

• Basic Research •

# Experimental study of CT-guided percutaneous ethanol ablation in rabbit renal VX2 tumor

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**[Abstract]** **Background and Objective:** CT-guided percutaneous ethanol ablation (PEA) has been widely used in treating solid tumors such as hepatoma, lung cancer, adrenal nonfunctional adenoma. This study was to explore the efficacy, safety and feasibility of CT-guided PEA in treating renal tumor in rabbit. **Methods:** Twenty-five rabbits carrying VX2 tumor were randomized into PEA group (15 rabbits) and control group (10 rabbits). After CT-guided PEA, the area of the largest cross section lipiodol deposition in PEA group was measured. After one week, the kidneys carrying VX2 tumor were removed, tumor size in both groups and the area of the largest cross section coagulation necrosis in PEA group were measured. Wound infection and the changes of living habits of the rabbits were observed after experiment. **Results:** A total of 25 VX2 tumors were developed in the 25 rabbits. The area of the largest cross section was 1.38-2.25 cm<sup>2</sup>, with an average of (1.61±0.04) cm<sup>2</sup>. There was no significant difference in tumor size between the two groups. After ablation, the area of lipiodol deposition in PEA group was 1.31-1.85 cm<sup>2</sup>, with an average of (1.56±0.05) cm<sup>2</sup>. At one week after ablation, the area of the largest cross section of tumors was significant smaller in PEA group than in control group [(1.58±0.03) cm<sup>2</sup> vs. (1.94±0.03) cm<sup>2</sup>,  $P<0.05$ ]; the area of coagulation necrosis in PEA group was 1.27-1.78 cm<sup>2</sup>, with an average of (1.54±0.04) cm<sup>2</sup>, and was similar to the area of lipiodol deposition ( $P>0.05$ ). Tumor tissue in ablation areas showed acidophilia changes and irregular coagulation necrosis. There was no obvious complication in PEA group. **Conclusion:** CT-guided PEA can effectively inactivate rabbit kidney VX2 tumors, and it is a safe and feasible treatment without obvious complications.

**Key words:** rabbit, VX2 tumor, ablation, ethanol, renal neoplasm

Following the development of microinvasive intervention technique, CT-guided percutaneous ethanol ablation (PEA) has been widely used in treating solid tumors such as liver cancer, lung cancer and adrenal nonfunctional adenoma, for its advantages such as less invasive, more indications, certain efficacy, low cost.<sup>1-4</sup> But the experiment and clinical application of PEA in treating renal solid tumors have not been reported yet. This study was to explore

the efficacy, safety and feasibility of CT-guided PEA in treating renal tumors by experiment in rabbit renal VX2 tumors and to provide theoretical basis and technical supports for clinical application of PEA in renal solid tumors.

## Materials and Methods

**Experimental animals.** Twenty-five healthy New Zealand white rabbits (animal certificate numbers: No. 0027906, No. 0028028, No. 0028202), aged 3-4 months, weighed 1.5-2.5 kg, either sex, and one rabbit with VX2 tumor were all provided by the Animal Center of Sun Yat-sen University. The rabbits were numbered randomly, raised separately in Animal Center.

**Reagents and instruments.** Sumianxin (1.5 mL/u) was produced by Animal Remedy Factory of Changchun. absolute ethanol (purity 99.7%, 10 mL/u) was produced by Pharmacy Department of General Hospital of Guangzhou Military Command. ultra liquid iodized oil (10 mL/u) was produced by Jiabai Company of France. 16G puncture trocar, 18G multipolar chemo-ablation needles, and gelatin sponge were used. Double spiral CT scanning machine was produced by Elscints Company.

**Preparation of rabbit renal VX2 tumor model.** Rabbit renal VX2 tumor model was prepared by embedding pieces of tumor tissue. The VX2 tumor-bearing rabbit received intramuscular injection of Sumianxin (0.2 mL/kg). After anesthesia, tumor mass was removed from the rabbit. Removing the necrosis and fascia, the fresh fish-like tissue, with abundant blood supply, at the tumor edge was selected, washed twice with normal saline, cut into pieces of 1 mm 1 mm 1 mm, and transplanted into the upper pole of the right kidney of the rabbits with about 2 cm in depth using a 16G puncture trocar. The rabbits received intramuscular injection of  $4 \times 10^5$  units of penicillin for three days.

**CT-guided percutaneous ethanol ablation in rabbit kidney VX2 tumors.** After tumor transplantation, the 25 experiment rabbits were raised separately for 2 weeks, and underwent enhanced CT scanning to confirm successful

transplantation and measure the size of tumors. Fifteen rabbits were selected randomly as PEA group, anesthetized with Sumianxin, and underwent CT-guided puncture the tumor, intratumoral injection of the mixture of absolute ethanol and lipiodol (10:1) using the 18G ablation needle till well-distributed deposit in tumor (Figs. 1-3). The largest section area of lipiodol deposition was measured. After PEA, the rabbits received intramuscular injection of  $4 \times 10^5$  units of penicillin for three days. The changes of rabbit behaviors, such as drinking and eating, motions and stimulation responses, were observed. The rest 10 rabbits received no treatment and were used as controls.

**Acquisition of specimen and making of the large slices.** At one week after PEA, the rabbits were killed by air embolism, tumor masses were removed and cut into large slices along the largest cross section. The tumor size in control group, the solidification necrosis area and size of tumors in PEA group were measured. After HE staining, the necrosis in the ablated lesions and peripheral tumor tissues were observed under light microscope.

**Statistical analysis.** Measurement data between the two groups were compared by t test using SPSS10.0 software package. A P value of  $< 0.05$  was considered significant. All data are presented as mean standard deviation.

## Result

At 2 weeks after tumor transplantation, enhanced CT scanning showed that tumor formed in all of the 25 rabbits with a success rate of 100%. All rabbits were alive, without complications such as infection and hemorrhage. The largest section areas of tumors were 1.38-2.25 cm<sup>2</sup>, with an average of  $(1.61 \pm 0.04)$  cm<sup>2</sup>, with clear boundary and homogeneous density. Before treatment, there was no significant difference in tumor size between the two groups ( $t=1.87$ ,  $P>0.05$ ). After CT-guided PEA, the high density area of lipiodol deposition was 1.31-1.85 cm<sup>2</sup>, with an average of  $(1.56 \pm 0.05)$  cm<sup>2</sup>. After treatment, all the rabbits resuscitated within 1 h, and were in a

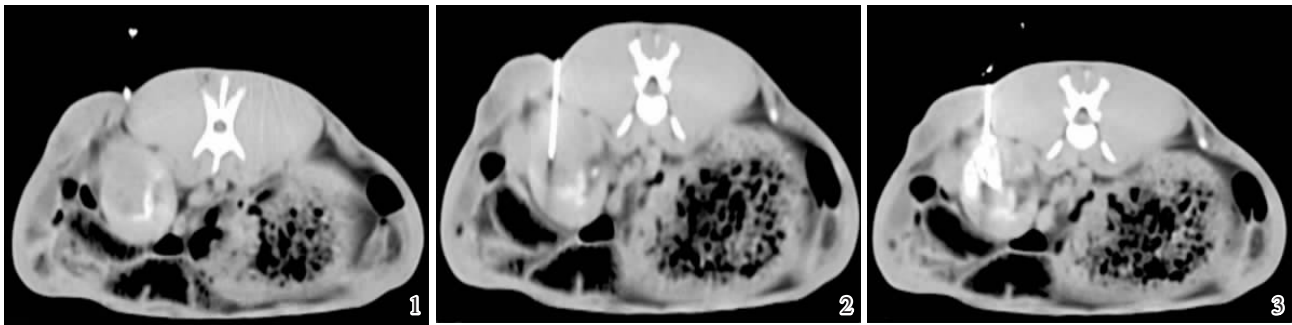


Figure 1 Enhanced CT scan shows a round tumor mass in the upper pole of the right kidney at two weeks after tumor implantation

Figure 2 CT-guided punctuation and percutaneous ethanol ablation (PEA) of the tumor in the right kidney

Figure 3 CT scan shows absolute ethanol and lipiodol deposition in the tumor

good condition. Some were nervous, with less motions, no intake, and a little tachypnea, but all recovered on the next day, without accidental death.

At 1 week after treatment, the tumors in PEA group grew slowly without obvious enlargement, while those in control group grew rapidly without obvious enlargement. The tumor size was significantly smaller in PEA group (including the area of coagulation necrosis) than in control group ( $t=6.04$ ,  $P<0.05$ ).

In gross specimens of PEA group, the ablated areas showed irregular morphology, with homogeneous gray coagulation necrosis in the center, a clear boundary to peripheral tissues, and a pink zone of hyperemia and edema in surrounding tissues; some tumors were not completely ablated, with some gray-yellow fish-like tissue surrounded the coagulation necrosis area (Fig.4). The size of coagulation necrosis area was  $1.27\text{--}1.78\text{ cm}^2$ , with an average of  $(1.54 \pm 0.04)\text{ cm}^2$ , which was similar to the size of lipiodol deposition area ( $t=0.66$ ,  $P>0.05$ ). With HE staining, the ablation areas showed nuclear chromolysis, cell disruption, and acidophilia, presented as an irregular coagulation necrosis area with no cellular structure, surrounded by granulation tissue hyperblastosis, inflammatory cell infiltration, neogenesis capillary network and a little fibrous connective tissue (Fig. 5).

In gross specimens of control group, the tumors were in irregular oval-like shape with clear boundary, presented as gray fish-like tissues with hard texture, and showed round nodules in

renal cortex with no envelope. With HE staining, invasive carcinoma nests with no obvious boundary to the renal parenchyma were observed, cancer cells lined up compactly with few fiber spacers and abundant neogenesis blood capillary, lymphocytes and plasmocytes dispersed in interstitial tissue, some infiltrated renal glomeruli around tumors, cancer cells were large, with dark staining and irregular disposition (Fig. 6).

## Discussion

VX2 tumor cell line is the 72<sup>nd</sup> generation originated from squamous cell carcinoma derived from Shope virus-induced rabbit papilloma. VX2 cells can be successfully implanted in many tissues or organs of rabbits, such as the muscle, skeleton, urinary bladder, mammary gland, liver, kidney, lung and uterus, to prepare animal model of corresponding tumor in situ.<sup>5,6</sup> In this study, we prepared rabbit VX2 tumor model by embedding tissue pieces. This operation is easy to handle, with a high success rate, short period, and low rates of subcapsular renal hematoma and extra-renal implantation, and is an ideal method of making tumor model. Besides, this model is a good model in large experimental animal for studying the biological characteristics, metastasis, imaging, treatment of and efficacy evaluation of renal tumors.<sup>7</sup> We used this method to prepare rabbit renal VX2 tumor model. All rabbits were alive, with no complications such as hemorrhage and infection.

Renal carcinoma is insensitive to radiotherapy

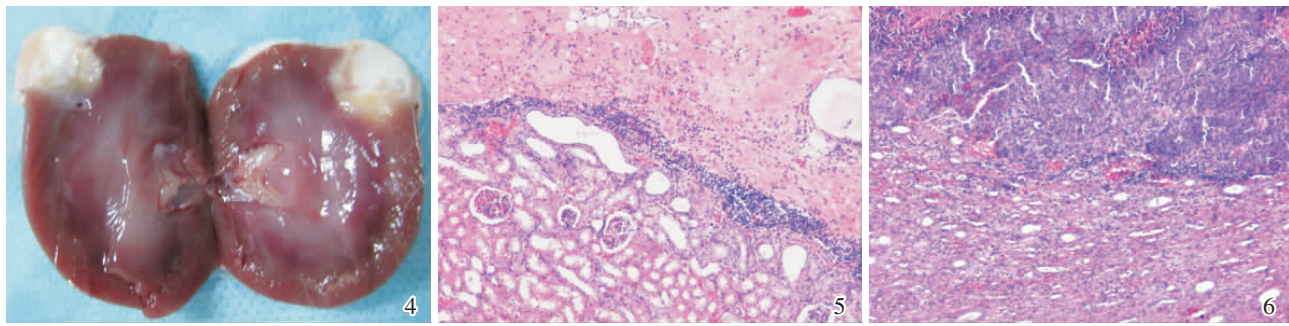


Figure 4 Morphology of the VX2 tumor-bearing kidney at one week after percutaneous ethanol ablation

The kidney is longitudinally incised. The tumor in the upper pole of the right kidney presents coagulatory necrosis in white-yellow, clear boundary with normal renal tissue.

Figure 5 Pathology of the tumor at one week after PEA treatment (HE ×200)

The right upper of the figure is the coagulation necrosis (in pink) of tumor tissue after PEA, and the left bottom is the normal renal tissue.

Figure 6 Pathology of the tumor in control group (HE ×200)

The cancer nests of VX2 tumor have clear margin.

and chemotherapy. At present, surgical resection still is an important treatment for renal cancer. But in some situations, such as renal carcinoma in solitary kidney or both kidneys, contralateral renal dysfunction, patients cannot tolerate operation, and patients requesting remaining kidney, either radical nephrectomy or nephron-sparing operation has some disadvantages, such as operation complexity, great difficulty, high risk, more intraoperative hemorrhage and more postoperative complications. Therefore, exploring mini-invasive treatment is quite important for renal tumors.

In recent years, following the development of mini-invasive techniques, the ablation treatment which directly inactivates tumors in situ by physics energy and chemical materials under imaging guidance develops very fast. It has advantages, such as exact location, mini-invasiveness, and less complications. Among the mini-invasive interventional treatments for renal carcinoma, cryoablation and radiofrequency are frequently used physical ablation therapies, microwave ablation and laser ablation are also used. Physical ablation, a mini-invasive local treatment for tumors, can preserve the nephron to maximal degree and decrease treatment risk when effectively treating renal tumors. But for some complex renal tumors such as lesions near to renal hilum, great vessels, or intestinal canal, and for patients at high risk, physical ablation

still has some risks or cause insufficient treatment due to irregular tumor shape, chemo-ablation can be more safe and effective in these cases.<sup>8,9</sup>

Absolute ethanol and acetic acid are the commonly used chemical ablation agents. CT-guided percutaneous ethanol ablation is to directly inject protein coagulant absolute ethanol into tumor tissue, result in rapid dehydration, protein degeneration, and coagulation necrosis of tumor cells, destroy tumor blood vessel endothelium and lead to thrombosis. Following the development of mini-invasive techniques, PEA has been extensively used to treat solid tumors, such as liver cancer, lung cancer and nonfunctional adrenal adenoma, due to it advantages of mini-invasiveness, extensive indications, certain efficacy and low costs. CT-guided PEA is less restricted by tumor location and adjacent organs, and treats tumors completely. In treating liver cancer when the lesions are close to liver capsule, diaphragmatic dome, gall bladder, intestine and great vessels, PEA is also safe and applicable.<sup>10,11</sup> Therefore, for it wide indications and safety, theoretically, PEA can be applied to treat renal carcinomas which are hard to be resected and insensitive to radiotherapy and chemotherapy, especially when they locate in the kidney center, or near the renal hilum, invade the renal envelope, close to great vessels and intestine, adhere to adjacent tissues. But the clinical application of PEA to treat renal



carcinoma has not been reported yet.

In 2003, Yasuyuki et al.<sup>12</sup> implanted human renal cell carcinoma OUR95 cells into the back of nude mice subcutaneously to establish experimental animal model, injected 95% absolute ethanol into the tumor and found that the tumor growth was inhibited obviously with no volume enlargement, no enhancement area on MRI images, no degeneration and necrosis area observed in histological examination. However, in this experiment, the tumor was subcutaneously implanted into the back of nude mouse, not in the kidneys, therefore, the safety, validity and feasibility of PEA in treating renal tumors need to be further explored. In our experiment, we used rabbit renal VX2 tumor model as research object, and applied CT-guided PEA to treat rabbit renal VX2 tumor.

CT can clearly show the anatomic structure of the abdomen and precisely locate the tumors, exactly guide the ablation needle to enter tumors and real-time monitor the dispersion scope of ablation agent, also can recognize complications such as hemorrhage and perforation in abdominal organs. Therefore, CT is a suitable guiding equipment for ablation of abdominal tumors. In addition, tumor tissues present as low density areas on CT image after PEA with absolute ethanol, without obvious difference in density to non-ablated tumor tissues, resulting difficulty in accurately assessing ablation area.<sup>13,14</sup> Mixing absolute ethanol and superliquefied lipiodol at a volume ratio of 10:1 to prepare ablation emulsion with the same physical and chemical characteristics, the ablated area of tumor presents high density on CT image, which helps assessing the dispersion scope of ethanol in tumors, observing whether it has leaked out of tumors, and evaluating the efficacy during re-examination.<sup>15,16</sup> In this study, we used CT-guided PEA. Before ablation, meglumine diatrizoate (1 mL/kg) was injected via the auricular vein for enhancement scanning that outlined tumors more clearly for accurate puncture. After contrast agent metabolism, the ablation emulsion was percutaneously injected for VX2 tumor ablation which showed high density as ablation scope. To avoid heterogeneous

dispersion of ethanol in tumors caused by fiber spacers, we used multipolar umbrella-shaped ablation needle which made ethanol dispersion more ideal. After PEA, the tumor tissues are inactivated in situ without marked change in tumor size in a short period, therefore, we did not statistically compare the tumor sizes of PEA group before and after PEA in this study. However, the tumors were obviously smaller in PEA group than in control group after one week.

Our results showed coagulation necrosis of tumor tissues in lipiodol deposition area after PEA. The lipiodol deposition areas in tumors on CT images were coincident with the coagulation necrosis areas in pathologic specimens. The tumors in PEA group grew more slowly than those in control group, without obvious adverse events and complications. Therefore, we consider that CT-guided PEA can effectively inactivate rabbit renal VX2 tumor tissue, it is safe and feasible, and does not cause obvious adverse events and complications.

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