Original Article
The efficacy of $^{99m}$Tc-MIBI imaging in $^{125}$I seed implantation treatment of rabbit VX2 transplanted liver cancer

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Abstract: Purpose: To evaluate the efficacy of $^{99m}$Tc-MIBI imaging in the evaluation of $^{125}$I radioactive particle implantation for treatment of rabbit VX2 transplanted liver cancer. Methods: Twelve New Zealand white rabbit VX2 liver cancer models were successfully prepared by tumor cell suspension method and randomly divided into a control group and treatment group. The treatment group received $^{125}$I particle implantation according to the TPS plan, and the control group received the same number of hollow particle implantation. $^{99m}$Tc-MIBI imaging was performed before and 7 d, 14 d, and 28 d after implantation. The target lesion (target, T) and normal liver tissue (nontarget, N) were determined by region of interest (ROI) technique. Radioactivity count was used to calculate the $^{99m}$Tc-MIBI uptake ratio (target-to-nontarget ratio, T/N) between the target lesion and normal liver tissue, thereby obtaining early ratio (ER) and delayed ratio (DR), respectively. The retention index (RI) was calculated. The mice were sacrificed after 28 days for histopathologic observation. Results: The T/N ratio, ER, and DR showed no statistical changes following the implantation time in the control group. In the treatment group, ER and DR gradually decreased after implantation of $^{125}$I seeds ($P < 0.05$). There was no significant difference in RI during different observation times between the treatment group and the control group. Compared with the treatment group, RI exhibited no statistical difference between before and 7 d, 7–14 d, and 14–28 d after implantation ($P > 0.05$). Conclusion: This method has value in evaluating the efficacy of $^{125}$I particles treatment of rabbit VX2 transplanted liver cancer. The T/N ratio is independent of the tumor diameter, but is related to the blood perfusion and metabolic state of the tumor. Implantation of $^{125}$I particles into the rabbit transplanted liver cancer can effectively inhibit tumor growth, thus is a safe and effective method.

Keywords: $^{99m}$Tc-MIBI imaging, liver cancer model, VX2 tumor, $^{125}$I particle, interstitial implantation, efficacy evaluation

Introduction

Primary liver cancer is a disease that seriously endangers human health and life. In China, more than 70% of clinical liver cancer cases are in advanced stage when diagnosed. The radioactive $^{125}$I interstitial implantation therapy has become an indispensable part of liver cancer treatment [1]. $^{125}$I seed implantation has been used for the treatment of tumors for more than 20 years. It is mainly used for the treatment of prostate cancer [2-7]. There are fewer applications for the treatment of liver cancer or liver metastasis. Martinez-Monge adopted $^{125}$I particles for liver metastases and found that the control rate of liver lesions and the survival rate of patients were significantly higher than in a control group with no obvious complications [8, 9]. Therefore, it is of great clinical significance to investigate whether $^{125}$I particles can be used alone or in combination in the treatment of liver cancer. Compared with other traditional imaging examinations such as CT and MRI, $^{99m}$Tc-MIBI tumor-positive imaging has the advantage of reflecting the function and metabolic activity of tumor cells, and is an important reference in evaluating the efficacy of $^{125}$I particles treatment in liver cancer [10,
This study aimed to explore the use of $^{99m}$Tc-MIBI tumor-positive imaging on the therapeutic effect of radioactive $^{125}$I particles in liver cancer.

**Materials and methods**

**Experimental animals and grouping**

12 healthy New Zealand white rabbits weighing 2.7 kg to 3.2 kg were purchased from Qingdao Institute of Pharmaceutical Sciences. The VX2 tumor tissue source used in the experiment was purchased from the Department of Interventional Radiology of Beijing Cancer Hospital. The tumor tissue was frozen in liquid nitrogen for half a year. The $^{125}$I particles were produced by Beijing Zhibo Hi-Tech Biotechnology Co., Ltd. The initial equivalent radioactivity of the single-sealed seed source was 0.7 mCi (25.9 MBq).

16 experimental rabbits were used to establish rabbit a VX2 liver transplantation tumor model by CT-guided tumor cell suspension puncture implantation. Two weeks after tumor implantation, 12 tumor models were selected from the successfully modeled rabbits. The standard contained a tumor diameter of about 2 cm and no complications. Twelve animal tumor models were randomly divided into a control group and treatment group, which was implanted by empty particles and 0.7 mCi $^{125}$I radioactive particles according to the TPS treatment plan.

**TPS treatment plan**

TPS software was jointly developed by Beijing University of Aeronautics and Astronautics and Guangzhou Aerovision Medical Imaging Technology Co., Ltd. The CT arterial phase enhancement images of the rabbits in the treatment group were introduced into the TPS software, and the region of interest was manually outlined on the tumor area layer by layer. Then, the simulated implantation of the particles was carried out, and the particle implantation pitch was 1 cm to obtain a particle distribution map. Considering the activity of the combined particles (the initial equivalent radioactivity of the $^{125}$I particles in this experiment was 0.7 mCi) and the prescription dose (120 Gy), the tumor dose-volume histogram was generated by the TPS plan after particle implantation. Finally, the $^{125}$I particle implantation therapy for tumor was generated.

**Particle implantation and post-implantation quality verification**

The rabbits were routinely sterilized and placed in a supine position on a special fixation plate. After CT scan, the TPS particle implantation plan was used as a guide to select the appropriate level, puncture point, needle direction, and needle depth for $^{125}$I particle implanting. CT scan was performed immediately after the particle implantation to verify whether the particle implantation was consistent with the TPS treatment plan. 400,000 IU/Kg of penicillin was intramuscularly injected daily for 3 consecutive days.

**$^{99m}$Tc-MIBI imaging method**

$^{99m}$Tc-MIBI labeling rate > 90%. The imaging instrument is the American Hawkeye SPECT/CT instrument, equipped with a low-energy universal collimator, with a peak energy of 140 Kev, a window width of 20%, and a matrix of 512 × 512. At the time of examination, the rabbit was placed in the supine position. The imaging agent $^{99m}$Tc-MIBI 185 MBq was injected into the ear vein. The liver early and delayed imaging was performed 15 minutes and 1 hour after the injection, and the collection count was 200 K.

**Image analysis:** Visual Reading Analysis: Two experienced nuclear medicine physicians determined whether the radioactive uptake of the lesion was higher than the surrounding normal liver tissue based on the image color (grey) order.

Semi-quantitative analysis: The region of interest (ROI) technique was adopted to measure the radioactivity of the target site (target, T) and normal liver tissue (nontarget, N). Radioactivity count was used to calculate the $^{99m}$Tc-MIBI uptake ratio (target-to-nontarget ratio, T/N) between the target lesion and normal liver tissue, thereby obtaining early ratio (ER) and delayed ratio (DR), respectively. The retention index (RI) was calculated. $RI = (DR - ER)/ER × 100%$.

**Statistical analysis**

Statistical analysis was performed on SPSS17.0 software. The measurement data were presented as mean ± standard deviation and compared by t-test. $P < 0.05$ was considered to be significant.
**Results**

**Survival rate**

Among the 12 rabbits, the control group began to lose weight, had decreased activity and eating in 14 days after treatment, and all died within 28-60 days. In the treatment group, poor spirits and reduced eating were observed in 28 days after the implantation of the particles.

**Table 1.** Animal weight changes (kg, $\bar{X} \pm S$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>7 d after treatment</th>
<th>14 d after treatment</th>
<th>28 d after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.64±1.12</td>
<td>2.23±10.28</td>
<td>1.88±21.97</td>
<td>1.28±5.97</td>
</tr>
<tr>
<td>Treatment group</td>
<td>2.63±0.67</td>
<td>2.61±0.68</td>
<td>2.26±0.49</td>
<td>2.01±0.25</td>
</tr>
</tbody>
</table>

$\Delta P < 0.05$ and $\Delta\Delta P < 0.01$.

**Table 2.** ER changes before and after treatment (T/N ratio, $\bar{X} \pm S$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>7 d after treatment</th>
<th>14 d after treatment</th>
<th>28 d after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.59±2.12</td>
<td>2.61±1.28</td>
<td>2.68±1.98</td>
<td>2.67±1.97</td>
</tr>
<tr>
<td>Treatment group</td>
<td>2.58±0.67</td>
<td>2.18±0.68</td>
<td>1.90±0.49</td>
<td>1.61±0.25</td>
</tr>
</tbody>
</table>

$\Delta P < 0.05$ and $\Delta\Delta P < 0.01$.

**Table 3.** DR changes before and after treatment (T/N ratio, $\bar{X} \pm S$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>7 d after treatment</th>
<th>14 d after treatment</th>
<th>28 d after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.82±2.12</td>
<td>2.83±1.28</td>
<td>2.89±1.98</td>
<td>2.91±1.97</td>
</tr>
<tr>
<td>Treatment group</td>
<td>2.82±0.62</td>
<td>2.37±0.68</td>
<td>2.06±0.49</td>
<td>1.75±0.25</td>
</tr>
</tbody>
</table>

$\Delta P < 0.05$ and $\Delta\Delta P < 0.01$.

**Figure 1.** Seeds were implanted for 28 days in the control group with $^{99m}$Tc-MIBI imaging. A. Early phase. B. 1 hour delayed phase. The tumor is close to occupying the whole liver tissue, the radioactive uptake of the tumor edge tissue is increased, and the necrotic area of the tumor center is a sparsely distributed area.

**Discussion**

Clinically, liver cancer treatment can be divided into two categories: one is endovascular intervention, including hepatic arterial infusion chemotherapy (TAE), hepatic artery embolization (TAE), and transcatheter arterial chemembolization (TACE); the other is non-vascular minimally invasive, including percutaneous chemical ablation, percutaneous physical ablation, and internal irradiation of radionuclides. Since the 1970s, TACE has become the classic treatment, which can relieve the symptoms to a certain extent. Furthermore, some patients obtain the opportunity of second-stage surgery. However, most patients have recurrence after the disease has stabilized for 1 to 3 months due to the formation of collateral circulation. Liver cancer is less sensitive to external exposure, and the radical dose of external irradiation is
I seed implantation in VX2 transplanted liver cancer

much higher than the tolerated dose of normal liver tissue. The internal irradiation implantation treatment of the radionuclide is to implant the radioactive source into the tumor or surrounding infiltrated tissue, and kill the tumor cells by the radiation released by the decay. It can be performed in vitro or under the help of imaging techniques. This treatment can select the desired site for irradiation, so that the cancer cells can receive continuous radiation for a long time, while the dose of surrounding normal liver tissue is small, which exceeds the total radiation dose of the whole liver caused by external irradiation therapy and insufficient radiation dose of tumor tissue.

Clinically, the prescription dose of $^{125}\text{I}$ particle interstitial tissue implantation for the treatment of liver cancer is not agreed upon. It was shown that the efficacy is related to the prescribed dose. Zhang reported [12] that the prescription dose of $^{125}\text{I}$ particle implantation for liver cancer patients with portal vein thrombosis was 130 Gy, and no complications were observed during follow-up for 6 months. Chen indicated [13] that the prescription dose for $^{125}\text{I}$ interstitial implantation of primary liver cancer is 60 Gy–80 Gy, and considered that the effect of $^{125}\text{I}$ particles interstitial implantation for the treatment of liver cancer is not good. In this experiment, since the carrier of the tumor is a New Zealand white rabbit, the overall tolerance is relatively worse than that of humans. In order to reduce the incidence of radiation-related complications, a prescription dose of 120 Gy was selected.

$^{99m}\text{Tc}$-MIBI is a lipophilic cationic imaging agent that is concentrated in cells by the Na$^+$/H$^+$ anti-transport system into the mitochondria. The mechanism by which $^{99m}\text{Tc}$-MIBI is acquired by malignant tumors is not fully understood and may be related to: (1) The feature of MIBI (its cationic and lipophilic); (2) The transmembrane potential of cell membrane and mitochondrial membrane, and cellular metabolic activity; 3) Increased blood flow and capillary permeability due to high metabolism of tumor lesions [14]. The T/N ratio is an index for quantitative analysis of the $^{99m}\text{Tc}$-MIBI concentration degree in tumors, which can reflect the relative metabolic activity of tumor cells and is not susceptible to other factors. The early phase T/N ratio (ER) of $^{99m}\text{Tc}$-MIBI mainly reflects the amount of $^{99m}\text{Tc}$-MIBI in VX2 tumor cells, and the delayed phase T/N ratio (DR) mainly reflects the mitochondrial residue after P-glycoprotein clearance. The RI mainly quantitatively reflects the amount of $^{99m}\text{Tc}$-MIBI taken by tumor cells after removing blood perfusion factor.

In the present study, there was no significant difference in ER and DR between the control groups over time ($t = 0.076, P > 0.05$). After $^{125}\text{I}$ seed implantation in the treatment group, ER and DR gradually decreased at the observation time points ($P < 0.05$). It is suggested that the T/N ratio is independent of the tumor diameter, but is related to the blood perfusion and metabolic state of the tumor. The T/N ratio decreased after particle implantation treatment, indicating that the tumor metabolic activity decreased after implantation. There was no difference in the RI between the two groups, revealing that RI could not reflect a change of tumor activity.

Table 4. RI changes before and after treatment (%, $\bar{x} \pm S$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>7 d after treatment</th>
<th>14 d after treatment</th>
<th>28 d after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.03±0.32</td>
<td>8.02±1.28</td>
<td>8.06±1.98</td>
<td>8.05±1.97</td>
</tr>
<tr>
<td>Treatment group</td>
<td>8.05±0.67</td>
<td>8.07±0.68</td>
<td>8.06±0.49</td>
<td>8.04±0.25</td>
</tr>
</tbody>
</table>

Figure 2. Seeds were implanted for 28 days in the treatment group with $^{99m}\text{Tc}$-MIBI imaging. A. Early phase. B. 1 hour delayed phase. The tumor size was significantly reduced and the T/N ratio decreased compared with the control group.
In sum, interstitial implantation of $^{125}$I particles is safe and reliable for the treatment of rabbit VX2 transplanted liver cancer. $^{99m}$Tc-MIBI imaging has great application value in evaluating the efficacy of $^{125}$I interstitial implantation for the treatment of rabbit VX2 transplanted liver cancer. Compared with CT examination, $^{99m}$Tc-MIBI imaging mainly reflects the changes in the function and metabolic activity of tumor cells. It has great guiding significance in evaluating the efficacy of tumor radiotherapy [15, 16]. However, it cannot accurately reflect the exact size, morphology, and environment of tumors. $^{18}$F-PET/CT fusion imaging can take into account the tumor anatomy and functional metabolism, but it is expensive and difficult to popularize. $^{99m}$Tc-MIBI SPECT/CT fusion imaging is cheaper and more advantageous. It is an effective way to observe the efficacy of $^{125}$I particles in the treatment of liver cancer.

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Disclosure of conflict of interest

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References