Effect of hyperthermia perfusion chemotherapy combined with systemic chemotherapy on the expression and secretion of malignant molecules in advanced bladder cancer

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ABSTRACT

Objective: To study the effect of hyperthermia perfusion chemotherapy combined with systemic chemotherapy on the expression and secretion of malignant molecules in advanced bladder cancer. Methods: Patients who were diagnosed with advanced bladder cancer in Gongan County People’s Hospital between March 2015 and December 2017 were chosen and randomly divided into two groups, experimental group accepted hyperthermia perfusion chemotherapy combined with systemic chemotherapy, and control group accepted routine infusion chemotherapy combined with systemic chemotherapy. The expression levels of proliferation and invasion genes in the lesions as well as the secretion of cytokines in the urine were measured before chemotherapy and 3 months after chemotherapy. Results: Livin, Bcl-2, TRAP1 and MMP9 mRNA expression in lesions as well as VEGF, TGF-β1, MCP-1 and CEACAM1 secretion in urine of both groups after chemotherapy were lower than those before chemotherapy whereas Bad, LRIG3, Beclin1, KLF4, CHD13 and E-cadherin mRNA expression in lesions as well as IFN-γ and IL-2 secretion in urine were higher than those before chemotherapy, and Livin, Bcl-2, TRAP1 and MMP9 mRNA expression in lesions as well as VEGF, TGF-β1, MCP-1 and CEACAM1 secretion in urine of experimental group were significantly lower than those of control group whereas Bad, LRIG3, Beclin1, KLF4, CHD13 and E-cadherin mRNA expression in lesions as well as IFN-γ and IL-2 secretion in urine were significantly higher than those of control group. Conclusion: Hyperthermia perfusion chemotherapy combined with systemic chemotherapy can be more effective than routine infusion chemotherapy combined with systemic chemotherapy to regulate the expression and secretion of malignant molecules in advanced bladder cancer.

1. Introduction

Bladder cancer is a common malignant tumor of urinary system, it is derived from the urothelial cells, and the malignantly transformed cells show invasive growth and are prone to abdominal dissemination and distant metastasis after invading bladder muscle layer. In clinical practice, the early diagnosis of bladder cancer is very difficult, and it is mostly in locally advanced stage at diagnosis, and has lost the indications of surgical resection[1,2]. Systemic chemotherapy and local perfusion chemotherapy are the main methods to treat advanced bladder cancer, which can effectively kill cancer cells, but also cause certain toxic and side effects as well as adverse reactions[3]. Hyperthermia perfusion chemotherapy is local therapy for bladder cancer developed in recent years, and it continuously and circularly perfuses the chemotherapy drugs that are heated to a certain temperature into the bladder, which not only kills local cancer cells through the effect of chemotherapy drugs, but also kills cancer cells directly through the heat effect on the one hand, and on the other hand, increases the sensitivity...
of cancer cells to chemotherapy drugs.[4] It has been reported that the adjuvant bladder hyperthermia perfusion chemotherapy after bladder cancer TURBT can prevent local recurrence of tumor, but the therapeutic effect for advanced bladder cancer has not been reported. In the following studies, we specifically analyzed the effects of hyperthermia perfusion chemotherapy combined with systemic chemotherapy on the expression and secretion of malignant molecules in advanced bladder cancer.

2. Case information and research methods

2.1. General case information

Patients who were diagnosed with advanced bladder cancer in Gongan County People’s Hospital between March 2015 and December 2017 were selected, all patients were diagnosed with bladder cancer by cystoscopic biopsy, imageological inspection confirmed the tumor stage was locally advanced stage, the expected survival time was more than 3 months, and the patients combined with chemotherapy contraindications and those who were treated with antitumor drugs before inclusion were ruled out. A total of 82 patients were enrolled, and the random number table method was used to divide them into two groups, each with 41 cases. There were 27 males and 14 females in the experimental group, they were 45-63 years old, and the maximum diameter of lesion was (2.73±0.41) cm; there were 29 males and 12 females in the control group, they were 43-66 years old, and the maximum diameter of lesion was (2.81±0.45) cm. There was no significant difference in the general information between the two groups (P>0.05).

2.2. Chemotherapy

Both groups of patients received systemic chemotherapy, and the method was as follows: intravenous drip of gemcitabine 1 000 mg/m² on day 1 and day 8 as well as intravenous drip of 30 mg/m² on day 2-4, 28 d as one cycle of chemotherapy, for a total of four cycles. On the basis of systemic chemotherapy, control group received perfusion chemotherapy, which was as follows: on the 6th day after systemic chemotherapy was started, epirubicin 20 mg was added in 100 mL of 5% glucose injection, which was provided by bladder perfusion, once a week, for a total of eight times. On the basis of systemic chemotherapy, experimental group received bladder hyperthermia perfusion chemotherapy, and the method was as follows: they were connected to coelom hyperthermia perfusion apparatus, epirubicin 20 mg was added in 1 500 mL of 5% glucose injection, the temperature was set to 43 °C, perfusion rate was 200 mL/min, and the perfusion lasted for 1 h and was conducted once a week for a total of eight times.

2.3. Laboratory detection methods

2.3.1. Gene expression detection

Before chemotherapy and 3 months after chemotherapy, cystoscopic biopsy was conducted respectively to get lesion tissues, the experimental steps of RNA extraction, cDNA synthesis and PCR amplification kits were followed to extraction the RNA within the lesions, synthesize cDNA, use cDNA as the template to configure PCR reaction system and conduct PCR reaction, and the reaction curves were referred to calculate the mRNA expression of Livin, Bcl-2, Bad, LRIG3, Beclin1, KLF4, CHD13, E-cadherin, TRAP1 and MMP9.

2.3.2. Cytokine secretion detection

Before chemotherapy and 3 months after chemotherapy, 10 mL of morning urine was collected respectively and centrifuged to get rid of the residue, and the experimental steps of Elisa kit were followed to determine VEGF, TGF-β 1, MCP-1, CEACAM1, IFN-γ and IL-2 secretion.

2.4. Statistical methods

Software SPSS 19.0 was used to input data, the difference in measurement data between the two groups was analyzed by t test and P<0.05 meant statistical significance in the differences.

3. Results

3.1. Proliferation gene expression in lesions

Before chemotherapy and 3 months after chemotherapy, analysis of proliferation genes Livin, Bcl-2, Bad, LRIG3 and Beclin1 expression in lesions of the two groups of patients was as follows: Livin, Bcl-2, Bad, LRIG3 and Beclin1 expression in lesions of both groups of patients were significantly different between before and after chemotherapy (P<0.05), and Livin and Bcl-2 mRNA expression in lesions of both groups after chemotherapy were lower than those before chemotherapy whereas Bad, LRIG3 and Beclin1 mRNA expression were higher than those before chemotherapy; Livin, Bcl-2, Bad, LRIG3 and Beclin1 expression in lesions were not significantly different between the two groups of patients before chemotherapy (P>0.05) whereas they were significantly different after chemotherapy (P<0.05), and Livin and Bcl-2 mRNA expression in lesions of experimental group were significantly lower than those of control group whereas Bad, LRIG3 and Beclin1 mRNA expression were significantly higher than those of control group.

Table 1.
Changes of proliferation gene expression in lesions before and after chemotherapy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>Livin</th>
<th>Bcl-2</th>
<th>Bad</th>
<th>LRIG3</th>
<th>Beclin1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>41</td>
<td>Before chemo</td>
<td>1.03±0.14</td>
<td>0.99±0.12</td>
<td>1.04±0.16</td>
<td>1.02±0.14</td>
<td>0.97±0.13</td>
</tr>
<tr>
<td>Control</td>
<td>41</td>
<td>Before chemo</td>
<td>1.02±0.14</td>
<td>0.97±0.13</td>
<td>1.05±0.14</td>
<td>1.01±0.15</td>
<td>0.98±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After chemo</td>
<td>0.42±0.07&quot;</td>
<td>0.39±0.05&quot;</td>
<td>2.51±0.41&quot;</td>
<td>1.98±0.22&quot;</td>
<td>2.25±0.32&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After chemo</td>
<td>0.75±0.09&quot;</td>
<td>0.54±0.07&quot;</td>
<td>1.52±0.18&quot;</td>
<td>1.45±0.17&quot;</td>
<td>1.61±0.25&quot;</td>
</tr>
</tbody>
</table>

*: Comparison between before and after chemotherapy within group, P<0.05; †: comparison between groups after chemotherapy, P<0.05.
Changes of cytokine secretion in urine before and after chemotherapy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>KLF4</th>
<th>CDH13</th>
<th>E-cadherin</th>
<th>TRAP1</th>
<th>MMP9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>41</td>
<td>Before chemo</td>
<td>1.05±0.14</td>
<td>0.95±0.12</td>
<td>1.03±0.14</td>
<td>1.01±0.14</td>
<td>0.96±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After chemo</td>
<td>2.58±0.35*</td>
<td>2.04±0.34*</td>
<td>2.75±0.37*</td>
<td>0.33±0.08*</td>
<td>0.47±0.06*</td>
</tr>
<tr>
<td>Control group</td>
<td>41</td>
<td>Before chemo</td>
<td>1.02±0.16</td>
<td>0.97±0.13</td>
<td>1.01±0.15</td>
<td>1.04±0.12</td>
<td>0.98±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After chemo</td>
<td>1.76±0.24*</td>
<td>1.56±0.22*</td>
<td>1.77±0.25*</td>
<td>0.61±0.08*</td>
<td>0.63±0.08*</td>
</tr>
</tbody>
</table>

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

Table 3.
Changes of cytokine secretion in urine before and after chemotherapy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>VEGF</th>
<th>TGF-β 1</th>
<th>MCP-1</th>
<th>CEACAM1</th>
<th>IFN-γ</th>
<th>IL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>41</td>
<td>Before chemo</td>
<td>1.65±0.22</td>
<td>0.82±0.11</td>
<td>176.2±22.3</td>
<td>121.3±14.8</td>
<td>0.76±0.08</td>
<td>1.21±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After chemo</td>
<td>0.62±0.09*</td>
<td>0.34±0.06*</td>
<td>85.5±10.3*</td>
<td>47.2±6.8*</td>
<td>2.43±0.33*</td>
<td>3.38±0.42*</td>
</tr>
<tr>
<td>Control group</td>
<td>41</td>
<td>Before chemo</td>
<td>1.62±0.19</td>
<td>0.85±0.10</td>
<td>180.1±19.4</td>
<td>122.8±15.1</td>
<td>0.80±0.10</td>
<td>1.17±0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After chemo</td>
<td>1.12±0.15</td>
<td>0.56±0.08</td>
<td>127.5±16.2</td>
<td>76.4±9.3</td>
<td>1.65±0.22</td>
<td>2.03±0.31</td>
</tr>
</tbody>
</table>

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

3.2. Invasion gene expression in lesions

Before chemotherapy and 3 months after chemotherapy, analysis of invasion genes KLF4, CDH13, E-cadherin, TRAP1 and MMP9 expression in lesions of the two groups of patients was as follows: KLF4, CDH13, E-cadherin, TRAP1 and MMP9 mRNA expression in lesions of both groups of patients were significantly different between before and after chemotherapy (P<0.05), and KLF4, CDH13 and E-cadherin mRNA expression in lesions of both groups after chemotherapy were higher than those before chemotherapy while TRAP1 and MMP9 mRNA expression were lower than those before chemotherapy; KLF4, CDH13, E-cadherin, TRAP1 and MMP9 mRNA expression in lesions were not significantly different between the two groups of patients before chemotherapy (P>0.05) whereas they were significantly different after chemotherapy (P<0.05), and KLF4, CDH13 and E-cadherin mRNA expression in lesions of experimental group were significantly higher than those of control group whereas TRAP1 and MMP9 mRNA expression were significantly lower than those of control group.

3.3. Cytokine secretion in urine

Before chemotherapy and 3 months after chemotherapy, analysis of cytokines VEGF (ng/mL), TGF-β 1 (ng/mL), MCP-1 (pg/mL), CEACAM1 (pg/mL), IFN-γ (ng/mL) and IL-2 (ng/mL) secretion in urine of the two groups of patients was as follows: VEGF, TGF-β 1, MCP-1, CEACAM1, IFN-γ and IL-2 secretion were higher than those before chemotherapy; VEGF, TGF-β 1, MCP-1, CEACAM1, IFN-γ and IL-2 secretion in urine were not greatly different between the two groups of patients before chemotherapy (P>0.05) whereas they were significantly different after chemotherapy (P<0.05), and VEGF, TGF-β 1, MCP-1 and CEACAM1 secretion in urine of experimental group were lower than those of control group whereas IFN-γ and IL-2 secretion were higher than those of control group.

4. Discussion

Systemic chemotherapy and bladder perfusion chemotherapy are the main therapies for advanced bladder cancer, and the intravenous and local bladder administration of chemotherapy drugs can effectively kill the cancer cells and prolong the survival time of patients with advanced bladder cancer. Epirubicin is the most common perfusion chemotherapy drug that can inhibit the activity of DNA polymerase during DNA replication, embed DNA double helix structure and cause DNA damage to ultimately result in the cell growth arrest and death. Hyperthermia perfusion chemotherapy is auxiliary therapy for bladder cancer developed in recent years, and the coelom hyperthermia perfusion apparatus is adopted to heat the chemotherapy drugs to a predetermined temperature and continuously perfuse them to the bladder, which can not only exert the killing effect of chemotherapy drugs on cancer cells, but also exert the damage effect of heat effect on cancer cells. Malignant tumor cells have high metabolism rate and are sensitive to local temperature variations, and the heat effect produced by hyperthermia perfusion chemotherapy in local lesions can cause tumor cell temperature to change and lead to cellular damage; in addition, the heat effect can also increase the sensitivity of cancer cells to chemotherapy drugs so as to enhance the killing effect of chemotherapy drugs on cancer cells[5,6]. It has been reported that bladder hyperthermia perfusion chemotherapy can reduce recurrence rate after bladder cancer surgery, but the effect on advanced bladder
cancer is not clear.

The proliferation activity of cancer cells in bladder cancer is very strong, and many kinds of proliferation genes have changed significantly. The coding products of Livin are small molecular apoptosis inhibitors, which inhibit cell apoptosis by antagonizing the activity of caspase[7]; Bcl-2 and Bad are the anti-apoptosis and pro-apoptosis members of Bcl-2 family respectively, the former blocks the apoptosis mediated by cytochrome C release within the mitochondria, and the latter promotes the apoptosis mediated by cytochrome C release within the mitochondrial[8,9]; LRIG3 is the LRIG family member that can close the biological effect of promoting cell growth mediated by EGFR to inhibit cell proliferation[10]; Beclin1 is the regulatory molecule of autophagy, which initiates autophagy to causes cell damage and induce apoptosis[11]. Analysis of the changes of above proliferation gene expression in bladder cancer lesions before and after chemotherapy showed that Livin and Bcl-2 expression in lesions decreased whereas Bad, LRIG3 and Beclin1 expression increased after chemotherapy, and Livin and Bcl-2 expression in lesions of experimental group after chemotherapy were significantly lower than those of control group whereas Bad, LRIG3 and Beclin1 expression were significantly higher than those of control group. This means that chemotherapy can down-regulate the pro-proliferation gene expression and up-regulate the anti-proliferation gene expression in bladder cancer lesions to inhibit the proliferation of cancer cells, and hyperthermia perfusion chemotherapy is better than routine perfusion chemotherapy to regulate proliferation gene expression and inhibit cancer cell proliferation.

The abdominal dissemination and distant metastasis of the lesion will appear when the bladder cancer enters the advanced stage, and the invasive growth of cancer cells is closely related to it. In the process of cancer cell invasion to the abdominal cavity and distant tissue, the cellular epithelial mesenchymal transition process is enhanced, and the extracellular matrix and basement membrane are over-degraded. KLF4 and CHD13 are the upstream regulatory molecules of cellular epithelial mesenchymal transition, the former increases the expression of epithelial gene E-cadherin to enhance epithelial characteristics, maintain intercellular polarity and inhibit cell invasion, and the latter is directly involved in the maintenance of cellular epithelial characteristics and intercellular adhesion, and can also inhibit cell invasion[12,13]; when epithelial phenotype is weakened, the invasion ability of cells is enhanced[14]. TRAP1 is the HSP super family member that conducts signal transduction through the downstream signaling molecule Smad2/3, increase the expression of MMP9, then hydrolyze extracellular matrix and basement membrane through the biological activity of MMP9, and promote cell invasion[15]. Analysis of the changes of above invasion gene expression in bladder cancer lesions before and after chemotherapy showed that KLF4, CHD13 and E-cadherin expression in lesions increased while TRAP1 and MMP9 expression decreased after chemotherapy, and KLF4, CHD13 and E-cadherin expression in lesions of experimental group after chemotherapy were significantly higher than those of control group whereas TRAP1 and MMP9 mRNA expression were significantly lower than those of control group. This means that chemotherapy can up-regulate anti-invasion gene expression and down-regulate pro-invasion gene expression in bladder cancer lesions to inhibit the invasion of cancer cells, and hyperthermia perfusion chemotherapy is better than routine perfusion chemotherapy to regulate invasion gene expression and inhibit cancer cell invasion.

The excessive proliferation and invasion of cancer cells in bladder cancer lesions not only involves the abnormal expression of corresponding regulatory genes, but is also related to the abnormal secretion and functional changes of various cytokines. VEGF, TGF-β 1, MCP-1 and CEACAM1 are cytokines that promote tumor growth, and VEGF can directly stimulate the endothelial cells to form vascular structures and provide adequate blood flow for tumor growth[16]; TGF-β 1 can promote epithelial mesenchymal transition of cells and enhance the movement performance of the cells[17]; MCP-1 and CEACAM1 have cell chemotaxis and adhesion effect, which can promote the chemotaxis, adhesion and localization of tumor cells to adjacent tissues, and facilitate the lesion metastasis[18]. IFN-γ and IL-2 are cytokines with anti-tumor effect, which are mainly from Th1 immune cells and can directly kill tumor cells by their biological effects[19,20]. Analysis of the change of above cytokine secretion in urine before and after chemotherapy showed that VEGF, TGF-β 1, MCP-1 and CEACAM1 secretion in urine decreased whereas IFN-γ and IL-2 secretion increased after chemotherapy, and VEGF, TGF-β 1, MCP-1 and CEACAM1 secretion in urine of experimental group after chemotherapy were significantly lower than those of control group whereas IFN-γ and IL-2 secretion were significantly higher than those of control group. This means that chemotherapy can decrease the pro-tumor cytokine secretion and increase the anti-tumor cytokine secretion in bladder cancer lesions to exert anti-tumor effect, and hyperthermia perfusion chemotherapy is better than routine perfusion chemotherapy to regulate cytokine secretion and inhibit tumor growth.

Above all, it can be concluded that hyperthermia perfusion chemotherapy combined with systemic chemotherapy is more effective than routine perfusion chemotherapy combined with systemic chemotherapy to adjust the expression of proliferation and invasion genes as well as the secretion of cytokines in bladder cancer lesions to inhibit cancer cell proliferation and invasion.
References


