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Original Article

Experimental study of image-guided percutaneous microwave ablation in rabbit lung VX2 tumor model

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Abstract: Purpose: To investigate the efficacy and safety of percutaneous microwave ablation. Methods: Twenty-six rabbits with lung VX2 tumor were randomly divided into experimental and control group. In the experimental group, microwave ablation guided by ultrasound or CT was performed based on location of the tumor. Enhanced CT scan was carried out immediately before and after the ablation for all animals. Two animals from each group were sacrificed immediately or 1 week after the ablation respectively and the others were followed for the rest of their lives. Results: CT scan revealed that the tumor was greatly reduced or ablated after ablation. Pathological examination immediately after ablation also confirmed the tumor reduction or ablation. The survival time of the animals in the experimental group was significantly longer than that in the control group. Conclusions: Microwave ablation is a safe and effective method for treating lung cancer in rabbits, showing potential clinical applicability.

Keywords: Microwave ablation, VX2 tumor, lung cancer

Introduction

Lung cancer is one of the most common malignancies worldwide, and the current therapies include surgery, radiation therapy and chemotherapy [1-3]. Surgical intervention is usually performed in patients with cancers at early-stage and remains the primary curative technique [1, 2]. Nevertheless, surgical treatment is frequently limited in clinical practice due to concomitant chronic obstructive bronchopneumopathy and/or other associated diseases [4]. Recently, the efficacy and safety of stereotactic radiation therapy (SRT) has been reported [5]. However, SRT has its side effects such as radiation pneumonitis, pulmonary fibrosis, radiation myelitis, mucocutaneous reactions (stomatitis, esophagitis, enteritis, cystitis) and weakness, fatigue, dizziness, headache, anorexia, nausea, vomiting and other reactions. In addition, the radio sensitivity is related with the tumor cell proliferation cycle and the pathological grade.

Under this condition, minimally invasive treatments often receive great attention as is happening in thermal ablation which includes radiofrequency ablation (RFA), microwave ablation (MA), laser ablation (LA), etc. Image-guided thermal ablation has opened a new era for treatment of solid tumors by “in situ” tumor destruction since its birth over a decade ago, especially for treatment of early-stage liver cancer or surgically nonresectable tumors. Because it is less traumatic than regular surgery with fewer and less severe complications, it has become accepted by more and more clinicians and surgeons, and its clinical use has expanded to treatment of renal, adrenal, lung, and bone malignancies. Theoretically, lung tumors can be treated with thermal ablation because the surrounding air in the adjacent normal parenchyma provides an insulating effect, thus facilitating energy concentration within the tumor tissues.

Radiofrequency ablation (RFA) is also a well-established modality in the treatment of nonresectable liver tumors and recently, several authors have evaluated RFA for the treatment of primary and metastatic lung cancer and it has been reported to be an effective, safe, and

easily available way for lung cancer treatment [6-10]. In the thermal ablation (thermal therapy), there are no such side effects of SRT.

Although experimental and clinical studies regarding percutaneous RFA of lung cancer have been reported, there have few reports on MA. MA is a relatively recent technology in the field of tumor ablation, and it has been proved to be a safe and effective way to treat various cancers by in vivo experiments. MA has several advantages in energy delivery. Most importantly, microwave propagation is not limited by charred tissue and, therefore, tissue temperatures can be elevated to very high levels (> 150°C) without impairing energy deposition. High temperatures are more likely to overcome vascular mediated cooling and create larger and more lethal ablation zones with shorter treatment times [11, 12]. RF current may cause pacemaker malfunction, so the patients with cardiac pacemaker were banned in RFA treatment. Thus, despite the RFA treatment of lung cancer has achieved good results; we still need a systematic study on microwave ablation on the treatment of lung cancer.

This study aims to further investigate the application of MA in treatment of lung cancer using the rabbit lung VX2 tumor model we have recently developed to evaluate the feasibility and safety of this technique. We hereby report the imaging and pathological changes, complications, and survival duration of the animals after MA. There have been few reports of microwave ablation for treatment of lung cancer. Considering the "oven effect" in the lung, whereby the air (high resistance) in the lung tissue surrounding the tumor (low resistance) traps heat within the targeted tumor by posing an insulating effect [13], we hypothesize that microwave ablation should also work well or even better than RFA for lung cancer treatment.

Materials and methods

Animals

Twenty-six New Zealand white rabbits, weighing 2.5-3.0 kg, that have been successfully implanted with VX2 tumors were randomly divided into experimental group (n = 14) and control group (n = 12). A pre-operative CT scan with contrast enhancement was performed for all animals in the experimental group. Ultrasound-guided (tumor involving the pleura, n = 10) or CT-guided (tumor without involving the pleura, n = 4) microwave ablation was then conducted, based on the location of the tumor. Animals in the control group did not receive any treatment.

Microwave ablation

KY-2000 Microwave Ablation System (Kangyou Applied Research Institute, Nanjing, China) with 2450 MHz microwave antenna with internal water-cooled shaft was used for tumor ablation. The output power can be adjusted from 10 to 100 watts in a continuous mode ranging from 0 to 6000 seconds. The length of the metal part of the antenna is 10 cm, and it has a diameter of 1.9 mm with a special coating on the surface that prevents tissue adhesion during high heat, and a sharp conical tip that is designed for percutaneous puncture into deep tissue. The internal shaft is continuously cooled by water circulation driven by BT01-100 peristaltic pump (Baoding Longe Constant Pump Co., Ltd, Hebei, China) to reduce the surface temperature of the antenna to prevent skin burn (30-35 ml/min; room temperature distilled water is used).

Before ablation, the animals were anesthetized by intramuscular injection of Sumianxin II (0.1~0.2 ml/kg). Each animal was then fixed on the operating table, and the skin was prepared for the thoracic surgical ablation. Acuson Sequoia 512 Ultrasound System was used for ultrasound-guided ablation (6L3 linear transducer with puncture guide, frequency bandwidth 4.0~6.0 MHz). Following determination of the direction and depth of needle placement by measuring the size and blood supply of the tumor, the microwave antenna was inserted into the center of the tumor with ultrasound guidance, and the tumor was ablated at 30 W for 3 min, with ultrasound monitoring during ablation. For CT-guided ablation, the direction and depth of needle placement were determined by the result of CT scan. After insertion of the microwave antenna, CT scan was conducted to confirm the position of the antenna in the tumor before ablation (**Figure 1**).

After ablation, the animals were given penicillin (200,000 U/day) for 3 days by intramuscular injection to prevent pulmonary infection. CT scan with contrast enhancement was conducted immediately and 3-5 days after ablation.

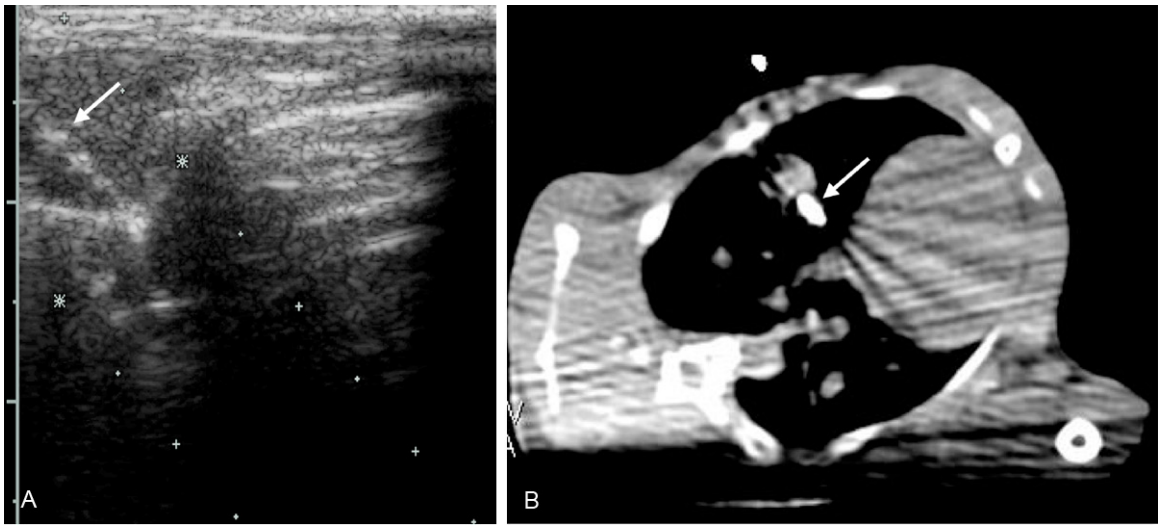

Figure 1. Image-guided MA. A. Ultrasound-guided MA (arrow: microwave antenna); B. CT-guided MA (arrow: microwave antenna inserted in the tumor).

Spiral CT scan

Lightspeed16 Multi-slice CT scanner (GE, USA) was used for the scanning and 3D reconstruction (pitch 1.375:1, and slice thickness/spacing 1.25 mm/1.0 mm). Non-ionic contrast medium Ultravist 300 was used for contrast enhancement (Bayer Schering Pharma) (3 ml/kg, 2.0 ml/s), and CT scan was conducted 30 sec, 1 min, and 3 min after injection of the contrast medium. The Hounsfield Units (HU) was calculated based on the center slices of the tumor. If the center of the tumor was low density, the HU were then calculated based on the rim area. The level of enhancement is calculated as HU of 30 sec, 1 min, and 3 min after injection of the contrast medium subtracted by HU of native scan.

Pathological examination

Two animals were randomly selected for each group and sacrificed immediately and 1 week after ablation, respectively. The whole right lung was removed and the area of ablation was measured. The samples were fixed in 4% paraformaldehyde solution for 48 hours, paraffin-embedded, continuously sectioned (5 um), H & E (hematoxylin & Eosin)-stained, and examined under optical microscope.

Observation of animal behavior and life span

After microwave ablation, the daily activity and eating behavior of the animals were observed. The life spans of the animals were compared between the experimental group and control group.

Statistical analysis

SPSS13.0 software was used for statistical analysis. Kaplan-Meier method was used for survival analysis. $P < 0.05$ was considered to be statistically significant.

Results

Out of 14 animals in the experiment group, 10 were given ultrasound-guided microwave ablation because the tumor had involved the pleura, and the other 4 had CT-guided ablation because the tumor was confined in the lung and the air in the lung would interfere with ultrasound imaging.

General observation and life span of the animals

All animals survived the surgery. The eating and activity of most animals were reduced during post-operative 1-3 days, but become better thereafter. Six animals had slight pneumothorax, 1 had very severe pneumothorax (lung compression over 80%), 1 had small amount of bleeding in the lung, and 1 had slight burn of the skin, but all those animals recovered in 3-5 days without any treatment.

The survival duration of the animals after the implantation of the tumor was 23-56 (mean 42.6 ± 9.7) days and 19-48 (mean 33.8 ± 9.3)

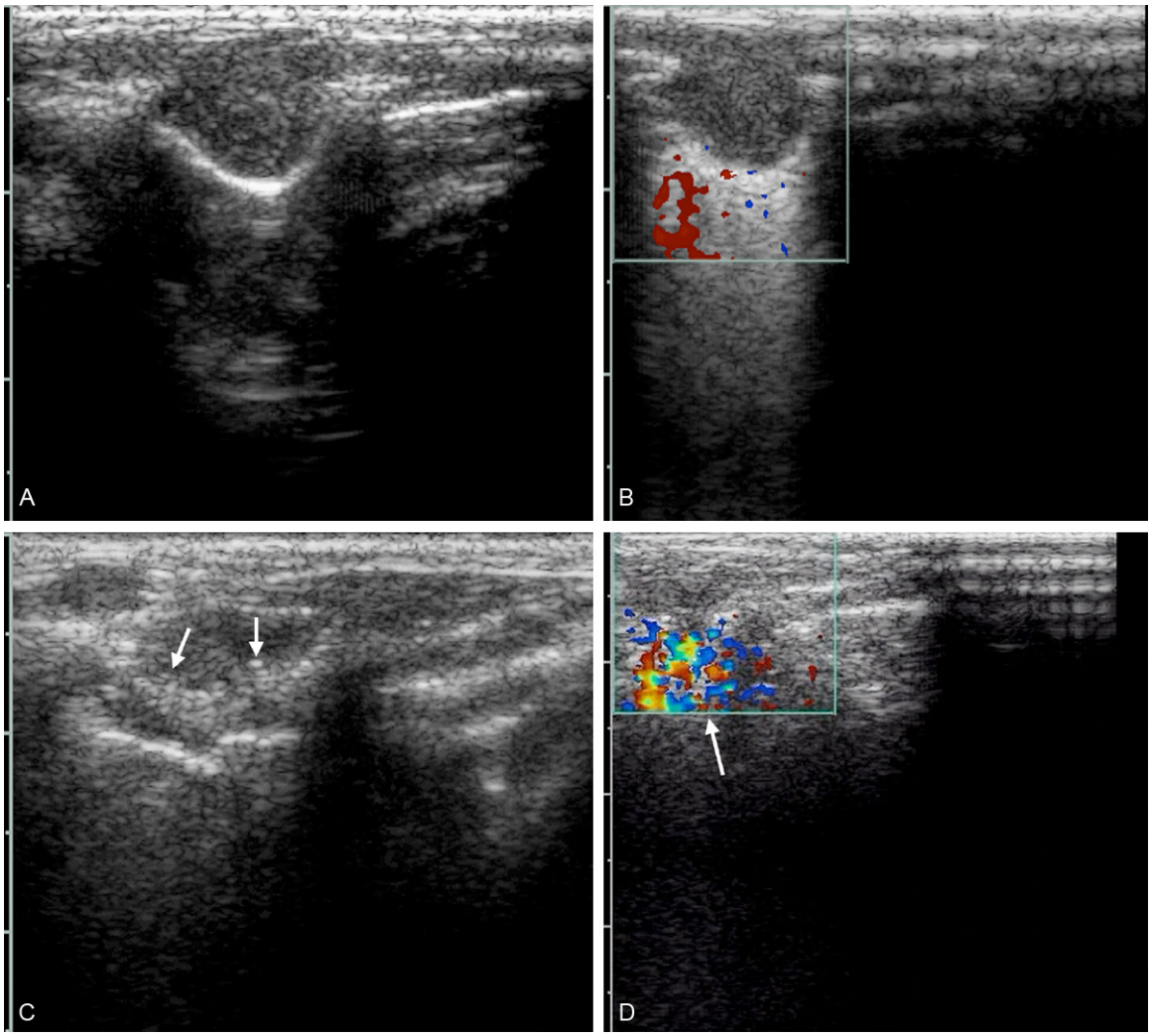

Figure 2. Ultrasound before and during MA. A. Gray scale ultrasound before MA: hypoechoic nodule with clear margin and even echo density inside the tumor; B. CDFI before MA: no significant blood flow signal. C. Gray scale ultrasound during MA: vacuole-like hyperechoic reflection (arrows); D. CDFI during MA: heat energy (arrow).

days in the experimental group and the control group, respectively, excluding the 4 animals in the experimental group that were sacrificed immediately and 1 week after ablation for pathological evaluation (censored data). Statistical analysis showed that the survival time of the animals in the experimental group was significantly longer than that in the control group (Log Rank test, P = 0.026).

Ultrasound

Before ablation, ultrasound showed a hypoechoic nodule (0.7 × 0.4 cm~1.1 × 0.8 cm) with clear margin and even echo density inside the tumor. No significant blood flow signal was observed in the tumor by Color Doppler Flow Imaging (CDFI) (**Figure 2A** & **2B**). During ablation, as soon as the microwave ablation started there appeared vacuole-like hyperechoic reflection around the microwave antenna spreading to the periphery, and the hyperechoic area was gradually enlarged with increase of the ablation time, which was clearly shown as heat energy by CDFI (**Figure 2C** & **2D**) (1.4 × 0.6 cm~1.6 × 1.0 cm).

CT scan

Before ablation, CT scan showed single round- or oval-shaped, or lobulated nodule with spiculated margin and slight to intermediate enhancement inside the tumor. After ablation, the lesion showed ground-glass opacity of var-

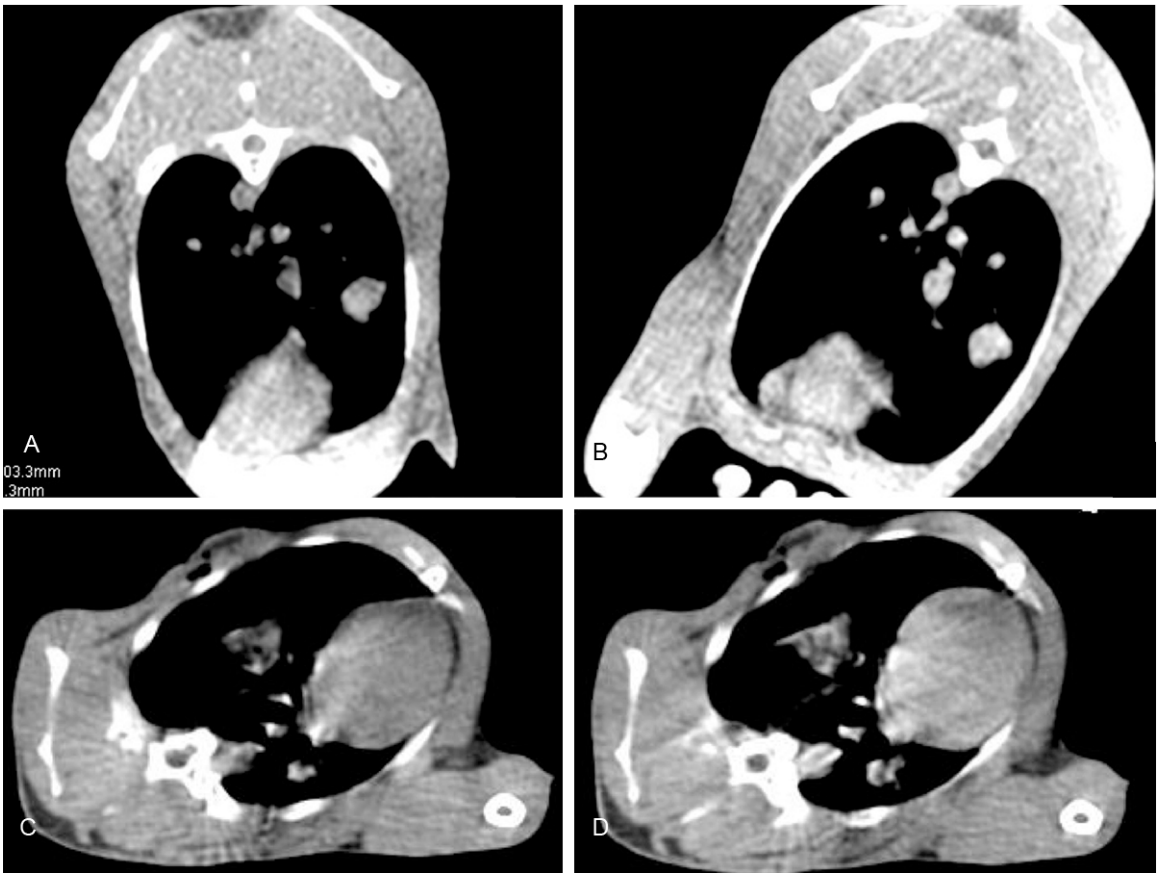

Figure 3. CT scan before and after MA (mediastinal window). A. Natural scan before MA: single round-shaped, lobulated nodule with spiculated margin; B. Enhancement before MA: slight to intermediate enhancement inside the tumor; C. Natural scan after MA: ground-glass opacity of varied densities with blurry margin; D. Enhancement after MA: no enhancement in the center with slight to intermediate enhancement in the peripheral rim.

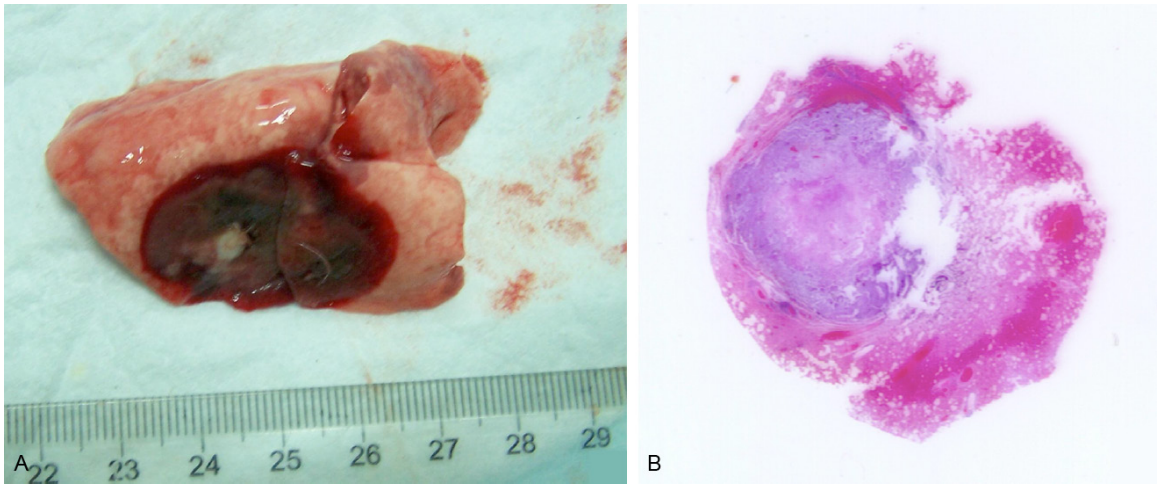

Figure 4. Gross pathological examination immediately after MA. A. Gross examination: whitish tumor nodule and peripheral rim of sable coagulation area surrounded by salmon pink hyperemic reaction zone with edema; B. H & E staining.

ied densities with blurry margin on natural CT scan, and there was no enhancement in the center but slight to intermediate enhancement in the peripheral rim (**Figure 3**).

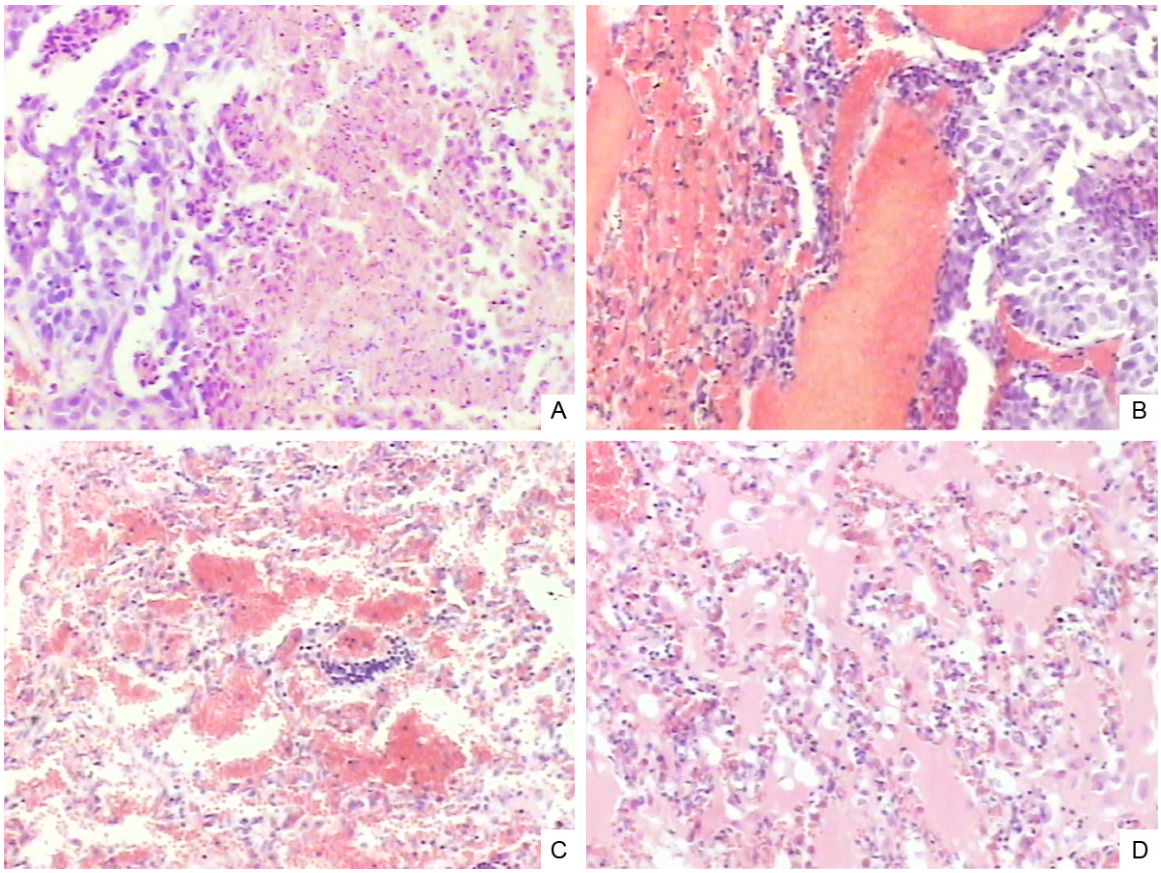

Figure 5. Microscopic examination immediately after MA (H & E, 100X). A. Coagulation necrosis in the center of tumor; B. Peripheral rim and hemorrhagic zone; C. Infiltration of lymphocytes in the hemorrhagic zone; D. Large amount of liquid exudates in the surrounding alveoli.

Pathological findings

Gross examination revealed a whitish nodule of the tumor, and a peripheral rim of sable coagulation area surrounded by salmon pink hyperemic reaction zone with edema immediately after ablation (**Figure 4**). The biggest ablation area was 2.9 × 1.6 cm. One week after the ablation, the necrotic area became yellowish, and the congestion and edema were significantly reduced in the peripheral rim, which gradually became indistinguishable from the surrounding normal lung tissue. Light microscopic examination showed coagulation necrosis in the center of tumor after ablation, with disrupted cell morphology and fragmented nuclei. The cell morphology was preserved in the peripheral rim, and a large number of erythrocytes and infiltration of lymphocytes were observed in the alveoli in the hyperemic reaction zone, which were surrounded by alveoli with large amount of liquid exudates (**Figure 5**). One week after the ablation, the tumor cells in the peripheral rim showed coagulation necrosis with fragmented nuclei, and the liquid exudates in the alveoli became much less, but lymphocytes were still seen. There were significant inflammatory cell infiltration and liquid exudates in the bronchial lumen (**Figure 6**).

Discussions

Surgical intervention in treating lung cancer patients is usually regarded as the preferred therapy. However, in clinical practice only about one-third of patients are eligible for surgical intervention because the majority of lung cancer patients are older than 70 to 75 years and frequently have concomitant cardiopulmonary and/or other diseases [14]. Unfortunately, chemotherapy and external beam radiation have not greatly improved the prognosis of patients with nonresectable disease, but contribute to substantial toxicity especially for those with

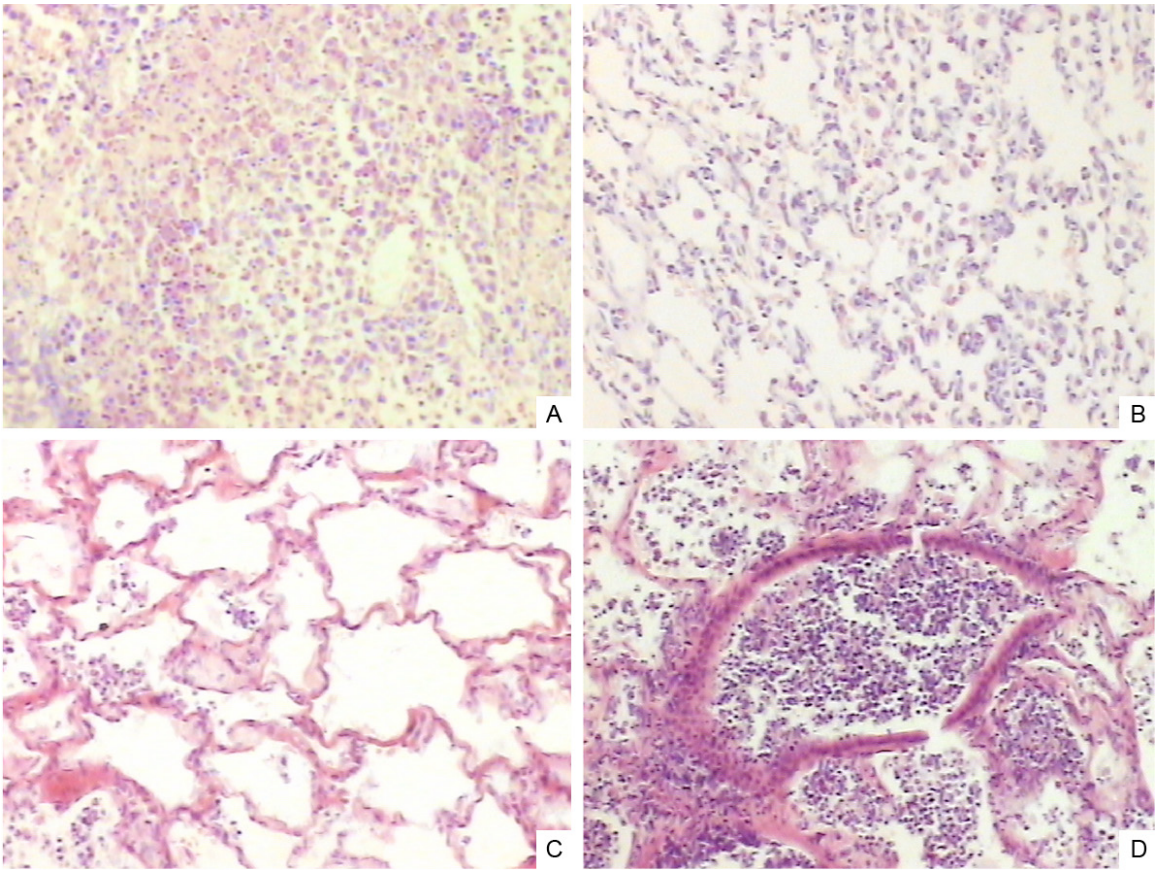

Figure 6. Microscopic examination 1 week after MA (H & E, 100X); A. Coagulation necrosis in the center of tumor with fragmented nuclei; B. Liquid exudates decreased in the surrounding alveoli; C. Infiltration of lymphocytes still seen; D. Inflammatory cell infiltration and liquid exudates in the bronchial lumen.

comorbidities [15]. Percutaneous image-guided ablation with thermal energy sources such as RF, MA, high-intensity focused ultrasonography (US) and laser have recently received increasing attention as minimally invasive strategies for local treatment of solid malignancies [16-19]. Lung tumors seem to be suitable for thermal ablation because the surrounding air in the adjacent normal lung parenchyma provides an insulation leading to energy concentration within the tumors [13].

For these reasons, we conducted Image-guided MA in 14 rabbits with implanted VX2 tumor. All animals survived the surgery, and the animals that have received MA demonstrated a significantly longer life span than the control animals that received no treatment. Our results have indicated that MA might be an effective and safe way of treating lung cancer.

The risk of complications may be minimized by reducing the number of puncture, based on our experience. In addition, a study on RFA suggests that some complications are related to the tumor location. For example, the thermal injury of bronchi during the ablation can induce the coagulation necrosis of bronchial wall and result in bronchial obstruction and obstructive pneumonic consolidation.

In this study, there were not any peri-operative treatment in these animals, and the emission power was 30 W and ablation lasted for 3 min. Gross examination revealed a whitish nodule of the tumor, and a peripheral rim of sable coagulation area surrounded by salmon pink hyperemic reaction zone with edema immediately after ablation. These findings confirmed the feasibility of MA. In addition, with respect to the normal morphology of the tumor cells in the peripheral rim under optical microscope after MA, which is probably resulted from the heat-fixing effect, those “normal-looking” cells actually have granular degeneration in both the

cytoplasm and nucleus with plasma membrane disruptions under electronic microscope, and they eventually undergo necrotic changes over time [20]. In clinical practice, CT or MRI is usually used for evaluation of the effect of the ablation [21], but sometimes it is probably hard to tell apart the inflammatory response from remaining tumor tissue by the enhancement in the peripheral rim. This problem may be solved by using PET (positron emission tomography).

One of the limitations of our study is that we did not evaluate the relationship between the output power and the size of ablation area. The reason that high output power was not used is that the rabbit lung is not big enough. The purpose of this study was to assess the feasibility and safety of percutaneous MA of the lung tumor. The safety of procedure cannot be ensured if the output power is too high. So the emission power was set at 30 W. However, the size of tumors in clinical cases is usually 3 cm or larger. Further studies are required to investigate the selection of appropriate output power for ablation according to the lesions. Another limitation is that it is difficult to evaluate the pain and fever in animal models, which, however are frequently encountered in the treatment of liver cancer. CT scan is a useful method to determine the location of probe and the complications.

Conclusion

Our study has demonstrated that it is feasible and safe to perform Image-guided percutaneous MA of the lung tumor. Although further study is still required in clinical trials, this method represents an effective but less invasive therapeutic technique in treating lung tumors.

Disclosure of conflict of interest

None.

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