# Original Article Brachytherapy with lodine-125 seeds strand for treatment of main portal vein tumor thrombi: an experimental study in a rabbit model

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Abstract: This study aims to establish an animal model of implanted main portal vein tumor thrombus (MPVTT) and to evaluate safety and efficacy of brachy therapy with lodine-125 (<sup>125</sup>I) seeds strand to treat MPVTT of rabbit. VX, tumor thrombus was implanted in main portal vein (MPV) of 32 New Zealand white rabbits. These rabbits were randomly divided into treatment group (Group T, T1-T16) and control group (Group C, C1-C16). <sup>125</sup>I seeds and blank seeds strand were implanted in MPV of rabbits in Group T and C, respectively. Changes of general condition, body weight and blood laboratory examination were monitored at every time point after procedure. 2 weeks later, 8 rabbits of each group were sacrificed for pathologic examination. The rest of rabbits were dissected postmortem, and therapeutic effects were evaluated on basis of multi-detector computed tomography and histopathology. Ki-67 labeling index (Ki-67 LI) and apoptosis index (AI) were compared between two groups. Overall survival period was recorded. At every time point after brachytherapy, more serious weight loss were detected in Group C. Results of liver function tests and blood cells counts showed no significant difference between two groups. Mean volume of tumor tissue within MPV were 565.40  $\pm$  220.90 mm<sup>3</sup> in Group T and 2269.90  $\pm$  437.00 mm<sup>3</sup> in Group C (P < 0.001). (Ki-67 LI) and AI were  $(4.14 \pm 1.84)$ % and  $(6.51 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % and  $(0.91 \pm 1.92)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in Group T, compared with  $(33.82 \pm 6.07)$ % in G, compared with (33.820.26)% in Group C, respectively (P < 0.001). Media survival time of rabbits were 39.50 ± 2.37 days in Group T and 27.38 ± 1.22 days in Group C, respectively (P = 0.001). In conclusion, injecting and suspensory fixing VX, tumor strip into MPV is a reliable method to establish MPVTT animal model. Brachytherapy with <sup>125</sup>I seeds strand was safe and effective to treat VX<sub>2</sub> tumor strand inoculated in the MPV of rabbit.

Keywords: Brachytherapy, Iodine-125 seed, VX, tumor, portal vein tumor thrombus

#### Introduction

Hepatocellular carcinoma (HCC) is a global health problem with increasing incidence [1]. About 12.5%-39.7% of patients with advanced HCC have portal vein invasion [2]. When the main portal vein (MPV) was involved by tumor thrombus, the patient's prognosis was extremely poor. Without treatment, the median survival time of these patients is only 2.7-4 months [2-4]. MPV tumor thrombus reduces the portal blood supply to hepatic parenchyma, increases the incidence of intrahepatic metastasis and aggravates portal hypertension. The therapeutic options for patients with HCC and MPV tumor thrombus are limited [1-4].

Systemic treatment with sorafenib, a multikinase inhibitor, has been shown to improve the overall survival of patients with advanced HCC complicated by macroscopic vascular invasion in randomized controlled clinical phase III trials [5, 6]. Unfortunately, the current cost of the drug precludes sorafenib from becoming a commonly used therapy for advanced HCC [7]. Radioembolization with transarterial infusion of yttrium-90 microspheres has been reported to treat HCC with portal vein thrombosis safely [8, 9]. But vttrium-90 microspheres have not been approved by FDA in our country. Although threedimensional conformal radiotherapy (3-DCRT) can provide an effective local control of tumor thrombus in portal vei [10-12], liver function impairment was not an uncommon issue presented in patients after 3-DCRT [12].

VX<sub>2</sub> carcinoma is an anaplastic squamous cell carcinoma derived from a virus-induced papil-





loma in wild rabbits [13]. Inoculation VX<sub>2</sub> carcinoma into rabbit liver has been used extensively as an animal model with hepatic hypervascular tumor for the study of HCC in human being. Recently, the safety and feasibility of brachytherapy with interstitial implantation of <sup>125</sup>I seeds to treat HCC has been demonstrated [14, 15]. We have demonstrated that the linear <sup>125</sup>I seeds strand brachytherapy is a safe and effective method for treating rabbits with implanted inferior vena cava tumor thrombus [16]. To our knowledge, no experimental study of using <sup>125</sup>I seeds strand to treat MPVTT has been reported in English literature so far. The

purpose of this paper was to evaluate the safety and efficacy of brachytherapy with  $^{125}\rm{I}$  seeds strand for treatment of VX $_2$  tumor inoculated in the MPV of rabbit.

#### Materials and methods

#### Animals

All experimental protocol was approved by the Institutional Animal Care and Use Subcommittee of our hospital and performed in accordance with the institutional guidelines. Thirty five healthy New Zealand white rabbits, weighing

# Brachytherapy with Iodine-125 for MPVTT animal model



**Figure 2.** Tumor strand and seeds strand and process of implanting tumor into portal vein. A and B. Show the tumor strand for implanted portal vein tumor thrombus. C-E. Show the process of Seeds Strand making. F. Open abdomen and expose main PV, white arrow shows inferior vena cava, black arrow shows portal vein. G. Put the protective sutures around PV. H. Purse-string suture was done on the anterior wall of PV (white arrow). I. Tighten the purse and tie a knot after the tumor strip injected into PV, pull the reservation suture out to make sure that the tumor strip is hanging against the inner wall of PV. White arrow shows preparation suture.



Withdraw the needle, tighten the purse and tie a knot, pull the reservation suture outward to make sure that the tumor strip is fixed firmly inside the lumen of PV

Figure 3. The schematic plot of Establishing implanted portal venous tumor thrombus.

about 1.8~2.2 kg, either gender, were used in this study. Three of them are used to be tumorbearing rabbit. The animals were housed individually and allowed free access to food and water. All procedure of this experimental was performed in animal laboratory of Zhongshan Hospital. Each rabbit was sedated and anesthetized with an intramuscular injection of the admixture of ketamine hydrochloride injection (4 ml/200 mg) and diazepam injection (2 ml/10 mg) at a dose of 1.5 ml/kg. Benzylpenicillin sodium antibiotic was given intramuscularly at a dose of 40,000 IU/kg twice a day, once before and 3 days after the operation. The flowchart of this study was presented in Figure 1.

# Preparation of VX, tumor strand

To establish the tumor-bearing rabbit, VX, tumor cell suspension, containing 1×107 cells in a volume of 0.5 ml, was injected intramuscularly into the hind legs of 3 rabbits. When grew to a size of about 2.0 cm in the longest diameter the VX<sub>2</sub> tumor was ready for harvest. Under sterile conditions, the VX<sub>2</sub> tumor was carefully dissected and excised from the hind limb of these rabbits. The periphery portion of the harvested tumor without macroscopic necrosis was cut into a tumor strip with diameter of 1.0 mm and length of 4.0 mm in a cold (4°C) phos-

phate-buffered saline (pH = 7.4). A 5-0 prolene surgical suture with length of 3.0 cm was used to penetrate through the end of VX<sub>2</sub> tumor strip. After that, the tumor strip with suture was put into an 18-Gauge needle for inoculation (Figure 2A, 2B).

# Establishing implanted MPVTT

32 rabbits were anesthetized as described above. The abdomen of rabbits was shaved, prepared with po vidone iodine and draped sterilely. A midline incision was made to expose the MPV. A purse with diameter of 2.0 mm was made on the anterior wall of MPV by a 6-0 prolene surgical suture. Through the center of purse, the VX<sub>2</sub> tumor strip with surgical suture was injected into MPV with an 18-Gauge needle. Then, the surgical suture penetrated through the end of tumor strip was pull outwardly and tighten to ensure the tumor strip to be fixed on the internal wall of MPV (Figure 2F-I). The schematic plot of this process shows in (Figure 3). The purse was sutured. Finally, the abdominal wall was closed in layers when no active bleeding was confirmed.

# Preparation of Iodine-125 seeds strand

Model 6711 <sup>125</sup>I seed used in this study is a cylindrical brachytherapy source encapsulated by titanium. Diameter and length of the titani-

purse





um capsule is 0.8 mm and 4.5  $\pm$  0.5 mm. Radioactivity of each seed is 25.9 MBq with a half-life of 59.4 days. The principal photon emissions are 27.4 keV x-ray, 31.4 keV x-ray, and 35.5 keV gamma ray. Half-value thickness of tissue for <sup>125</sup>I seeds is 17 mm, and the incipient dose rate is 7cGy/h. The <sup>125</sup>I seeds were stored in the lead-shielded pots. The <sup>125</sup>I seeds were deposited in the nuclear medicine department; those were disposed by professional personnel. Three model 6711 <sup>125</sup>I seeds or three fake seeds without radioactivity were arranged linearly and sealed into a 4-F sterile catheter continuously to construct a <sup>125</sup>I or blank seeds strand, respectively (Figure 2C-E). According to the formula offered by the American Association of Medical Physics [17], the cumulative radiation dose provided by three <sup>125</sup>I seeds arranged linearly was 9.0, 16.0 and 23.0 Gy at 7, 14 and 21 days after implantation, respectively.

#### Implantation of seeds strand

After confirming the implanted MPVTT by MDCT (**Figure 4A**), 32 rabbits with implanted MPVTT were randomly assigned into the therapeutic group (Group T, N = 16) and the control group (Group C, N = 16). Group T accepted <sup>125</sup>I seeds strand brachytherapy. Under sterile condition, the MPV with implanted tumor thrombosis was exposed. Using 5-0 prolene surgical suture, the

**Figure 4.** The process of seeds strands implantation and overall survival of rabbits after therapy. A. The transverse section CT imaging at the 2<sup>nd</sup> weeks after implanting PVTT shows round filling defects in PV (white arrow). B. White arrow shows the main portal vein tumor thrombus. C. White arrow shows seeds strand sutured on the outer wall of the PVTT with 5-0 sewing threads along the long axis. D. Overall survival of rabbits after therapy. The live time was evidently longer in the group T than in group C. The significant difference was observed between the groups (P = 0.001).

 $^{125}$ I and blank seeds strand was fixed on the surface of VX $_2$  tumor strip inoculated in the MPV of Group T and C, respectively (**Figure 4B**, **4C**).

#### Laboratory data and general condition

Blood samples were collected from heart before and at 1, 2, and 3 weeks after the intervention. The white blood cell count (WBC) and blood platelets (PLT) were measured by an autoanalyzer. Plasma aspartate aminotransferase (AST), albumin (ALB), and total bilirubin levels were tested by a biochemical autoanalyzer. The general condition was monitored every day, including mental status, food intake, activity, defecation, and survival time were monitored after implanting seeds strand. The body weight was measured every week. The data was recorded by a recorder who was blinded to the animal grouping.

#### MDCT imaging of the PVTT and staging

All 32 rabbits were subject to MDCT scans at 2 weeks post-implanting PVTT under anesthesia. The scanning parameters were 120 kV, 150 mA/sec, 5.0 mm slice thickness, 1.0 mm reconstruction slice thickness, 0.625 mm reconstruction intervals. Scan position is supine. Push through ear margin vein to inject

Table 1. Weight and outcome of therapy\*

		Group T	Group C	Р
BW (kg)	Preoperative	1.92 ± 0.12	1.90 ± 0.15	0.838
	Postoperative-1 w	$1.91 \pm 0.08$	1.87 ± 0.12	0.291
	Postoperative-2 w	$1.79 \pm 0.07$	$1.71 \pm 0.11$	0.073
	Postoperative-3 w	1.72 ± 0.09	$1.48 \pm 0.14$	0.001
V <sub>x</sub> (mm <sup>3</sup> )	Preoperative	43.04 ± 6.31	42.86 ± 5.56	0.932
	Postoperative-1 w	145.71 ± 45.42	238.24 ± 48.05	0.000
	Postoperative-2 w	344.65 ± 110.90	821.83 ± 183.09	0.000
	Postoperative-3 w	565.40 ± 220.90	2269.90 ± 437.00	0.000
Ki-67 LI		4.14% ± 1.84%	33.82% ± 6.07%	0.001
AI		6.51% ± 1.92%	0.91% ± 0.26%	0.001

\*Pre-BW, body weight before Seeds Strand implanted; Post-BW, body weight at every week after Seeds Strand implanted; Vx, tumor thrombus volume at every week after Seeds Strand implanted; Ki-67 Ll, Ki-67 labeling index; AI, apoptosis index.

contrast agent Ultravist Injection (300 mg/ml), the dose of contrast agent is 3.0 ml/kg, injection rate I ml/s, delay time of arterial phase enhancement 20 s, Portal vein phase enhancement delay time is 45 s. Tumor length (L) and width (W) were measured in the CT scan images by using the 3D-MPR in advantage workstation. Tumor volume was evaluated according to the following formula [18]: Tumore volume (mm<sup>3</sup>) = L(mm) × W<sup>2</sup>(mm<sup>2</sup>)/2.

MDCT scans and 3D MPR were acquired at 1, 2, 3 week post-implanting seeds strand in all rabbits.

# Pathological examination

At 14<sup>th</sup> day, 16 rabbits (8 animals were selected randomly from each group) were sacrificed by intravenously injection of overdose Ketamine (0.8 mL/kg). And this can minimize suffering of the rabbit. Compare the tumor growth and metastasis between the two groups by macroscopic examination, and evaluate the correlation of pathology and imaging examination.

The remaining 16 rabbits (8 from each group) were kept for survival observation. Survival time was calculated from the day of <sup>125</sup>I seeds strand implantation to natural death. The cause of death is extensive invasion and widespread metastasis. And they were dissected postmortem. Whole liver, MPV and surrounding duodenum were carefully dissected and excised for macroscopic examination. The number and maximal diameter of liver metastatic nodules were measured. The representative samples

were fixed in 4% formaldehyde for 24 hours and embed in paraffin. 5-µm paraffin sections were adhered to glass slides for microscopic examination. Hematoxylin and eosin (H&E) staining, immunocytochemical detection of Ki-67 protein expression and terminal deoxynucleotidyl transferase (TdT) mediated dU-TP-biotin nick end labeling (TUNEL) assay for apoptosis detection were performed. Standard procedures that described by Jones HB [19] and Loo DT [20], were used

for Ki-67 protein expression detection and TUNEL assay, respectively. According to the formula provided by Jones HB [19], the Ki-67 LI and AI were calculated by using: LI (%), or AI (%) = total of labeled cell/(total of labeled + unlabeled cell). Microscopic examination was performed by two independent pathologists who were blinded to the animal data. Sections with discrepant were re-evaluated to reach a final agreement.

# Statistical analysis

The quantitative data were presented in the form of mean values  $\pm$  standard errors. The *t* test and Wilcoxon rank test were used to determine statistical significances of the differences in the body weight, plasma biochemical levels, the volume of tumor thrombus, AI, and Ki-67 LI between two groups. Survival time was analyzed by the Kaplan-Meier curves. A *P* value of less than 0.05 indicated a significant difference. Statistical software (SPSS version 13.0, SPSS, Chicago, Illinois) was used for analysis.

# Results

# General conditions, laboratory tests and survival time

No intraoperative complications occurred. There were no perioperative deaths. The success rate of operation to establish PVTT and implant seeds strand was 100%. Rabbits gradually resumed eating and routine activities at 12 hour after operation. The body weight baseline was no statistical difference between the

		TB (µmol/L)	ALT (U/L)	ALB (g/L)	WBC (×109)	PLT (×10 <sup>9</sup> )
Preoperative	Т	18.7 ± 7.3	64.0 ± 25.9	27.9 ± 4.9	7.3 ± 4.2	267.6 ± 122.4
	С	18.4 ± 5.7	67.2 ± 36.6	29.2 ± 3.5	7.1 ± 3.5	279.3 ± 132.5
	Р	0.368	0.491	0.124	0.467	0.258
Postoperative-1 w	Т	17.0 ± 1.2	68.6 ± 10.6	27.9 ± 2.2	6.6 ± 1.4	251.5 ± 50.8
	С	17.2 ± 1.1	73.3 ± 13.5	26.5 ± 3.1	7.4 ± 1.8	230.9 ± 42.8
	Р	0.601	0.275	0.176	0.170	0.225
Postoperative-2 w	Т	19.6 ± 3.0	88.3 ± 17.6	26.7 ± 3.5	6.8 ± 1.4	258.5 ± 132.3
	С	21.9 ± 8.6	92.6 ± 24.6	24.4 ± 3.2	7.1 ± 1.8	275.3 ± 127.7
	Р	0.110	0.609	0.069	0.508	0.437
Postoperative-3 w	Т	24.5 ± 7.5	106.5 ± 31.9	23.1 ± 2.3	6.7 ± 1.3	241.1 ± 61.9
	С	31.3 ± 8.0	124.1 ± 51.8	20.2 ± 4.2	7.5 ± 1.1	227.3 ± 93.4
	Р	0.101	0.427	0.115	0.216	0.733

Table 2. The Comparison of laboratory tests\*

\*Operation: Seeds Strand implanted.

Table 3. Survival data

Survival time (d)					Mean $\pm$ SD				
Т	51	29	45	42	40	38	36	35	39.50 ± 2.37
С	28	23	32	24	26	29	25	32	27.38 ± 1.22
Ρ	0.001								

two groups (*P*>0.05). At the third week after seeds strand implantation, the mean Body weight in Group T was much higher than that in Group C and more serious weight loss was detected in Group C (**Table 1**). Results of liver function tests and blood cells counts showed no significant difference between two groups at every time point (**Table 2**). Mean and media survival time of rabbits were ( $39.50 \pm 2.37$ ) days (95% Cl 32.97~43.13 days) in Group T and ( $27.38 \pm 1.22$ ) days (95% Cl 23.43~28.57 days) in Group C, respectively (*P* = 0.001) (**Table 3**; **Figure 4D**).

# MDCT imaging

The baseline tumor thrombus volume was no statistical difference between the two groups. MDCT scan revealed that the PVTT increased gradually in both groups (**Figure 5**). All rabbits in group C and T showed liver metastases at the  $2^{nd}$  week after intervention. Whereas the number and maximum diameter of intrahepatic metastatic nodules in group T were less than that of in Group C. The volume of PVTT after intervention in group T is smaller than that of group C obviously (**Figure 6**), and there was a statistically significant difference between group T and group C at the  $3^{rd}$  week after intervention (P < 0.05) (**Table 1**).

# Macroscopic findings

At the 2<sup>nd</sup> week after seeds strand implantation, intrahepatic metastatic nodules were detected in all rabbits (**Figure 6**). Mean number and maximum diameter of intrahepatic metastatic nodules in Group T were obviously less than that in Group C, respectively. No ulcer or necrosis was detected within the surrounding duodenum.

# Microscopic examination

In Group T, massive necrotic area and tumor cells arranged sparsely in MPV tumor tissue was presented (Figure 7). Hyperchromatic and shriveled nucleus were detected within most tumor cells. MPV intima and media were damaged and loosened. Swelling and necrotic cells were detected within the hepatic parenchyma 10 mm from <sup>125</sup>I seeds strand implantation (Figure 8). No obvious pathological changes were detected within the surrounding duodenum and hepatic artery (Figure 8). In Group C, scattered necrotic cells within MPV tumor tissue were found. Polyploidy nucleus arranged disorderly were found in most tumor cells. Tumor cells arranged intensively was observed within the MPV media. Cells with positive Ki-67 protein expression were more in Group C and more TUNEL positive cells were found in Group T (Figure 7). Ki-67 labeling index was  $(4.14 \pm$ 1.84)% in Group T and (33.82% ± 6.07)% in Group C, respectively (t = 11.47, P < 0.001). Apoptosis Index was (6.51 ± 1.92)% in Group T, compared with  $(0.91 \pm 0.26)\%$  in Group C. respectively (t = 7.07, P < 0.001) (**Table 1**).



**Figure 5.** The CT imagings before and  $1^{st}$ ,  $3^{rd}$  weeks after implanting seeds strand (A-F--Group T; G-L--Group C). (A and G) Show the filling defects in portal vein (black arrow) before implanting seeds strand in Group T and C. (D and J) Show there is no liver metastasis before implanting seeds strand in Group T and C. (B, C and H, I) Show the filling defect in portal vein (black arrow) in Group T was larger than that in Group C at  $1^{st}$  and  $3^{rd}$  week after implanting seeds strand. (C, I) PVTT present nonuniform enhances. (E, F and K, L) Show the liver findings in Group T and C. The number of intrahepatic metastasis nodules in Group T is obviously less than in Group C.

#### Discussion

These data in 32 animal models demonstrate the safety and efficacy of brachytherapy with <sup>125</sup>I seeds strand to treat MPVTT in rabbit. Lindsay, et al. [21] established an animal model with MPVTT in rhesus successfully by using chemical induction of carcinogen. However, the formation rate of MPVTT reported by Lindsay was only 35% (14/40). Moreover, the procedure of induction used by Lindsay was complex and the time taken for MPVTT formation was long. Thus, this animal model with MPVTT has not been used extensively. Genda T, et al. [22] injected Li7 and KYN-2 tumor cell into the liver parenchyma of nude mouse. Although intrahepatic vascular invasion was detected no MPVTT was presented. Wan, et al. [23] created an animal model with portal vein tumor thrombus in rabbits by injection VX<sub>2</sub> cell suspension into portal vein by percutaneouse puncture with ultrasound guidance or direct puncture during laparotomy, using these two methods, the formation rate of PVTT was 89% and 94%, respectively. In our study, the pre-prepared VX<sub>2</sub> tumor strip was injected into the MPV of rabbits directly and then was fixed on the inner wall of MPV. MPVTT formation rate was 100%. This method enabled the  $VX_2$  tumor strip to extend along within the lumen of main portal vein, which simulated the circumstance presented in human being with HCC complicated by MPVTT preferably.

Brachytherapy with interstitial implantation of <sup>125</sup>I seeds has been used previously in the treatment of HCC. Compared with external radiotherapy, brachytherapy has the following advantages: (1) Gamma rays emitted by <sup>125</sup>I have a short radiation distance; a high-dose irradiation can be kept within the tumor area with limited damage to surrounding normal tissue; (2) <sup>125</sup>I seeds have a long half-life (59.4 days); a sustained radiation can inhibit the replication of tumor cells and induce tumor cell apoptosis [15]; (3) Radiation emitted by <sup>125</sup>I seeds fixed on the surface of tumor strand inoculated in MPV greatly reduces the motion effects from respiration. (4) A low dose rate of radiation has been reported to decrease the incidence of metastasis by altering the immunophenotype of tumor cells [24, 25]. Puncture to target lesion was the method most often used to implant <sup>125</sup>I seeds [14, 15, 17]. Potential risks exist when using an 18-gauge needle to puncture the tumor tissue in MPV directly, such



**Figure 6.** The CT imagings and Macroscopic findings at the  $2^{nd}$  week after implanting seeds strand. A-C and D-F. Show the CT imagings and macroscopic findings of PVTT, liver metastasis and lung in Group C, respectively. G-I and J-L. Show the CT finding in Group T. The volume of PVTT and the number of intrahepatic metastatic nodules in Group T is obviously less than that in Group C.



**Figure 7.** The HE staining, proliferating cell (PCNA) and apoptosis (TUNEL) of PVTT. (A and D) Show the H&E staining (×100) manifest in Group C and T, a shows tumor cell eumorphism in PV. (D) Shows an abundance of amorphous

structures, and sparse tumor cell among these. (B and E) Show the Ki-67 protein expression that is a great quantity anachromasis brown cell in Group C, but a small quantity in Group T ( $\times$ 200). (C and F) Show the tumor cell apoptosis manifest, (C) shows a great quantity Calamine blue tumor cell, a small quantity buffy tumor cell nucleus in Group C, (F) shows an abundance of puse apoptotic bodies among tumor cell in Group T ( $\times$ 200).



**Figure 8.** The pathological manifestation of PV and surrounding tissue in 2 week after intervention. A. Swelling and necrotic cells were detected within the hepatic parenchyma 10 mm from <sup>125</sup>I seeds strand (×200 HE). B. The liver tissue that far away from <sup>125</sup>I seed more than 10 mm showed normal (×200 HE). C and D. No obvious pathological changes was detected within the surrounding duodenum and hepatic artery (×200 HE). E and F. (×25 and ×200 HE). Black arrows show portal vein (endothelial cells). Blue arrows show tumor thrombus in PV.

as injury to portal vein, bleeding, increasing the incidence of intraperitoneal tumor spread and displacement of  $^{125}\mathrm{I}$  seeds implanted. In this study, three  $^{125}\mathrm{I}$  seeds were arranged linearly and sealed into a 4-F sterile catheter continu-

ously to construct a <sup>125</sup>I seeds strand. Then, the <sup>125</sup>I seeds strand was fixed on the surface of tumor strand inoculated in MPV by surgical suture. Using this technique, the serious complications related to direct puncture to tumor tissue in the MPV would be avoided and the <sup>125</sup>I seeds implanted would be kept in position without displacement.

Nuclear Ki-67 protein expression is closely associated with cell proliferation. Higher Ki-67 labeling index reflects the existence of biological aggressive phenotypes and poor overall survival rates of HCC [26, 27]. Apoptosis, or programmed cell death has been linked to many disease states, including cancer [26, 27]. One of the biochemical hallmarks of apoptosis is the generation of free 3'-hydroxyl termini on DNA via cleavage of chromatin into single and multiple oligonuleosome-length fragments. The TUNEL assay demonstrates this biochemical hallmark by labeling the exposed termini of DNA, thereby enabling visualization of nuclei containing fragmented DNA [26, 27].

Ling, et al. [25] indicate when the doubling time of tumor cell is five minutes, the effective time of implanted <sup>125</sup>I is 120 days. The doubling time is 30 days; the effective time is 275 days. So the major limitations of this experimental study were small animal number with short-term follow-up period and the half-life of <sup>125</sup>I seeds, 59.4 days, seemed to be long compared to the doubling time of VX, tumor cells. This limited our ability to maximize the survival benefit from this promising therapy. We plan an experimental study next to evaluate the efficacy of using high dose rate brachytherapy to treat VX<sub>2</sub> tumor strand inoculated in MPV. VX $_2$  tumors have a higher growth rate than that of HCC, its doubling time is 34.5 hours [28], and thereby the therapeutic effect was decreased. The volume doubling time of human hepatocellular carcinoma is 176 days [29], so <sup>125</sup>I is suitable for the therapy of HCC. Second, we did not use other methods to treat the tumor because our aim was to evaluate the effect of linear <sup>125</sup>I seeds strand in treating MPVTT. Some differences might occur in the stability or effects of the agents in the presence of other therapeutic method. Third, we did not use stent to relieve the obstruction of MPV (which is used as a rule) because there was no eligible stent to implant into the PV of rabbit, and the <sup>125</sup>I seeds strand was sutured on the outer wall of the MPV instead of inserted into the MPV. This affected the overall survival time in two groups.

In our study, 7, 14 and 21 days after <sup>125</sup>I seeds strand was implanted all rabbits in Group T were alive and the results of laboratory tests

showed no significant difference between two groups. No abnormal pathological findings were detected in the surrounding duodenum. These confirmed the safety of brachyterapy with <sup>125</sup>I seeds strand to treat VX<sub>2</sub> tumor strand inoculated in the MPV of rabbit. 14 days after brachytherapy more serious weight lose was presented in Group C than that in Group T. Quantitative macroscopic examination revealed that the degree of intrahepatic metastasis and volume of tumor tissue within MPV in Group C was more serious and larger than those in Group T. According to the results of microscopic examination, more Ki-67-positive cells were found in Group C and more apoptotic body was presented in Group T, respectively. Survival time of rabbits in Group T was much longer than that in Group C. All these indicated that brachytherapy with <sup>125</sup>I seeds strand could decrease the incidence of intrahepatic metastasis, inhibit the proliferation of tumor cells, promote apoptosis, and improve the overall survival of rabbits with VX<sub>2</sub> tumor strand inoculated in MPV. In the patient of advanced HCC with PVTT, the symptoms of PV obstruction would be relieved by the metal stent placement, the growth of PVTT would be inhibited effectively by the radiation from <sup>125</sup>I seeds strand. And the primary tumor in liver could be treated by TACE, radiofrequency ablation (RFA) or microwave ablation. Luo et al. [30] and Li et al. [31] have achieved promising results clinically. And we will further evaluate interventional brachytherapy as an additional tool in tumor thrombus therapy concepts.

In conclusion, Injecting and suspensory fixing VX<sub>2</sub> tumor strip into MPV is a reliable method to establish MPVTT animal model. It was safe and efficacy to use brachytherapy with <sup>125</sup>I seeds strand for treating MPVTT.

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# Disclosure of conflict of interest

None.

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