Influence of Aidi injection combined with paclitaxel and platinum drugs on malignant molecule expression in malignant ascites of patients with advanced gastric cancer

Fan Yang, Xiao-Hua Wang

Oncology Department, Suning Central Hospital in Sichuan Province, Suning City, Sichuan Province, 629000, China

ARTICLE INFO

Article history:
Received 8 Sep 2017
Received in revised form 12 Sep 2017
Accepted 19 Sep 2017
Available online 28 Sep 2017

Keywords:
Gastric cancer
Aidi injection
Malignant ascites
Proliferation gene
Invasion gene
Autophagy gene

ABSTRACT

Objective: To explore the influence of Aidi injection combined with paclitaxel and platinum drugs on malignant molecule expression in malignant ascites of patients with advanced gastric cancer.

Methods: A total of 80 patients with advanced gastric cancer complicated by malignant ascites who were treated in the hospital between January 2015 and December 2016 were divided into control group and observation group by random number table, each with 40 cases. Control group were treated with paclitaxel and platinum drugs, and observation group were treated with Aidi injection combined with paclitaxel and platinum drugs. The differences in the malignant molecule expression in malignant ascites were compared between the two groups of patients before and after treatment.

Results: Before treatment, the proliferation, invasion and autophagy gene expression in malignant ascites were not statistically different between the two groups of patients. 1 week after treatment, EZH2, I2PP2A, Gal-1, HPA-1, MTA1, TROP2, BNIP3 and LC3 mRNA expression in malignant ascites of both groups of patients were lower than those before treatment while PTPN13, TRIM28, SOX7, Syndecan-1 and Beclin1 mRNA expression were higher than those before treatment, and EZH2, I2PP2A, Gal-1, HPA-1, MTA1, TROP2, BNIP3 and LC3 mRNA expression in malignant ascites of observation group were lower than those of control group while PTPN13, TRIM28, SOX7, Syndecan-1 and Beclin1 mRNA expression were higher than those of control group.

Conclusion: Aidi injection combined with paclitaxel and platinum drugs can effectively inhibit the gastric cancer cell proliferation and invasion, and regulate cell autophagy activity in the malignant ascites of patients with advanced gastric cancer.

1. Introduction

Malignant ascites can appear in patients with advanced gastric cancer, it is the sign of poor final treatment outcome and short survival time, and how to effectively control the disease in such patients is the emphasis and difficulty of the current clinical research[1,2]. The main method to treat patients with advanced cancer is to use chemotherapy drugs to kill cancer cells or reduce cancer cell activity and inhibit disease progression, paclitaxel is the diterpenoid alkaloids compound with antitumor activity, and oxaliplatin is the third generation of platinum chemotherapy drug and inhibits DNA synthesis and exert anti-cancer effect[3,4]. The combination of these two chemotherapy drugs has been proven to be able to inhibit the progress of various advanced tumors, but there are still the increase of malignant ascites and the aggravation of cachexia in some patients. Aidi injection the Chinese patent medicine with clearing away heat and toxic materials as well as eliminating blood stasis and stagnation, it has been successfully applied in the adjuvant treatment of colorectal cancer and gynecologic malignant tumors[5,6], it was introduced in the treatment of advanced gastric cancer patients with malignant ascites in this study, and the effect of Aidi injection combine with conventional chemotherapy on malignant degree of malignant ascites was discussed in order to lay practical foundation for future similar patients, now reported as follows.
2. Information and methods

2.1 Case information

A total of 80 patients with advanced gastric cancer complicated by malignant ascites who were treated in Suining Central Hospital in Sichuan Province between January 2015 and December 2016 were selected as the research subjects. Inclusion criteria: (1) signing informed consent; (2) pathologically diagnosed with advanced gastric cancer and with gastric cancer cells in ascites; (3) completing the treatment, cooperating with the related inspection, and with complete data. Exclusion criteria: (1) allergic to Aidi injection, paclitaxel and platinum drugs; (2) combined with primary malignant tumor in other tissue viscera; (3) combined with serious autoimmune diseases; (4) combined with severe heart, liver and kidney insufficiency. The random number table method was used to divide the patients into control group and observation group, 40 cases in each group. Control group included 30 men and 10 women that were 36-73 years old; observation group included 28 men and 12 women that were 38-73 years old. There was no statistically significant difference in general information between the two groups of patients ($P>0.05$).

2.2 Therapy

Control group received paclitaxel and platinum drug therapy, and the details were as follows: paclitaxel, intravenous drip, 135 mg/m$^2$, on d1; oxaliplatin, intravenous drip, 130 mg/m$^2$, on d2, 21 d as one course of treatment, for continuous 3-5 courses of treatment. Observation group received Aidi injection combined with paclitaxel and platinum drug therapy, specifically as follows: paclitaxel and oxaliplatin administration in same way as that of control group as well as Aidi injection 50 mL in 500 mL of 5% glucose liquid, intravenous drip, once/d, continuous 7 d of treatment and 14 d of rest as one course of treatment, for continuous 3-5 courses of treatment.

2.3 Ascites sample obtaining

Before treatment and 1 week after treatment, 10 mL of ascites was collected from two groups of patients and centrifuged at 4 ℃ and low speed to take the supernatant liquid and cryopreserve it in cryogenic environment for further test.

2.4 Malignant molecule expression

Fluorescence quantitative PCR method was used to detect malignant molecule expression in ascites supernatant liquid, and the specific steps and target molecules were as follows: splitting cells with Trizol reagent precipitating total RNA cleaning RNA precipitation and drying it at room temperature → detecting RNA purity by UV absorption spectrometry → synthesizing sample cDNA with reverse transcription kits amplifying the following with fluorescence quantitative PCR kits: proliferation genes: EZH2, PTPN13, TRIM28 and I2PP2A; invasion genes: Gal-1, SOX7, Syndecan-1, HPA-1, MTA1 and TROP2; autophagy genes: Beclin1, BNP3 and LC3 obtaining PCR amplification curve calculating target gene mRNA expression.

2.5 Statistical processing

Proliferation genes, invasion genes, autophagy genes and other measurement data were in terms of mean ± deviation and compared by t test. Statistical software was SPSS22.0 and statistic $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Proliferation gene expression

Comparison of proliferation genes EZH2, PTPN13, TRIM28 and I2PP2A mRNA expression in malignant ascites between two groups of patients before and after treatment was as follows: before treatment, differences in EZH2, PTPN13, TRIM28 and I2PP2A mRNA expression in malignant ascites were not significant between the two groups of patients ($P>0.05$); 1 week after treatment, EZH2 and I2PP2A mRNA expression in malignant ascites of both groups of patients were lower than those before treatment while PTPN13 and TRIM28 mRNA expression were higher than those before treatment, and EZH2 and I2PP2A mRNA expression in malignant ascites of observation group were lower than those of control group ($P<0.05$), shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>EZH2</th>
<th>PTPN13</th>
<th>TRIM28</th>
<th>I2PP2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>40</td>
<td>Before treatment</td>
<td>98.26±10.17</td>
<td>99.53±10.28</td>
<td>99.64±10.25</td>
<td>101.24±12.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after treatment</td>
<td>71.53±8.34*</td>
<td>120.36±13.42*</td>
<td>115.28±14.74*</td>
<td>85.23±9.18*</td>
</tr>
<tr>
<td>Observation group</td>
<td>40</td>
<td>Before treatment</td>
<td>99.45±10.32</td>
<td>100.63±11.36</td>
<td>99.17±9.74</td>
<td>99.85±10.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after treatment</td>
<td>50.78±6.14*</td>
<td>137.49±15.88*</td>
<td>143.98±17.21*</td>
<td>67.48±8.32*</td>
</tr>
</tbody>
</table>

Note: comparison of indexes within same group between before and after treatment, *$P<0.05$; comparison of indexes between observation group and control group 1 week after treatment, $P<0.05$.  

Table 1.

Comparison of proliferation gene expression in malignant ascites between two groups of patients before and after treatment.
Comparison of invasion gene expression in malignant ascites between two groups of patients before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>Gal-1</th>
<th>SOX7</th>
<th>Syndecan-1</th>
<th>HPA-1</th>
<th>MTA1</th>
<th>TROP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>40</td>
<td>Before treatment</td>
<td>99.72±10.84</td>
<td>100.48±12.52</td>
<td>98.25±10.17</td>
<td>101.25±13.47</td>
<td>99.47±10.51</td>
<td>98.36±10.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after treatment</td>
<td>85.36±9.11</td>
<td>115.36±13.42</td>
<td>123.17±14.39</td>
<td>90.47±9.62</td>
<td>88.64±9.12</td>
<td>84.26±9.11</td>
</tr>
<tr>
<td>Observation group</td>
<td>40</td>
<td>Before treatment</td>
<td>99.64±10.91</td>
<td>99.75±10.48</td>
<td>99.63±9.51</td>
<td>100.48±12.31</td>
<td>100.19±12.31</td>
<td>99.17±10.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after treatment</td>
<td>74.93±8.62</td>
<td>129.84±14.55</td>
<td>141.33±15.73</td>
<td>81.35±9.47</td>
<td>76.32±8.21</td>
<td>72.75±8.39</td>
</tr>
</tbody>
</table>

Note: comparison of indexes within same group between before and after treatment, *P* < 0.05; comparison of indexes between observation group and control group 1 week after treatment, #P < 0.05.

Comparison of autophagy gene expression in malignant ascites between two groups of patients before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>Beclin1</th>
<th>BNIP3</th>
<th>LC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>40</td>
<td>Before treatment</td>
<td>99.72±10.54</td>
<td>100.59±11.53</td>
<td>101.26±13.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after treatment</td>
<td>116.83±14.52</td>
<td>91.52±10.52</td>
<td>89.63±9.71</td>
</tr>
<tr>
<td>Observation group</td>
<td>40</td>
<td>Before treatment</td>
<td>99.69±9.74</td>
<td>99.97±10.54</td>
<td>100.58±10.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after treatment</td>
<td>130.27±15.18</td>
<td>78.61±8.57</td>
<td>75.27±8.61</td>
</tr>
</tbody>
</table>

Note: comparison of indexes within same group between before and after treatment, *P* < 0.05; comparison of indexes between observation group and control group 1 week after treatment, #P < 0.05.

### 3.2 Invasion gene expression

Comparison of invasion genes Gal-1, SOX7, Syndecan-1, HPA-1, MTA1 and TROP2 mRNA expression in malignant ascites between two groups of patients before and after treatment was as follows: before treatment, the differences in Gal-1, SOX7, Syndecan-1, HPA-1, MTA1 and TROP2 expression in malignant ascites were not significant between the two groups of patients (*P* > 0.05); 1 week after treatment, Gal-1, HPA-1, MTA1 and TROP2 mRNA expression in malignant ascites of both groups of patients were lower than those before treatment while SOX7 and Syndecan-1 mRNA expression were higher than those before treatment, and Gal-1, HPA-1, MTA1 and TROP2 mRNA expression in malignant ascites of observation group were lower than those of control group while SOX7 and Syndecan-1 mRNA expression were higher than those of control group (*P* < 0.05), shown in Table 2.

### 3.3 Autophagy gene expression

Comparison of autophagy genes Beclin1, BNIP3 and LC3 mRNA expression in malignant ascites between two groups of patients before and after treatment was as follows: before treatment, differences in Beclin1, BNIP3 and LC3 mRNA expression in malignant ascites were not significant between the two groups of patients (*P* > 0.05); 1 week after treatment, Beclin1 mRNA expression in malignant ascites of both groups of patients were higher than those before treatment while BNIP3 and LC3 mRNA expression were lower than those before treatment, and Beclin1 mRNA expression in malignant ascites of observation group was higher than that of control group while BNIP3 and LC3 mRNA expression were lower than those of control group (*P* < 0.05), shown in Table 3.

### 4. Discussion

Paclitaxel plus cisplatin drugs chemotherapy is a routine method in treatment of patients with advanced cancer, previous study has pointed out that the scheme can weakened the cancer cell activity to some extent, but it has limitations and is unable to effectively reverse the disease progress, and it is necessary to combine the drugs with other mechanisms of action to expand its curative effect. Aidi injection is a Chinese patent medicine made from cantharides, astragalus, ginseng, acanthopanax and so on. Cantharides counteracts toxic substances and erodes wound as well as removes the blood stasis and stagnation; astragalus replenishes qi to secure the exterior as well as induces diuresis and drains toxin; ginseng nourishes qi and strengthens the spleen as well as nourishes blood and calms heart; acanthopanax replenishes qi to secure the exterior as well as regenerates tissue to heal wound, and the combination of main drugs can eliminate fatigue and remove stagnation as well as benefit qi and remove virus, and help regulate the immune function and promote the death of cancer cells[7,8]. In this study, Aidi injection combined with conventional chemotherapy was used to treat patients with advanced gastric cancer combined with malignant ascites, and the effects of combined drug administration on the malignant degree of ascites were discussed.

The proliferation activity of cancer cells in malignant ascites can directly determine the rate of disease progression and the final outcome, and the abnormal expression of multiple proliferation genes is closely related to the formation of malignant ascites. EZH2 can promote the proliferation of tumor cells, and its high expression is one of the important markers of poor prognosis of patients with gastric cancer[9]; PTPN13 has antitumor effect in gastric cancer tissue and gastric cancer cells, and the low expression of PTPN13 indicates...
bad prognosis[10]; the expression of TRIM28 in gastric cancer tissue is higher than that in normal tissue, which plays an important role in the proliferation of gastric cancer cells; I2PP2A can promote the proliferation of gastric cancer cells through MMP-9 pathway, and the decrease of its expression can directly inhibit the proliferation rate of gastric cancer cells. In the study, analysis of the changes in proliferation gene expression in malignant ascites before and after treatment showed that EZH2 and I2PP2A mRNA expression in malignant ascites of both groups of patients decreased significantly while PTPN13 and TRIM28 mRNA expression were higher after treatment. This indicates that both treatments can inhibit the activity of pro-proliferation genes and increase the expression of anti-proliferation genes; further compared with those of control group, EZH2 and I2PP2A mRNA expression in malignant ascites of observation group were lower while PTPN13 and TRIM28 mRNA expression were higher after treatment, confirming that the Aidi injection combined with conventional chemotherapy can more effectively inhibit the invasion activity of gastric cancer cells, and reduce the malignant degree of tumor.

Autophagy is also involved in the occurrence and development of gastric cancer, the present study has shown that autophagy gene Beclin1 expression in gastric cancer tissue is significantly lower than that in tissue adjacent to carcinoma, indicating that the gene has negative regulating effect on gastric cancer progress. BNIP3 can balance the cellular survival status by regulating cell autophagy levels, and studies have shown that if the cancer cells are in relatively hypoxic condition, the expression of BNIP3 gene in the cells decreases significantly[18,19]. LC3 is involved in the formation of the autophagosome, the autophagy mediated by it plays an important role in the occurrence of gastric cancer, and previous study have shown that its expression in gastric cancer tissue is significantly higher than that in tissue adjacent to carcinoma[20]. In the study, analysis of the changes in autophagy gene expression in malignant ascites before and after treatment showed that Beclin1 mRNA expression in malignant ascites of both groups of patients increased while BNIP3 and LC3 mRNA expression decreased after treatment. This shows that both treatments have moderating effect on cell autophagy activity. Further compared with those of control group, Beclin1 mRNA expression in malignant ascites of observation group was higher while BNIP3 and LC3 mRNA expression were lower after treatment, confirming that the Aidi injection combined with conventional chemotherapy can more effectively adjust the autophagy activity of gastric cancer cells, and inhibit the gastric cancer cell growth.

Thus, Aidi injection combined with paclitaxel and platinum drug therapy can effectively inhibit the gastric cancer cell proliferation and invasion in ascites, and effectively regulate its autophagy activity, it is more outstanding than conventional chemotherapy in curative effect, and it is worthy of popularization and application in clinical practice in the future.

References


