety of malignant tumor cells, and can inhibit cell proliferation and induce cell apoptosis [6]; ginseng polysaccharides and astragalus polysaccharides play an promoting role in the antitumor immune response of the body. In the development and change of malignant tumors, the cancer cells can synthesize a variety of molecules and release them into the blood circulation, and the serum levels of corresponding molecules can reflect tumor load [8,9]. CEA, CA199, CCSA-3 and CCSA-4 are the clinical common colon cancer marker molecules, and the CEA and CA199 are used for the screening and evaluation of a variety of malignant tumors of digestive tract [10]; CCSA-3 and CCSA-4 are the newly discovered antigens in recent years that are specifically highly expressed in colon cancer. In order to define the killing effect of Aidi injection combined with FOLFOX4 on the cancer cells in lesions of patients with advanced colon cancer, serum levels of above tumor markers were analyzed at first in the study, and the results showed that serum CEA, CA199, CCSA-3 and CCSA-4 levels of combined chemotherapy group were significantly lower than those of FOLFOX4 group. This means that Aidi injection combined with FOLFOX4 chemotherapy has better killing effect on colon cancer cells than FOLFOX4 chemotherapy alone, the tumor load is lower and serum tumor markers levels were lower after chemotherapy.

In recent years, studies about colon cancer progression believe that the tumor stem cells within colon cancer lesions are the biological basis that causes cancer cell proliferation and tumor lesion growth [11,12]. CD133, Musashi-1, Piwi12, Nanog and Sox-2 proteins in Aidi injection are the common traditional Chinese medicine preparation for auxiliary treatment of advanced malignant tumors, the cancer cells can synthesize a variety of molecules and release them into the blood circulation, and the serum levels of corresponding molecules can reflect tumor load. CEA, CA199, CCSA-3 and CCSA-4 are the clinical common colon cancer marker molecules, and the CEA and CA199 are used for the screening and evaluation of a variety of malignant tumors of digestive tract; CCSA-3 and CCSA-4 are the newly discovered antigens in recent years that are specifically highly expressed in colon cancer. In order to define the killing effect of Aidi injection combined with FOLFOX4 on the cancer cells in lesions of patients with advanced colon cancer, serum levels of above tumor markers were analyzed at first in the study, and the results showed that serum CEA, CA199, CCSA-3 and CCSA-4 levels of combined chemotherapy group were significantly lower than those of FOLFOX4 group. This means that Aidi injection combined with FOLFOX4 chemotherapy has better killing effect on colon cancer cells than FOLFOX4 chemotherapy alone, the tumor load is lower and serum tumor markers levels were lower after chemotherapy.

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molecules can promote and maintain the characteristics of tumor stem cells in colon cancer lesions. CD133 is a kind of cell surface adhesion molecule, also the earliest discovered stem cell surface marker, and closely related to the self-renewal characteristics and multi-directional differentiation potential of stem cells; Musashi-1 is the first discovered Musashi family member and can enhance self-renewal ability of stem cells through the positive regulation of Notch and Wnt/β-catenin pathway[13]; PiwiII2 is a member of Piwi family which can regulate the Bcl-XL and CyclinD1 signal pathway through the RNA silencing mechanisms, and maintain stem cell proliferation potential[14]; Nanog and Sox-2 are the key molecules to maintain the self-renewal characteristics and multi-directional differentiation potential of stem cells, and can promote continuous stem cell proliferation and differentiation to cancer cell[15,16]. In order to define the stem cell characteristics in colon cancer lesions after aidi injection combined with FOLFOX4 therapy, the expression levels of above stem cell marker molecules were analyzed in the study, and the results showed that CD133, Musashi-1, PiwiII2, Nanog and Sox-2 protein content in tumor lesions of combined chemotherapy group were significantly lower than those of FOLFOX4 group. This means that Aidi injection combined with FOLFOX4 chemotherapy has better inhibiting effect on the stem cell characteristics in colon cancer lesions than FOLFOX4 chemotherapy, and the expression levels of stem cell marker molecules were lower after chemotherapy. Immune escape is the important pathological feature in the occurrence and development of malignant tumors and the body's antitumor immune response is in inhibited state. During intravenous chemotherapy, although chemotherapeutics can kill cancer cells, they will also affect the immune cell differentiation and maturation, and further inhibit anti-tumor immune response. T lymphocytes, B lymphocytes and NK cells are the important effector cells of antitumor immune response in the body, T and B lymphocytes are mainly involved in specific immune response, and NK cells are mainly involved in nonspecific immune response[17,18]. The side effect of conventional intravenous chemotherapy on the body can suppress the differentiation and maturation of a variety of immune cells and also directly kill the differentiated and matured immune cells, which will weaken the antitumor immune response and increases the chances of immune escape of cancer cells. The ginseng polysaccharides and astragalus polysaccharides in Aidi injection have immunomodulatory activity and have significant enhancement effect on specific and nonspecific immune response. In order to define the antitumor immune response in patients with colon cancer after Aidi injection combined with FOLFOX4 treatment, the expression levels of above immune cell marker molecules in peripheral blood and tumor lesions were analyzed in the study, and the results showed that the mean fluorescence intensity of CD3, CD4, CD8, CD16 and CD56 in peripheral blood mononuclear cells and tumor lesions of combined chemotherapy group were significantly higher than those of FOLFOX4 group. This means that after aidi injection combined with FOLFOX4 chemotherapy, the antitumor immune response in patients with colon cancer is better than that after FOLFOX4 chemotherapy alone, which also further reflects that Aidi injection can reduce the inhibiting effect of chemotherapy on antitumor immune response and improve the antitumor immune response during chemotherapy.

To sum up, it shows that Aidi injection combined with FOLFOX4 chemotherapy has better killing effect on advanced colon cancer cells than FOLFOX4 chemotherapy alone, and the chemotherapy regimen can reduce the tumor marker levels, inhibit tumor stem cell characteristics and also enhance antitumor immune response.

References


