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Lymph node mapping in rabbit liver cancer with nanocarbon and methylene blue injecta

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ABSTRACT

Objective: To discuss the value of lymph node mapping in rabbit liver cancer with nanocarbon and methylene blue injecta. **Methods:** Rabbit liver cancer model was established by transplanting VX2 cells with laparotomy in celiac planting method. Twenty Japan white rabbits were divided into two groups randomly. Each group had 10 rabbits. Lymph node mapping in two groups rabbit liver cancer were observed. Two groups rabbit liver cancer and local lymph nodes were removed. The number and location of local lymph nodes were recorded, and then the samples were obtained from both groups. **Results:** The lymph nodes dyed time was (100.50±29.92) s in nanocarbon group, and (11.20±4.18) s in methylene blue group with statistical significance between two groups (P=0.000). In the comparison of lymph node fading time, nanocarbon group was (2.22±0.74) h, methylene blue group was (1.63±0.54) h, nanocarbon group was longer than the methylene blue group, but without statistical significance (P=0.058). The accuracy was 87.5% (35/40) in methylene blue group, while, the nanocarbon group was 87.2% (34/39) , with statistical significance (P=1.000). **Conclusions:** Experimental results show that application of nanocarbon injection and methylene blue injection during resection of liver cancer and local lymph nodes in rabbit liver cancer model has obvious tracer function in liver cancer and lymphatic drainage. It can reduce the complexity and risk of the operation, and avoid the blindness in the process of traditional lymph node dissection surgery. Besides, they can effectively reduce the number of residual lymph nodes after operation. It can achieve the lymph node dissection more thoroughly, promptly, easily and safely.

1. Introduction

Primary carcinoma of liver (hereafter referred to as liver cancer) is one of the most life-threatening diseases, ranking fifth in malignancies and third in the caused of cancer-related death[1]. Except for liver transplant, surgical removal is still the preferred treatment tactic for patients with liver cancer. The reported 5-year recurrence rate after radical resection of liver cancer is up to 80% and the rate for small hepatocellular carcinoma ranges from 40% to 50%[2]. Previously, local lymph node metastasis was rarely included in the studies of treatments and prognostic factors of liver cancer[3]. Actually, 25%-50% lymph fluid of liver is drained into ductus thoracicus. Intrahepatic lymphatic system, extrahepatic lymphatic drainage and the distribution of lymph nodes are complex, which allow the metastasis of liver cancer[5,6].

In China, surgical removal is currently the preferred treatment for liver cancer, except for liver transplant. However, micrometastasis of liver cancer is difficult to identify and the clearance rate of locally metastatic lymph nodes is low, which causes recurrence after radical resection of liver cancer[7]. Regional lymph node metastasis

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staging is of importance for solid tumor grade and prognostic factor. Lymph node dissection is one of the vital steps in radical resection of solid tumors, while its role for liver cancer is still ambiguous. Rarely, lymph node metastasis is considered in the analysis of prognostic factors of liver cancer and the studies of local lymph node metastasis of liver cancer are limited, which is attributed to the shortage of prospective randomized controlled trials to verify that systematic lymph node dissection is conducive for patients with liver cancer. People have not reached the consensus over whether patients should undergo radical resection of liver cancer as well as lymph node dissection[8,9].

To solve the problems above, there is a trend that the tracer technique is applied to reduce blindness of lymph node dissection during the resection of liver cancer. In this study, we chose to transplant VX2 cells into Japan white rabbits with celiac planting method in order to simulate the growth pattern and blood supply of human liver cancer, which established the rabbit liver cancer model. The rabbits were divided into experimental group with nanocarbon injecta and control group with methylene blue injecta. The two tracers were both injected around the liver cancer intraoperatively to mark lymph nodes. This study is aimed to compare the efficiency and effectiveness of nanocarbon injecta and methylene blue injecta that trace the lymphatic drainage of rabbit liver cancer intraoperatively. Additionally, we also intend to investigate the way that nanocarbon injecta labels the metastatic lymph nodes of liver cancer and its feasibility of clinical application

2. Materials and methods

2.1. Experimental animal, cancer cell and model establishment

A total of 22 Japan white rabbits of either gender, aged 3–4 months and of 2.5–3.0 kg in weight, were enrolled in this study.

They were raised separately for 1 week before the experiment. Two healthy white rabbits were taken and injected into the muscle of their hind legs with 1.0 mL VX2 cell suspension of 1×10^6 /mL in cell count. The tumor-bearing rabbit model was established when spherical mass of approximately 2.0–2.5 cm in diameter could be palpable at the injection site. The masses were obtained and cut into much smaller masses of 1 mm×1 mm×1 mm in size. The remaining 20 rabbits were weighed and randomized divided into two groups with 10 rabbits for each group. One group is considered as the nanocarbon group and the other one the methylene blue group.

All the rabbits underwent laparotomy with abdominal median incision after anaesthesia. Tissue forceps were used to obliquely puncture where the left lobe of liver was thicker to form a blind track. Two masses were picked and crammed into the other end of the blind track before closing the abdominal cavity. Twenty one days after the implantation surgery, a spherical mass of 0.8–1.0 cm in diameter were formed within the left lobe of liver.

2.2. Operation procedure of nanocarbon group

The open surgery was performed through the initial approach after anesthesia. The nanocarbon injecta of 0.1 mL was slowly injected into 2–3 sites around the tumor within the left lobe of liver with 1 mL syringe.

It could be observed during the operational process that, promptly after the injection, the injection sites were stained into black and the region within 0.5–1.5 cm of the border of the tumor appeared black with the injecta diffusing into normal liver tissue.

Linear black staining appeared within the connective tissue of the hepatogastric ligament after roughly 1.5–2.0 min and simultaneously diffused into the hepatogastric ligament and hepatoduodenal ligament. Blackened lymph nodes could obviously seen along the linear black staining.

Simulated resection of liver cancer and lymph node dissection was performed after 30 min when black-stained lymph nodes had not changed significantly. The liver cancer was removed with 5 mm as its surgical margin. During the surgery, it can be easily seen that the region within 3–5 mm of the tumor was dyed black. The black-stained area in the shape of crab legs that centers on the tumor and radiates out in all directions is considered as the lymph vessels accompanied with the channels in the Glisson's capsule[10–15]. The local lymph nodes that were visible or dyed black around the liver were removed.

2.3. Operating procedure of methylene blue group

The numbers and sites of the removed lymph nodes around the liver, as well as their stain fading time were recorded for the two groups.

2.4. Pathological examination

The specimen of the liver cancer from the surgery was observed to see whether there was tumor capsule, satellites lesions, portal vein tumor thrombus and cancer cells within 1 mm around the surgical margin. Also, the specimen of the lymph nodes was examined to see if metastasis had occurred.

2.5. Statistical analysis

Statistical analysis was performed using the SPSS 11.01 for WINDOWS.

A *t* test was used to compare the number of lymph nodes, staining time and stain fading time between the nanocarbon group and the methylene blue group.

P value of less than 0.05 was considered significant. With regard to lymph nodes, a χ^2 test was applied to compare the results of detection rate, accuracy, sensitivity, specificity, false negative rate, false positive rate, positive predictive value and negative predictive rate within each group.

3. Results

3.1. Statistical results

A total number of 39 lymph nodes were dissected in the nanocarbon group with the mean number of detected lymph nodes per rabbit at 1.70±0.95. Pathologically, 16 lymph nodes were confirmed as metastatic and 23 as non-metastatic. There were 17 stained lymph nodes, with 14 as metastatic and 3 as non-metastatic, and 22 uncolored lymph nodes, with 3 as metastatic and 20 as non-metastatic. The accuracy was 87.2% (34/39).

A total number of 40 lymph nodes were dissected in the methylene blue group with the mean number of detected lymph nodes per rabbit at 1.40±0.70. Pathologically, 12 lymph nodes were confirmed as metastatic and 28 as non-metastatic. There were 14 stained lymph nodes, with 10 as metastatic and 4 as non-metastatic, and 26 uncolored lymph nodes, with 2 as metastatic and 24 as non-metastatic. The accuracy was 87.5% (35/40).

Totally, 11 indices (namely, detection rate, accuracy, sensitivity, specificity, false negative rate, false positive rate, positive predictive value, negative predictive rate, number of the detected lymph nodes, staining time and stain fading time) were analyzed to compare the two staining agents. The results showed that the staining time was (100.50±29.92) s for the nanocarbon group and (11.20±4.18) s for the methylene blue group, which was statistically significant (*P*=0.000). As for the stain fading time, it was (2.22±0.74) h for the nanocarbon group and (1.63±0.54) h for the methylene blue group. Clearly, the stain fading time of the nanocarbon group was longer than that of the methylene blue group. Nevertheless, this difference had no significance. Other indices were not significantly different.

3.2. Pathological examination results of liver cancer

It could be observed from the specimen of the liver cancer that the surgical margins for the two groups were grey-white, like "fish flesh", hard, well-defined and not encapsulated. The HE staining microscopy of the tissue slices showed that the cancer cells with mitotic figures were round, spindle or irregular and of nested or dispersed distribution. The poor-defined interstitial tissue with less connective tissue was infiltrated by lymphocytes and plasma cells (Figure 1). There were no cancer cells within 1 mm around the surgical margins of every specimen. The positive rate of cancer cells around the surgical margin was 0.

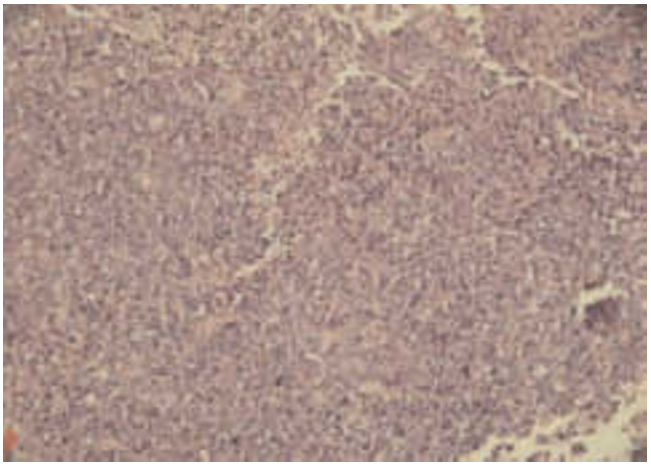

Figure 1. Liver cancer of nanocarbon and methylene blue injection experiment (HE×200).

3.3. Pathological examination results of lymph nodes

The pathological results of the dissected lymph nodes were showed in Figure 2.

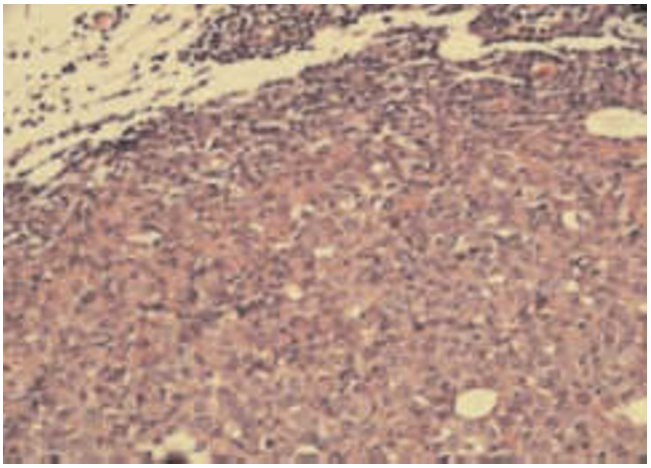

Figure 2. Lymph node infiltration by cancer (HE×400).

4. Discussion

In the experiments, the maximum and minimum number

of lymph nodes detected around the liver was 6 and 2, respectively. The stained lymph nodes were largely found around the common hepatic artery within the hepatogastric ligament, around the left gastric artery and within the hepatoduodenal ligament. When dissecting the lymph nodes for the methylene blue group, the operators found more difficulty. After injecting methylene blue, most of the lymph nodes were covered by the blue stained adipose tissue and the lymph nodes were close to the important channels, which made it time-consuming and laborious to identify and dissect the lymph nodes and also, had the potential risk of carelessly injuring the organs. By contrast, the lymph nodes were dyed black after injecting nanocarbon, while the adipose tissue around the lymph nodes was uncolored. The colors in sharp contrast shortened the duration of the surgery as the lymph nodes were easily identified.

It was proved by normality test that the numbers of the dissected lymph nodes of the two groups were normally distributed as the P values were both over 0.05. The homogeneity of variance was satisfied ($P>0.05$). And unpaired t test revealed that there was no significant difference ($P>0.05$). These analysis results indicate that both nanocarbon and methylene blue are effective as a tracer in detecting lymph nodes.

To eradiate the risks of being uncompleted and unsafe, it is imperative to convert the liver cancer resection and lymph node dissection from empirical and blind operations into standard and visible ones.

In this experiment, VX2 cells were implanted into peritoneal cavity to establish the rabbit liver cancer model, which simulated the clinical characteristics of patients with liver cancer. Operators tried to apply intraoperative tumor tracing technique under direct vision into radical liver cancer resection. Objective observation and statistical results of the two tracers have revealed that nanocarbon can precisely define the resection range of liver cancer and expose lymphatic drainage and lymph nodes.

The application of nanocarbon as a tracer is positively significant for the status quo of liver cancer resection and reduction of postoperative recurrence and residual metastatic lymph nodes. In addition to tracer, nanocarbon may carry anti-cancer drugs to be a medium of targeted therapy for liver cancer, which can be used for oriented chemotherapy targeted at residual and recurrent foci. This technique will pioneer a new path for clinically comprehensive treatment of liver cancer to improve the living standard of patients and prolong their life expectancy.

Conflict of interest statement

We declare that we have no conflict of interest.

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