Effect of surgical resection combined with intraperitoneal fluorouracil implants placement on the long-term advanced gastric cancer recurrence and malignant molecule expression

Qiang Lu¹, Yao-Xiang Zou¹,², Xu-Dong Ruan²

¹. General Surgery, the Affiliated Dongnan Hospital of Xiamen University, Zhangzhou, Fujian, 363000
². Department of Oncology, the Affiliated Dongnan Hospital of Xiamen University, Zhangzhou, Fujian, 363000

ARTICLE INFO

Article history:
Received 14 Apr 2017
Received in revised form 17 Apr 2017
Accepted 19 Apr 2017
Available online 24 May 2017

Keywords:
Advanced gastric cancer
5-fluorouracil implants
Tumor markers
Tumor stem cell

ABSTRACT

Objective: To study the effect of surgical resection combined with intraperitoneal fluorouracil implants placement on the long-term advanced gastric cancer recurrence and malignant molecule expression. Methods: A total of 68 patients with postoperative advanced gastric cancer recurrence who were treated in our hospital between May 2015 and October 2016 were selected, and according to the different methods of intraoperative 5-fluorouracil administration, the patients with advanced gastric cancer recurrence were divided into implants group and control group. 1 day, 1 week and 2 weeks after operation, the liver and kidney function indexes were determined; at recurrence, tumor marker levels in serum as well as stem cell-related molecule expression in recurrent lesions were determined. Results: Differences in serum ALT, AST, BUN and Scr levels were not statistically significant within group and between groups 1 day, 1 week and 2 weeks after operation; at recurrence, serum OPN, VEGF, CEA and CA50 levels of implants group were significantly lower than those of control group, and Lgr5, CD44, Tff2, Musashi-1, Notch1, Wnt and β-catenin expression in recurrent gastric cancer lesions were significantly lower than those of control group. Conclusion: Surgical resection combined with intraperitoneal fluorouracil implants placement can reduce the malignant degree of recurrent gastric cancer lesions and inhibit the characteristics of tumor stem cells.

1. Introduction

Gastric cancer is one of the most common malignant tumors of digestive system, and surgical resection is the preferred treatment. Although radical operation for gastric cancer can guarantee the negative margin, the surrounding infiltrated small lesions and cancer stem cells will become the pathologic basis of distant tumor recurrence. In radical operation for gastric cancer, conventional abdominal cavity flushing with 5-fluorouracil can cause damage to small lesions and cancer stem cells to a certain extent, but influenced by shorter drug effect time, intraoperative abdominal cavity flushing with 5-fluorouracil has limited value for preventing long-term gastric cancer relapse[1]. 5-fluorouracil implants developed in recent years is the sustained-release drug form of 5-fluorouracil, can locally constantly release drugs and maintain drug concentration above the lowest concentration for tumor suppression, and has sustained killing effect on small lesions and cancer stem cells[2,3]. Existing domestic research has shown that 5-fluorouracil implants can reduce the local recurrence after radical operation for gastric cancer[4], but the malignant degree of recurrent lesions and the properties of stem cells are not yet clear. In the following study, the effect of surgical resection combined with intraperitoneal fluorouracil implants placement on the long-term advanced gastric cancer recurrence and malignant molecule expression was analyzed.

2. Research subjects and groups

2.1. Research subjects

Patients with postoperative advanced gastric cancer recurrence who were treated in our hospital between May 2015 and October 2016 were selected, and the inclusion criteria were: (1) receiving radical
operation for gastric cancer in our hospital; (2) with intraoperative abdominal cavity flushing by 5-fluorouracil or intraperitoneal 5-fluorouracil implants placement; (3) diagnosed with gastric cancer recurrence in situ by pathological biopsy; (4) with complete medical records. The medical records were reviewed, a total of 68 cases were included, and the patients with postoperative advanced gastric cancer recurrence were divided into implants group and control group according to different ways of intraoperative 5-fluorouracil delivery. Implants group, a total of 31 cases, included 19 male cases and 12 female cases that were 45-67 years old; control group, a total of 37 cases, included 22 male cases and 15 female cases that were 42-69 years old. The two groups of patients were not significantly different in general data ($P>0.05$).

2.2. Research methods

Two groups of patients received radial operation for gastric cancer performed by the same group of surgeons, both received laparotomy, both distal and proximal incisal margins were more than 3-5 cm from tumor, and 2-3 cm below pylorus was selected for conventional closing and gastrojejunostomy. After surgical resection was completed, 4 000 mL 43-45℃ distilled water was used to flush abdominal cavity, then 600 mg 5-fluorouracil was added in 100 mL 43-45℃ distilled water, the solution was poured into the abdominal cavity and kept there for 20 min, and then aspirator was used to drain all peritoneal lavage fluids; on the basis of abdominal cavity flushing with 5-fluorouracil, the implants group received 5-fluorouracil implants placement, a total of 600 mg was placed, the placement sites were around the tumor lesion and abdominal aorta, and the drug in each placement site should not be more than 150 mg.

2.3 Serum index detection

1 day, 1 week and 2 weeks after operation, peripheral blood samples were collected to separate serum, and automatic biochemical analyzer was used to detect the contents of ALT, AST, BUN and Scr; in the case of recurrence, peripheral blood samples were collected to separate serum, and enzyme-linked immunosorbent assay kits were used to determine OPN, VEGF, CEA and CA50 contents.

2.4 Malignant molecule expression detection in lesions

In the case of recurrence, the recurrent gastric cancer lesions were collected, added in moderate amount of PBS buffer and grinded, the obtained tissue suspension was centrifuged at 4℃ and 12 000 r/min for 20 min, the tissue precipitation was abandoned, the supernatant was kept, and enzyme-linked immunosorbent assays kits were used to determine Lgr5, CD44, Tff2, Musashi-1, Notch1, Wnt and β-catenin contents.

2.5 Statistical methods

SPSS 17.0 software was used to input the expression of serum indexes and malignant molecules in lesions, differences in above data between two groups was by t test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Liver and kidney function indexes within 2 weeks after operation

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Point in time</th>
<th>Liver function</th>
<th>Kidney function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ALT (U/L)</td>
<td>AST (U/L)</td>
</tr>
<tr>
<td>Implants</td>
<td>31</td>
<td>1 d after operation</td>
<td>28.59±5.34</td>
<td>18.92±2.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after operation</td>
<td>32.14±4.62</td>
<td>20.12±2.35</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>2 weeks after operation</td>
<td>31.94±5.58</td>
<td>20.33±2.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 d after operation</td>
<td>29.03±4.51</td>
<td>19.34±2.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week after operation</td>
<td>31.48±3.86</td>
<td>20.21±2.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 weeks after operation</td>
<td>30.58±4.52</td>
<td>20.58±3.26</td>
</tr>
</tbody>
</table>

Comparison of liver and kidney function indexes between two groups of patients within 2 weeks after operation.

At recurrence, analysis of serum tumor markers OPN (μg/L), VEGF (ng/mL), CEA (μg/L) and CA50 (IU/mL) levels between two groups of patients was as follows: serum OPN, VEGF, CEA and CA50 levels of implants group were significantly lower than those of control group. Differences in serum OPN, VEGF, CEA and CA50 levels of implants group were significantly lower than those of control group. Differences in serum OPN, VEGF, CEA and CA50 levels were statistically significant between two groups of patients at recurrence ($P<0.05$), shown in Table 2.

3.2 Serum tumor marker levels at recurrence

Analysis of tumor stem cell marker molecules Lgr5, CD44, Tff2 and Musashi-1 expression in recurrent gastric cancer lesions between two groups of patients was as follows: Lgr5, CD44, Tff2 and Musashi-1 expression in recurrent gastric cancer lesions of implants group were significantly lower than those of control group,
Groups of patients (gastric cancer lesions were statistically significant between two control group, shown in Table 4. Differences in Lgr5, CD44, Tff2, Musashi-1, Notch1, Wnt and β-catenin expression in recurrent gastric cancer lesions were statistically significant between two groups of patients (P<0.05).

4. Discussion

Abdominal cavity flushing with 5-fluorouracil in radical operation for gastric cancer is commonly used to kill tiny residual lesions and prevent tumor recurrence, but 5-fluorouracil is absorbed quickly and takes effect for only six to eight hours in the abdominal cavity. 5-fluorouracil implant is a newly developed sustained-release drug form of 5-fluorouracil and can continue to release drugs for 300-500 h. Intra-abdominal placement of 5-fluorouracil implants can constantly release 5-fluorouracil and prolong the drug action time, but it also can increase the chemotherapy drug damage to normal tissues and organs[5,6]. 5-fluorouracil implants continue to release drug for about 300-500 h, or two weeks, and the viscera damage within 2 weeks of 5-fluorouracil implants placement was analyzed in the study. Liver and kidney are the target organs commonly involved by side effects of chemotherapy drugs, liver damage can cause the release of AST, ALT and other liver enzymes into the blood circulation, and kidney damage will affect the creatinine and urea nitrogen discharge and lead to the increased BUN and Scr contents. Serum ALT, AST, BUN and Scr levels were not significantly different between two groups of patients 1 day, 1 week and 2 weeks after operation. This means that intra-abdominal 5-fluorouracil implants placement will not increase?

It has been confirmed that 5-fluorouracil implants can prevent the gastric cancer recurrence after radical operation[4], but the malignant degree of recurrent lesions is not yet clear. In the process of gastric cancer recurrence in situ, the proliferation of cancer cells can cause increased synthesis and secretion of a variety of proteins and peptides. VEGF is the cytokine involved in angiogenesis regulation, and it is closely related to tumor recurrence[7]; OPN is a secreted calcium-binding glycoprotein that regulates cell migration and invasion, and it is associated with the invasive tumor growth[8]; the CEA and CA50 are the common markers for malignant digestive tract tumor screening and tumor load evaluation[9,10]. In order to define the effect of intraperitoneal 5-fluorouracil implants placement on the malignant degree of recurrent gastric cancer lesions, serum tumor marker levels were analyzed in the study, and the result showed that serum OPN, VEGF, CEA and CA50 levels of implants group were significantly lower than those of control group. This means that intraperitoneal 5-fluorouracil implants placement can not only prevent the long-term gastric cancer recurrence after radical surgery, but can also inhibit the malignant degree of recurrent gastric cancer lesions.

Cancer stem cells in gastric cancer lesions and surrounding tissues are the pathological basis of tumor recurrence, cancer stem cells are with self-renewal and multi-directional differentiation potential, and they can differentiate into tumor cells with different malignant degree and form tumor lesions[11], Lgr5, CD44, Tff2 and Musashi-1 are the marker molecules that reflect the characteristics of stem cells within the tumor lesion, and Lgr5 is a kind of G protein-coupled receptor that is used to mark gastric epithelial progenitor cells and can adjust progenitor cell differentiation into various mucosal epithelial cells[12]; CD44 is a transmembrane glycoprotein that can be used as a cell membrane receptor to deliver a variety of external

Table 2.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>OPN</th>
<th>VEGF</th>
<th>CEA</th>
<th>CA50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implants group</td>
<td>31</td>
<td>33.51±4.59</td>
<td>126.23±19.51</td>
<td>24.21±3.52</td>
<td>115.56±17.62</td>
</tr>
<tr>
<td>Control group</td>
<td>37</td>
<td>47.69±7.59</td>
<td>193.43±23.41</td>
<td>36.71±5.24</td>
<td>178.94±24.25</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>7.595</td>
<td>7.918</td>
<td>7.182</td>
<td>8.785</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3.

Comparison of stem cell marker molecule expression in recurrent gastric cancer lesions between two groups of patients (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Lgr5</th>
<th>CD44</th>
<th>Tff2</th>
<th>Musashi-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implants group</td>
<td>31</td>
<td>1.84±0.25</td>
<td>1.21±0.19</td>
<td>1.03±0.16</td>
<td>1.56±0.22</td>
</tr>
<tr>
<td>Control group</td>
<td>37</td>
<td>3.32±0.54</td>
<td>2.58±0.39</td>
<td>2.18±0.32</td>
<td>2.93±0.50</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>8.592</td>
<td>11.384</td>
<td>12.018</td>
<td>8.145</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 4.

Comparison of stem cell-related signaling pathway molecule expression in recurrent gastric cancer lesions between two groups of patients (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Notch1</th>
<th>Wnt</th>
<th>β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implants group</td>
<td>31</td>
<td>3.14±0.52</td>
<td>2.37±0.35</td>
<td>1.27±0.20</td>
</tr>
<tr>
<td>Control group</td>
<td>37</td>
<td>5.79±0.80</td>
<td>6.42±0.91</td>
<td>3.28±0.52</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>8.280</td>
<td>15.822</td>
<td>18.481</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
signals and regulate cell differentiation, proliferation, adhesion and migration[13]; Tff2 is also a gastric epithelial progenitor cell marker that can adjust progenitor cell differentiation into parietal cells, mucous cells and solid cells[14]; Musashi-1 is a transcription factor that regulates cell differentiation, and it can induce pseudopod-like metaplasia and mucous metaplasia[15]. In the study, analysis of cancer stem cell marker molecule expression in recurrent gastric cancer lesions proved that Lgr5, CD44, Tff2 and Musashi-1 expression in recurrent gastric cancer lesions of implants group were significantly lower than those of control group. This means that intraperitoneal 5-fluorouracil implants placement can inhibit the characteristics of tumor stem cells in the process of gastric cancer recurrence.

The characteristics of cancer stem cells and the expression of related marker molecules are regulated by Notch1, Wnt and various other signaling pathways. Notch1 signaling pathway is mediated by transmembrane receptor Notch1 as well as the ligand Jagged1, Jagged2, DLL1 and DLL3, the activated Notch1 can cause intracellular fragments to split and form NICD, and the latter is the active form of Notch1 receptor and can regulate the expression of various downstream stem cell marker molecules[16]; Wnt signaling pathway is mediated by Wnt molecules as well as downstream Frizzled and LRPS/6, and Wnt can form complexes with Frizzled and LRPS/6 to hinder β-catenin phosphorylation, cause its accumulation within the cells, and then regulate the expression of a variety of stem cell marker molecules through the TCF/LEF transcription factors[17]. In the study, analysis of tumor stem cell signaling pathway molecule expression in recurrent gastric cancer lesions proved that Notch1, Wnt and β-catenin expression in recurrent gastric cancer lesions of implants group were significantly lower than those of control group. This means that intraperitoneal 5-fluorouracil implants placement can inhibit the activation of tumor stem cell-related signaling pathways in the process of gastric cancer recurrence.

To sum up, it is believed that intraperitoneal fluorouracil implants placement can not only prevent the long-term gastric cancer recurrence after radical surgery, but can also inhibit the malignant degree of recurrent gastric cancer lesions, and inhibiting cancer stem cell characteristics and related signaling pathway activation is the possible molecular mechanism for intraperitoneal fluorouracil implants placement to reduce the malignant degree of recurrent gastric cancer lesions.

References


