Effect of the combination treatment of high-intensity focused ultrasound and cryocare knife in advanced liver cancer

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Summary

Purpose: To analyze the effect of the combination treatment of high-intensity focused ultrasound (HIFU) and cryocare knife in advanced liver cancer.

Methods: 74 patients with advanced liver cancer were divided into the observation group (40 cases) and the control group (34 cases). Patients in the control group were treated with simple transcatheter arterial chemoembolization (TACE), while those in the observation group were given the combination treatment of HIFU and cryocare knife. Therapeutic effects were compared.

Results: The total effective rate of the observation group was significantly higher than that of the control group (p<0.05). After treatment, both groups showed decreased level of AFP and increased level of ALT. Besides, the observation group had significantly lower levels of AFP and ALT than the control group (p<0.05). Comparison of albumin levels before and after treatment revealed no difference between groups. After treatment, both groups showed increased percents of CD3+ and CD4+ T lymphocytes and natural killer (NK) cells with the observation group having obviously higher percents than the control group (p<0.05) After treatment, CD8+ T lymphocyte of the observation group reduced, while that of the control group increased (p<0.05). After treatment, both groups showed decreased levels of hyaluronic acid (HA), laminin (LN), collagen (IV-C) an III procollagen (PCIII), and the levels of the observation group were obviously lower than those of the control group (p<0.05). Both groups were followed with a median follow-up time of 25.0 months. The observation group had significantly more prolonged median survival time, decreased recurrence rate and improved survival rate (p<0.05).

Conclusion: The combination treatment of HIFU and cryocare knife can improve the short and long-term treatment outcome of advanced liver cancer.

Key words: advanced liver cancer, cryocare knife, high-intensity focused ultrasound, transcatheter arterial chemoembolization

Introduction

Currently, 5-year survival rate of advanced liver cancer is less than 30% [1], and how to prolong patient survival is a current research hotspot. TACE is often applied for the treatment of advanced liver cancer and has positive effect on shrinking tumor volume and reducing tumor cell activity [2]. However, TACE is not radical treatment. Recent recurrence rate after TACE is high, so combination with other treatments is necessary for enhancing the overall treatment effect [3]. HIFU, as a new noninvasive treatment method, takes advantage of the excellent characteristics of ultrasound to focus energy on liver tumor and generate instantaneous high-temperature effect, so that the tumor target tissue suffers coagulative necrosis, realizing noninvasive tumor resection of [4]. Cryocare knife is a kind of non-invasive cryosurgery device, which can destroy the normal metabolism of tumor tissue by local ultralow temperature and lead to irreversible death of tumor.
[5]. HIFU and cryocare knife have been applied to various tumor treatments [6]. This study has combined HIFU and cryocare knife to treat advanced liver cancer, analyzed its short and long-term effects and provided references for the selection of reasonable therapy in clinical practice.

Methods

General information

Seventy-four patients who were diagnosed with advanced liver cancer and received treatment for the first time from Jan 2013 to Jan 2016 in our hospital were consecutively enrolled. Inclusion criteria: (1) Patients with confirmed advanced liver cancer by biopsy of hepatic tissue; (2) The patients had no operative indications; (3) The patients had treatment indications of TACE, HIFU and cryocare knife; (4) medical history data were complete. Exclusion criteria: (1) severe impairment of liver function; (2) history of severe cardiovascular and cerebrovascular disease within 3 months; (5) primary surgery within 1 month; (4) coagulation disorders and diseases of the immune system; (5) Primary malignancies of other organs; (6) serious infections and trauma.

This research has been approved by Ethics Committee of our hospital and patients and their families provided written informed consent. According to different therapy methods, patients were divided into the control group (34 cases) and the observation group (40 cases).

The control group included 21 males and 13 females with mean age 58.3±7.6 years (range 45-70). The range of tumor diameter was 1.0 to 4.5 cm (mean 2.8±1.2). The number of tumor lesions was 1-3 (mean 1.5±0.3). According to TNM stage, there were 15 cases with IIb stage, 12 cases with III stage and 9 cases with IV stage.

The observation group included 23 males and 17 females with mean age 59.7±7.8 years (range 47-72). The range of tumor diameter was 1.3-4.8 cm (mean 2.9±1.4) and the number of tumor lesions ranged from 1 to 3 (mean 1.4±0.5). According to TNM stage, there were 12 cases with IIb stage, 16 cases with III stage and 12 cases with IV stage. The baseline data of the two groups were comparable.

Treatment methods

The patients in the control group were given TACE treatment, and details were as follows: Coeliac artery and hepatic arterial angiography were performed after femoral artery puncture and encheiresis by Seldinger technology [3] to identify the main ultra selective tumor blood supply artery. Five to ten ml of mixed chemotherapy liquid (8 ml iodized oil + 750 mg fluorouracil+40 mg fkomrubicin) were injected slowly. The tumor blood supply vessels were oppressed using gelatin sponge. Imaging evaluation was conducted two weeks after treatment. Patients with poor outcome were to be evaluated every four weeks.

Patients in the observation group received combination treatment of HIFU and cryocare knife, and details were as follows: HIFU, a kind of focused ultrasound oncotherapy system, could locate the tumor target section and determine the therapeutic area of every layer automatically according to MRI and ultrasonography (US) results. The probe could attach tumor tissue of each layer from far to near until the tumor tissue suffers coagulation necrosis. In the process of treatment, computer image processing system calculated the treatment effect in real time according to US of the target section and change of tissue echogenicity, and timely adjusted the therapeutic dose of US during the process.

Cryoablation knife cryoaulation: before the operation, tumor location, entry point and entry direction were confirmed with the aid of CT scanning. Lidocaine was used for local anesthesia and intensified anesthesia. Patients were lying on the unaffected side. Cryoablation knife which matched tumor size was selected. After sterilization and drape, skin was cut from the reserved puncture point and cryoaulation knife was placed to the expected position under CT guidance. Argon was introduced (knife point temperature was cooled below -140°C). Cryoablation was kept for 10-15 min (specific time depended on the tumor size). The ideal cryoaulation range was over the tumor margin. Argon was stopped to import helium hot medium. Temperature recovered to 20-30°C. The above thermocycling was repeated several times until final rewarming was finished. Local bandage, hemostasis and electrocardiograph monitoring were used.

Observation methodology

Therapeutic effects were compared. According to standards set in 1.1 version of Response Evaluation Criteria in Solid Tumors (RECIST), complete remission (CR) refers to dissapearance of all target lesion(s) and pathology suggests short diameters of all lymph nodes are reduced to <10mm; partial response (PR) means that the diameter sum of target lesion(s) are at least 30% less than the baseline; progressive disease (PD) means that the diameter sum of a target lesion is increased by at least 20%, and the absolute value of diameter sum is increased by at least 5mm, or new lesion(s) is (are) found; stable disease (SD) indicates a situation between PD and PR. Effective rate= (CR+ PR+ SD) / Cases ×100%.

Serum AFP and liver function tests (ALT and albumin level) before treatment and 3 months after treatment were compared.

The numbers of T lymphocyte and NK cells before treatment and 3 months after treatment were com-
pared. Two ml fasting venous blood was collected, and monoclonal antibodies of CD3 +, CD4 +, CD8 +, CD56 were added after heparin anticoagulation. One ml of red blood cell dissolved solution (preheated at 37°C) was added after incubation at room temperature. After complete dissolution, flow cytometry was performed to detect CD3 +, CD4 +, CD8 + lymphocytes and the percentage of NK cells (CD56).

Fibrosis before treatment and 3 months after treatment was compared. Two ml fasting venous blood was collected and the supernatant was extracted after centrifugation at 2000g for 15 min. Hyaluronic acid (HA), laminin (LN), collagen (IV-C) and III procollagen (PC III) levels of the two groups after treatment were tested by radioimmunoassay, and Sn-695B-type smart γ gauge (No.1 Ri Huan Device, Shanghai Institute of Nuclear Research) and its corresponding kits were applied.

The complications rate, median survival, recurrence rate and overall survival rate were followed up and compared. Patients were followed-up for 5-40 months (median 25).

Statistics
Software SPSS 23.0 was used to process and analyze data which were presented as mean±standard deviation. For comparison between groups independent t sample was used and for intra-group comparison paired t test was performed. Enumeration data were presented as percents and comparison between groups was performed using x² test. Ranked data were detected by nonparametric rank-sum test. For survival the Kaplan-Meier method was performed and differences were compared with log-rank test. A p value<0.05 was considered to be statistically significant.

Results

Comparison of treatment efficacy
The total effective rate of the observation group was considerably higher than that of the control group (p<0.05; Table 1).

Comparison of serum AFP and liver function tests
After treatment, both groups showed decreased level of AFP and increased level of ALT. Of note, the observation group had significantly lower levels of AFP and ALT than the control group (p<0.05). Comparison of albumin levels before and after treatment revealed no difference (Table 2).

Comparison of T lymphocytes and NK cells
After treatment, both groups showed increased proportions of CD3+ and CD4+ T lymphocytes and NK cells. Of note, the observation group had obviously higher proportion than the control group (p<0.05). After treatment, CD8+ T lymphocytes of the observation group reduced, while those of the control group increased (p<0.05; Table 3).

Comparison of HA, LN, IV-C and PCIII
After treatment, both groups showed decreased levels of HA, LN, IV-C and PCIII, and the levels of the observation group were considerably lower than those of the control group (p<0.05) (Table 4).

Table 1. Comparison of treatment efficacy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>CR n (%)</th>
<th>PR n (%)</th>
<th>SD n (%)</th>
<th>PD n (%)</th>
<th>Effective rate n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>40</td>
<td>7(17.5)</td>
<td>20(50.0)</td>
<td>7(17.5)</td>
<td>6(15.0)</td>
<td>34(85.0)</td>
</tr>
<tr>
<td>Control group</td>
<td>34</td>
<td>2(5.9)</td>
<td>10(29.4)</td>
<td>10(29.4)</td>
<td>12(35.3)</td>
<td>22(64.7)</td>
</tr>
<tr>
<td>Z/x²</td>
<td></td>
<td>8.424</td>
<td></td>
<td></td>
<td></td>
<td>4.112</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.038</td>
</tr>
</tbody>
</table>

CR: complete remission, PR: partial response, SD: stable disease, PD: progressive disease

Table 2. Comparison of serum AFP and liver function tests

<table>
<thead>
<tr>
<th>Groups</th>
<th>AFP(μg/L)</th>
<th>ALT(U/L)</th>
<th>Albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>Observation group</td>
<td>852.6±52.7</td>
<td>423.6±45.7</td>
<td>84.7±12.2</td>
</tr>
<tr>
<td>Control group</td>
<td>846.2±62.3</td>
<td>567.4±52.3</td>
<td>83.2±12.5</td>
</tr>
<tr>
<td>t</td>
<td>0.126</td>
<td>4.628</td>
<td>0.137</td>
</tr>
<tr>
<td>P</td>
<td>0.924</td>
<td>0.035</td>
<td>0.867</td>
</tr>
</tbody>
</table>

AFP: alpha fetoprotein, ALT: alanine transaminase
Comparison of long-term survival outcomes

No patient in both groups suffered serious complications like serious infection, bleeding, fever, jaundice and diarrhea and their condition was stable after symptomatic treatment for the complications. In the observation group median survival time was prolonged and the recurrence rate decreased compared with the control group. Differences were statistically significant (p<0.05) (Table 5).

Discussion

By taking advantage of the favorable directivity, penetrability and focusability of ultrasound, HIFU successfully focuses low energy ultrasound on deep lesions, generating instant high temperature effect and cavitation effect, and promoting irreversible coagulation necrosis of tumor tissue [7]. Cryocare knife can kill tumor cells by physical trauma resulting from freezing the water in tumor cells [8]. Recent researches show that HIFU treatment contributes to enhancement of cancer patients’ immunity [9], and cryocare knife also has positive immunomodulatory effect [10]. Post-treatment percents of CD3+ and CD4+ T lymphocytes and NK cells of the observation group were higher than those of the control group, but the percent of CD8+ T lymphocyte was reduced. This may be related to relatively great rate of killing tumor cells and alleviating the inhibition effect of body immunity system.

In China, most patients with liver cancer suffer from chronic hepatitis B and liver cirrhosis, and their fibrosis index level is pretty high. Research has found that the treatment of liver cancer is often accompanied by fluctuation of the fibrosis index level, which is correlated to the effectiveness of treatment [11]. When liver cancer occurs, hepatic stellate cells (HSC) will proliferate quickly, and transition to myofibroblast and synthesis of extracellular matrix are enhanced. Liver cancer cells’ invasion nearby or to other organs leads to

Table 3. Comparison of T lymphocyte and NK cell level

<table>
<thead>
<tr>
<th>Groups</th>
<th>CD3+ Before treatment</th>
<th>CD3+ After treatment</th>
<th>CD4+ Before treatment</th>
<th>CD4+ After treatment</th>
<th>CD8+ Before treatment</th>
<th>CD8+ After treatment</th>
<th>NK Before treatment</th>
<th>NK After treatment</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>34.5±6.6</td>
<td>55.8±7.2</td>
<td>21.4±5.3</td>
<td>39.5±4.2</td>
<td>25.6±4.5</td>
<td>22.8±3.6</td>
<td>25.7±4.3</td>
<td>33.5±3.7</td>
<td>0.325</td>
<td>0.765</td>
</tr>
<tr>
<td>Control</td>
<td>35.6±7.2</td>
<td>46.3±7.5</td>
<td>22.3±5.5</td>
<td>35.4±4.6</td>
<td>25.3±4.6</td>
<td>28.7±3.9</td>
<td>26.6±4.2</td>
<td>30.6±3.4</td>
<td>0.274</td>
<td>0.786</td>
</tr>
</tbody>
</table>

Table 4. Comparison of fibrosis index (ng/ml)

<table>
<thead>
<tr>
<th>Groups</th>
<th>HA Before treatment</th>
<th>HA After treatment</th>
<th>LN Before treatment</th>
<th>LN After treatment</th>
<th>IV-C Before treatment</th>
<th>IV-C After treatment</th>
<th>PCIII Before treatment</th>
<th>PCIII After treatment</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>356.4±52.6</td>
<td>195.7±35.4</td>
<td>234.6±54.7</td>
<td>125.8±26.4</td>
<td>176.4±24.3</td>
<td>93.7±10.9</td>
<td>356.7±54.6</td>
<td>105.8±21.7</td>
<td>0.524</td>
<td>0.569</td>
</tr>
<tr>
<td>Control</td>
<td>348.7±54.5</td>
<td>261.8±37.6</td>
<td>225.7±35.2</td>
<td>151.9±27.6</td>
<td>165.9±23.8</td>
<td>131.2±15.8</td>
<td>352.9±35.7</td>
<td>171.5±28.6</td>
<td>0.346</td>
<td>0.725</td>
</tr>
</tbody>
</table>

Table 5. Comparison of long-term survival outcomes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Median survival (months)</th>
<th>Recurrence n (%)</th>
<th>Survival n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>40</td>
<td>16.4</td>
<td>15 (37.5)</td>
<td>28 (70.0)</td>
</tr>
<tr>
<td>Control</td>
<td>34</td>
<td>10.2</td>
<td>21 (61.8)</td>
<td>16 (47.1)</td>
</tr>
</tbody>
</table>

Comparison of long-term survival outcomes

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HSC apoptosis, increase of collagenase activity, increase of collagen degradation and rising level of fibrosis index in blood [12]. This study has concluded that HA, LN, IV-C and PCIII levels of the observation group after treatment were lower than those of the control group, suggesting that the combination treatment of HIFU and cryocare knife can effectively kill hepatoma cells and restrain its injury to normal HSC, reduce collagen degradation and prevent fibrosis products from entering into the bloodstream. Therefore, the fibrosis index in circulating blood has inner link with the treatment effect on liver cancer, which indirectly demonstrates the effectiveness of the combination treatment in the observation group.

Research results have shown that the total effective rate of the observation group was higher compared with the control group. The total effective rate of the observation group was 85.0%, CR 17.5%, PR of 50.0%. AFP and ALT of the observation group after treatment were lower than those of the control group. Comparison of albumin revealed no differences. AFP level is an important indicator that influences survival prognosis. Its increase suggests that both therapeutics can damage liver function, but the degree of injury in the observation group is milder and albumin level might be correlated with exogenous supplementation. Patients in both groups suffered no serious complications, suggesting the treatment is safe. The median survival time of the observation group was about 16.4 months, the recurrence rate about 37.5%, and the increased survival rate about 70.0%. All the above results suggest that the combination treatment of HIFU and cryocare knife can effectively kill liver cancer cells, delay the proliferation and metastasis from the residual cancer cells, and offers a relatively good advantage in optimizing the long-term outcomes of advanced liver cancer.

Conflict of interests

The authors declare no conflict of interests.

References