Effects of circulation hyperthermic perfusion chemotherapy on tumor marker content and PI3K/Akt/mTOR pathway function of gastric cancer peritoneal effusion patients

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ABSTRACT

Objective: To study the effects of circulation hyperthermic perfusion chemotherapy on tumor marker content and PI3K/Akt/mTOR pathway function of gastric cancer peritoneal effusion patients.

Methods: 80 cases of gastric cancer peritoneal effusion patients in our hospital from May 2013 to August 2014 were enrolled and randomly divided into two groups. Observation group received circulation hyperthermic perfusion chemotherapy; control group received conventional perfusion chemotherapy. Then blood tumor markers, LAG3 and HSP content, PI3K-AKT-mTOR signal molecules were assayed. Results: (1) tumor markers: DDK1, EXOSC2 contents and PGR ratio of observation group were lower than those of control group; PGI and PGII contents were higher than those of control group; (2) LAG3 and HSP contents: HSP27 and HSP90 contents of observation group were lower than those of control group; sLAG-3 content was higher than that of control group; (3) signal molecules: mRNA contents of PI3K, Akt and mTOR molecules of observation group were lower than those of control group. Conclusion: Circulation hyperthermic perfusion chemotherapy is helpful to kill tumor cells, reduce tumor marker releasing into blood, regulate LAG3 and HSP expression and inhibit PI3K/Akt/mTOR pathway function; it’s an ideal method for treating peritoneal effusion.

Gastric cancer is one of the most common malignant tumors of digestive system. Patients who developed to advanced disease have higher incidence of ascites, which will accelerate disease progression and need to take timely and effective treatment measures. Traditional intraperitoneal chemotherapy, to a certain extent, can kill cancer cells and control the malignant effusion formation, but the overall effect is not ideal. Thermo chemotherapy is a newly developed chemotherapy, which is a combination of thermotherapy and chemotherapy. First, tumor and normal tissues have different tolerance to temperature. Second, there exists sensitizing effects of hyperthermia on chemotherapeutic drugs to enhance the killing effect of the later to malignant tumor cells[1]. In the following study, we analyzed the effects of cyclic heat perfusion chemotherapy on gastric cancer patients’ peritoneal effusion marker content and its effects on PI3K/Akt/mTOR pathway function.

1. Materials and methods

1.1 Materials

80 gastric cancer cases with peritoneal effusion from May 2013 to August 2014 in our hospital were brought into the study. All patients were confirmed the diagnosis of advanced gastric cancer by pathological examination, ascites puncture and biopsy confirmed the existing of malignant ascites. None of them has undergone chemotherapy before diagnosis. All patients were informed of risk matters and signed the informed consent. According to the treatment methods, patients were randomly divided into two groups, 40 cases in each group. Patients in the observation group received peritoneal circulation hyperthermic perfusion chemotherapy, including 14 male cases and 26 female cases with average age 63.29 ± 7.82.
Patients in the control group received routine peritoneal perfusion chemotherapy, including 12 male cases and 28 female cases with average age $63.72 \pm 7.98$. There were no statistically significant differences between the two groups of patients with general information ($P>0.05$).

1.2 Treatment method

Two groups of patients were identified peritoneal effusion position by color Doppler ultrasound, and then did abdominal puncture and peritoneal catheter to empty intraperitoneal effusion before perfusion chemotherapy. The control group accepted the following therapy: dissolve 30 mg/m2 of cisplatin into 2000 ml sterile saline, fill the mixed solution into the abdominal cavity, then inject dexamethasone 5 mg and lidocaine 2 ml; Closed the infusion pipeline and persistent for 1 hour, during the transformation change posture. Do the above therapy 1 time a week. The observation group accepts the following methods: take the same methods to infuse cisplatin, connect pipeline to extracorporeal circulation then do circulation hyperthermic perfusion chemotherapy. The temperature is set at 45 $\degree$C, and the setting time is 60min.

1.3 Detection indexes

Before treatment and 4 weeks after treatment, peripheral blood was collected from two groups of patients. ELISA was used to determine DDK1, EXOSC2, PGI, PGII, HSP27, HSP90, sLAG-3 content. Use Trizol lysate to extract total RNA, reverse transcript mRNA to cDNA, and detect PI3K, Akt, mTOR content by fluorescence quantitative PCR. In the control group mRNA content in blood was set as 100. The mRNA contents of the observation group and the control group were calculated before treatment and that of the observation group after treatment respectively.

1.4 Statistical method

The SPSS19.0 was used to input data. Measurement data went through t test. Differences were considered to be statistically significant at a level of $P<0.05$.

2. Results

2.1 Tumor markers

After perfusion chemotherapy, both groups of patients' blood were collected to detect tumor markers contents. T test analysis showed that DDK1, EXOSC2 content and PGR ratio of patients in the observation group were lower than those in the control group, while PGI and PGII were higher. The difference was statistically significant ($P<0.05$).

2.2 LAG3 and HSP

The author measured the contents of LAG3 and HSP in blood respectively before and after the treatment. The results of ELISA showed that before treatment, no differences were found between the two groups of patients with sLAG3 and HSP content ($P>0.05$). After the treatment, patients in the observation group had lower HSP27 and HSP90 and higher sLAG-3 than the control group. The differences were statistically significant ($P<0.05$).

2.3 Signal pathway function

PI3K-AKT-mTOR signal pathway function was detected

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<tr>
<th>Table 1</th>
<th>comparison of tumor makers contents between two groups of patients</th>
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<tbody>
<tr>
<td></td>
<td>DDK1(μg/L)</td>
</tr>
<tr>
<td>Observation group</td>
<td>1.48±0.21</td>
</tr>
<tr>
<td>Control group</td>
<td>3.09±0.45</td>
</tr>
<tr>
<td>$T$</td>
<td>11.492</td>
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<tr>
<td>$P$</td>
<td>&lt;0.05</td>
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<tr>
<th>Table 2</th>
<th>Comparison of LAG3 and HSP contents between two groups of patients</th>
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<tr>
<td></td>
<td>sLAG-3(μg/L)</td>
</tr>
<tr>
<td>Observation group</td>
<td>165.64±21.44</td>
</tr>
<tr>
<td>Control group</td>
<td>166.29±19.38</td>
</tr>
<tr>
<td>$T$</td>
<td>0.284</td>
</tr>
<tr>
<td>$P$</td>
<td>&gt;0.05</td>
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respectively before and after the treatment. The results of fluorescent quantitative PCR analysis showed that, before the treatment no difference was found between two groups of patients \( (P > 0.05) \). After the treatment, mRNA contents of PI3K, Akt and mTOR in serum of patients in the observation group were lower than those in the control group. There were statistically significant differences \( (P < 0.05) \).

Figure 1: the effect of different treatment methods on PI3K-AKT-mTOR signal molecules in the blood. Before the treatment there was no difference in PI3K-AKT-mTOR signal pathway. After the treatment, circulation hyperthermic perfusion chemotherapy can inhibit the expression of molecules of I3K-AKT-mTOR signaling pathways. " compared with the control group, the difference was statistically significant, \( P < 0.05 \).

3. Discussion

Thermo chemotherapy is a newly developed therapy, which combines hyperthermia and chemotherapy. Under normal conditions, the chemotherapy drug can penetrate the tumor tissue by about 3mm deep, while in the hyperthermia conditions the depth increases to 5mm, which is conducive to the increase of effective drug concentration in tumor lesions, and also ensure the deep tumor cells to be killed by the chemotherapy drugs. In clinical practice, tumor marker content can accurately reflect the cancer cell activity, and indirectly reflect the killing effect of different treatments on cancer cells. In this study, we chose the following tumor markers: Dickkopf-1 (DKK1) can promote the proliferation of cancer cells by interaction with LRP5/LRP6 with its C-terminal. Dickkopf-1 (DKK1) can promote the proliferation of cancer cells. In this study, we chose the following tumor markers: 

- Pepsinogen (PG) is a kind of transmembrane glycoprotein, which is a costimulatory molecule belonging to the immunoglobulin family and has a negative regulatory role. It can bind a ligand molecule MHCII and inhibit T cell activation and proliferation. It is related to tumor occurrence and immune escape. When the peptide chain between membrane type LAG-3 molecule’s outer membrane segment and the trans-membrane region fractured under the action of proteases soluble LAG-3 molecules are formed, in the blood circulation it can compete with the membrane type of LAG-3 to bind MHCII molecules, thus inhibit immune activities mediated by LAG-3 and then induce helper T cells and regulatory T cells activation, which has significantly anti-tumor effect. Heat shock protein (HSP) is also called stress protein, which is a kind of highly conservative form of protein in eukaryotic cells. It mainly plays the role of molecular chaperone, which is of important value to maintain stability of protein and DNA structure and thus can make the cell maintain survival under lethal damage conditions. When the body is stimulated by physical and chemical factors such as inflammation, trauma, tumor and infection, a large number of HSP will express and help to maintain the internal environment homeostasis. In the process of carcinogenesis of gastric mucosa epithelial cells, HSP expression increases and participates in various malignant biological behaviors of gastric cancer cells. HSP27 and HSP90 are now known to be two kinds of HSP molecules most closely related to gastric cancer. The former is involved in cell proliferation, differentiation and other signals transduction pathways. The latter is involved in protein folding and subunit assembly and regulates cell survival. Through the analysis of the expression of the above two kinds of tumor molecular we found that patients in the observation group had lower HSP27, HSP90 than the control group, while the content of sLAG-3 was higher. This indicated that circulation hyperthermic perfusion chemotherapy contributed to the regulation of LAG-3 and HSP expression.

Recent studies suggest that the incidence of malignant tumors and the abnormal expression of various molecules in cells are related to abnormal activation of many signal pathways. Phosphoinositide 3-kinase (PI3K)-protein kinase B (PKB, also known as AKT) - the mammalian target of rapamycin (mTOR) are the important signaling pathways at downstream of factor growth receptor tyrosine kinase. PI3K-AKT-mTOR is considered to be most closely related to the occurrence of gastric cancer. PI3K can catalyze the produce of the

**Figure 1**: The effect of different treatment methods on PI3K-AKT-mTOR signal pathways in the blood. Before the treatment, there was no difference in PI3K-AKT-mTOR signal pathway. After the treatment, circulation hyperthermic perfusion chemotherapy can inhibit the expression of molecules of I3K-AKT-mTOR signaling pathways. Compared with the control group, the difference was statistically significant, \( P < 0.05 \).
second messenger molecule phosphatidylinositol 3, 4, 5-triphosphate (PIP3), and activate the downstream AKT, and then through AKT activate mTOR. The pathway mainly mediates anti-apoptotic and pro-survival effects and involves in malignant tumor cells proliferation, migration and other processes [13]. Domestic study of fei RH [14] has found that PI3K/mTOR dual-inhibitor PF-04691502 can induce apoptosis of gastric cancer cell line, which will further clarify the relationship between PI3K/mTOR signaling pathway and the occurrence and development of tumor. Recent in vivo experiment also finds that the PI3K inhibitor LY294002 can reduce the tumor volume, reduce local micro-vessel density and cancer cell invasion depth, as well as inhibit the expression of VEGF and MMP [15]. In the above study, the author analyzed the influence of circulation hyperthermic perfusion chemotherapy on PI3K/Akt/mTOR pathway function. The comparison of the expression of signal molecules in the blood between the two groups of patients showed that the mRNA contents of PI3K, Akt and mTOR in the observation group were lower than those in the control group. This suggested that the circulation hyperthermic perfusion chemotherapy helped to inhibit the activation of PI3K/Akt/mTOR signaling pathway.

References: