Effect of gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization on serum indexes in patients with hepatocellular carcinoma

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ABSTRACT

Objective: To study the effects of Gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization on serum indexes in patients with hepatocellular carcinoma. Methods: 90 cases of hepatocellular carcinoma patients were enrolled and randomly divided into two groups. Observation group received gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization, control group received gemcitabine conventional perfusion chemotherapy combined with carboplatin chemotherapy embolization. Malignant biological indicators of serum and liver tissue apoptosis regulation of gene expression of the two groups were compared. Results: (1) Serum malignant biological indicators: serum DKK1, TK1, HIF-1 alpha mRNA and protein content of the observation group were lower than that of the control group; (2) Promoting apoptosis gene: MTS1 in liver tissue, Caspase 3 and Bax mRNA and protein contents of the observation group was higher than that of the control group; (3) Apoptosis suppressor genes: liver cancer tissues Plk1, Bcl - 2 and Survivin mRNA and protein contents of the observation group was higher than that of the control group. Conclusion: Gemcitabine hot perfusion chemotherapy plus carboplatin chemotherapy embolism helps to inhibit tumor biological behavior, induce liver cancer cells apoptosis, and it is an ideal treatment for primary liver cancer.

1. Introduction

Primary Liver Cancer is a kind of deadly malignant tumor. Because of its high recurrence and low survival rate, it has been one of the focuses of clinical researches these years. Hepatic artery infusion chemotherapy combined with embolization is the most common interventional treatment, which can make chemotherapeutic drugs kill hepatocyte and block blood supply effectively. Hyperthermia is a new treatment method developed in recent years. Combined with perfusion chemotherapy, heat perfusion chemotherapy can induce hypoxic necrosis of cancer cells. In following research, we analyzed the effects of Gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization on serum indexes in patients with hepatocellular carcinoma.

2. Materials and methods

2.1. General materials

70 cases of patients with hepatocellular carcinoma in our hospital from June 2012 to December 2013 were enrolled. All patients were diagnosed of hepatocellular carcinoma. Understanding the treatment risks and research matters, they signed informed consents. According to different treatments, they were randomly divided into two groups, each with 35 cases. Observation group, including 25 males and 10 females of ages (63.45±8.27), received gemcitabine heat perfusion chemotherapy combined with carboplatin...
chemotherapy embolization. Control group, including 23 males and 12 females of ages (63.89±8.34), received conventional gemcitabine perfusion chemotherapy combined with embolization. There were no statistical differences between the two groups' general data (P>0.05).

2.2. Treatment methods

Observation group received gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization. Details were as follows: we used seldinger method to puncture through femoral artery, infused contrast agents to make sure the blood supply by using hepatic artery catheterization; and the supply vessels of tumors were inserted and treated with gemcitabine heat perfusion chemotherapy. Mixed 1 000 mg/m² gemcitabine with 1 200 mL normal saline, and then perfuse into tumor thermosterapeutic machine. The temperature was set at 51 °C, and the rate was 0.5-0.8 mL/s. When the gemcitabine heat perfusion chemotherapy was done, we mixed 200 mg/m² gemcitabine, 200 mg/m² carboplatin and 10 mL ultra-fluid lipiodol and treated with hepatic arterial embolization. Control group received conventional gemcitabine perfusion chemotherapy combined with embolization. Details were as follows: Mixed 1 000 mg/m² gemcitabine with 1 200 mL normal saline, and then perfuse into hepatic artery. The method of embolization was the same as observation group.

2.3. Determination methods

2.3.1. The mRNA content detection in HCC tissues and blood samples

Collected samples of blood and HCC tissues, injected in Trizol lysate and extracted total RNA and immediately synthesized into the first atrand of cDNA by reverse transcription reaction; and then amplified DKK1, TK1, HIF-1 , MTS1, Caspase-3, Bax, Plk1, Bcl-2, Survivn and β-actin through fluorescence ration PCR. Real-time PCR showed that the mRNA contents of observation group serum DKK1, TK1, HIF-1 were measured by ELISA.

2.3.3. The protein content detection in serum

Blood samples were centrifuged at 3 000 rmp for 10 min and contents of DKK1, TK1, HIF-1 were measured by ELISA.

2.4. Statistical methods

SPSS18.0 statistical software was used to input and analyze data, and counted data were measured by t-test. Results with P<0.05 were considered to be statistically significant.

3. Results

3.1. Serum hepatocellular carcinoma biological index

Real-time PCR showed that the mRNA contents of observation group serum DKK1, TK1 and HIF-1 are (25.59±3.45), (36.67±5.49) and (33.18±4.95) respectively, which are lower than the mRNA contents of control group serum (100.00±17.78), (100.00±15.91), (100.00±16.37). The difference has statistical significance (P<0.05) (Figure 1).

3.2. The promoting apoptosis gene in HCC tissues

Real-time PCR showed that the mRNA contents of observation group HCC tissues MTS1, Caspase-3 and Bax are (213.09±27.85), (226.88±31.52) and (249.41±31.46) respectively, which are higher than the mRNA contents of control group HCC tissues (100.00±15.92), (100.00±14.36), (100.00±16.24). The difference has statistical significance (P<0.05) (Figure 2).
tumors. When the local temperature of tumors was up to 40-46 °C, Hyperthermia is considered as a new treatment for malignant tumors. Secreted tumor necrosis factor (TNF) can interfere with cell cycles, but also induce hypoxia. The hepatoma cells' degree of malignancy is high, and its proliferation, angiogenesis and invasion are key factors in recurrence and metastasis of tumor. The evaluation of hepatic carcinoma malignant behavior will be useful to the prognosis. Dickkopf1 (DKK1), a kind of secretory diabetes, which includes a signal peptide sequence and two cysteine conserved domains. DKK1 carboxyl terminal can interact with LRP6 domain and promote the proliferation and metastasis of hepatoma cells; and TK1 can catalyze reversible transformation of the thymus cytosine deoxynucleotidyl to phosphate deoxynucleotidyl, which will induce proliferation and metastasis of hepatoma cells. HIF-1 is a kind of transcription factor, which can integrate with HRE in COX2 to start the transcriptional process. It can effectively express downstream platelet growth factor, basic fibroblast growth factor and epidermal growth factor, which will promote local neovascularization in tumors. By comparing the indexes of serum malignant behavior of two groups of patients, the study found that the mRNA and protein contents of DKK1, TK1 and HIF-1 in observation group patients’ serum are lower than the control group’s, which proved the inhibition effects of gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization on proliferation, metastasis and angiogenesis of hepatoma cells.

The change of malignant biology behavior index in hepatocellular carcinoma serum is closely related to viability of hepatoma cells. The hepatoma cell activity grows stronger, the malignant biology behavior changes faster. The viability of hepatoma cells is regulated by promoting apoptosis gene and inhibitor of apoptosis gene. Multiple tumor suppressor 1 (MTS1), which is also called p16 gene, is a member of cyclin-dependent kinase inhibiting protein gene family. It can integrate competitively with cyclin D1, and CDK4 will have negative regulation on cell cycle. The increase of MTS1 gene expression will promote apoptosis, and which will cause malignant proliferation of cells when MTS1 has mutations. Caspase family is a member of promoting apoptosis gene whose function is clearest. It includes 14 members from Caspase1 to Caspase14, which have specific regulatory function on the start up of apoptosis and its carry-out. Caspase-3 is an effector molecule of apoptosis in Caspase family. It can integrate protein to complete endonuclease reaction with DNA cAMP dependent protein kinase and sterol regulatory element-1(SRE-1), so that the cells can join apoptosis process directly.

Bax has high homology with anti-apoptosis gene Bcl-2, and it has specific apoptosis-promoting effect, which realized by hetero dimer formed from Bax and Bcl-2. In the malignant process, low Bax expression will weaken ability of Bax integrates with Bcl-2, which inhibit apoptosis process. Through analyzing the expression of promoting apoptosis gene in hcc tissues, we can know that mRNA and protein contents of p16, Caspase-3 and Bax in observation group are higher than control group’s. That is to say, gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization is helpful to induce the expression of promoting apoptosis gene and induce apoptosis of hepatoma cells.

Except for above promoting apoptosis gene, inhibitor of apoptosis gene also takes part in the regulation of hepatoma cells vitality. Polo-like kinase 1 (Plk1), which is a member of mitotic serine/threonine kinases, is a promoting proliferation molecular discovered

Figure 2. The effect of gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization on the promoting apoptosis gene in HCC tissues. *P<0.05 compared to control.

3.3. The inhibitor of apoptosis gene in HCC tissues

Real-time PCR showed that the mRNA contents of observation group HCC tissues Plk1, Bcl-2 and Survivn are (21.45±3.42), (33.39±5.03) and (29.04±3.49) respectively, which are lower than the mRNA contents of control group HCC tissues (100±11.91), (100±14.12), (100±17.64). The difference has statistical significance (P<0.05). And ELISA showed that the protein contents of observation group HCC tissues Plk1, Bcl-2 and Survivn are (28.48±4.18), (31.18±4.77) and (22.48±3.49) respectively, which are lower than the protein contents of control group HCC tissues (100±11.91), (100±14.12), (100±17.64). The difference has statistical significance (P<0.05) (Figure 3).

Figure 3. The effect of gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization on the inhibitor of apoptosis gene in HCC tissues. *P<0.05 compared to control.

4. Discussions

Hyperthermia is considered as a new treatment for malignant tumors. When the local temperature of tumors was up to 40-46 °C, the metabolic rate of cancer cell will increase, which will result in hypoxic necrosis. The gemcitabine heat perfusion chemotherapy will not only interfere with cell cycles, but also induce hypoxia. The hepatoma cells' degree of malignancy is high, and its proliferation, angiogenesis and invasion are key factors in recurrence and metastasis of tumor. The evaluation of hepatic carcinoma malignant behavior will be useful to the prognosis. Dickkopf1 (DKK1) and thymidine kinase (TK1) are discovered recently closely related with proliferation and metastasis of hepatic carcinoma. DKK1 is a kind of secretory diabetes, which includes a signal peptide sequence and two cysteine conserved domains. DKK1 carboxyl terminal
in recent years. Plk1 is high conservative in mammals and directly takes part in the regulation of cell cycles. In the cell cycle of G0, G1 and S, the expression level of Plk1 was low; the expression started to rise in G2 and reached the peak in M. The expression of Plk1 is synchronized to variation of cell cycles, and when we use siRNA to suppress the expression of Plk1, the proliferation of cells was significantly inhibited. Bcl-2 is one of apoptosis modulating proteins through mitochondrial pathway, which plays a specific role in inhibiting apoptosis and promoting proliferation. The realization of the effect relies on formation of Bcl2-Bcl-2 homo-dimer. The Bcl-2 in malignant tumor cells increased expression and forms homo-dimer, and the apoptosis resistant ability of cells improved. Survivin is a new kind of inhibitor of apoptosis protein. The gene analysis showed that promoter region of Survivin includes cell cycles dependent domain and cell cycles homologous domain. The analysis from proteomics showed that there are BIR domains in the n-terminal of Survivin, which include amino acid residues of Trp-, Pro-, Cys-, and it can directly inhibit promoting apoptosis molecular Caspase. Through analyzing the expression of inhibitor of apoptosis gene in hcc tissues, we can know that mRNA and protein contents of Plk1, Bcl-2 and Survivin in observation group are higher than control group’s. That is to say, gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization is helpful to inhibit the expression of anti-apoptosis gene and lower the anti-apoptosis activities of hepatoma cells.

In a word, gemcitabine heat perfusion chemotherapy combined with carboplatin chemotherapy embolization, which is helpful to inhibit biological behavior of tumor, induce apoptosis of hepatoma cells, is an ideal therapy of primary liver cancer.

References


