Technology in Cancer Research and Treatment
ISSN 1533-0346
Volume 11, Number 4, August 2012

Experimental Study on Pulmonary Cryoablation in a Porcine Model of Normal Lungs

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This study was supported by the Hai zhu District scientific and technological plan (No. 7-2010-6-2012) "Comprehensive research of lung cancer cryotherapy", China.

Objective of this study is to analyze the range of necrosis after using different freezing times and freeze-thaw cycles during percutaneous cryosurgery, in order to create a suggestion for optimizing the technique for lung cryoablation. Six healthy pigs were given a CT scan and histological investigation after percutaneous cryosurgery on both lungs. Three cryoprobes were inserted into both the left and right lungs of each pig, respectively. Cryoablation was performed with two cycles of an active 10-minute freezing using argon in the left lung, each freeze followed by an active 5-minute thaw using helium. In contrast to the left lung cryoablation, the right lungs underwent 3 cycles of freeze/thaw, the first and second cycles consisted of an active 5-minute freezing followed by an active 5-minute thaw, and the third cycle of 10-minute freezing and an active 5-minute thaw. The CT imaging change of an ice ball was continuously observed. The lung tissues were taken 4 hours after cryosurgery on day 3 and on day 7, respectively, for pathological observation. One pig presented acute symptoms including bradycardia and hypothermia 30 minutes after cryosurgery, and died 4 hours after the freezing, and the other 5 pigs experienced a weak condition for 4-6 hours and then exhibited relatively normal behavior and regularly took food. The freezing area (ice ball) on CT imaging during the cryoablation grew gradually in relation to the increase over time, and along with the increase in the number of cycles. The size of the cryolesion on the lung samples became larger than the ice ball during cryosurgery, regardless of whether 2 or 3 freeze-thaw cycles were performed. The area of necrosis histologically gradually increased for the time being. Percutaneous cryosurgery on the lung can achieve complete ablation of targeted tissue. Three freeze-thaw cycles are recommended, and the range of cryoablation may not be mandatory "1 cm safe border" during cryosurgery in order to avoid harming the organ and tissue which is close to the cancer. Correct use of the technique is especially important to treat the lung neoplasms, especially the malignant tumors, which are close to the heart and large vessels.

Key words: Pulmonology; Lung; Surgical oncology; Minimal invasive surgery; Percutaneous cryosurgery; Cryoablation; Cryotherapy; Experimental study.

Introduction

A new way of thinking must determine the present and future of cancer research and treatment options. The use of low temperatures to destroy normal and pathological tissues, the basis of cryosurgery, is now being successfully applied in many branches of medicine, included the treatment of primary lung cancer and secondary pulmonary metastases (1, 2).

The use of cryosurgery in thoracic medicine is a relatively recent development compared to other medical specialties where the lesions are easily accessible.

Abbreviations: Radiofrequency (RF); Ultrasonography (US).

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In fact, thoracic medicine is reliant on endoscopes with a very narrow bore in order to treat tracheal or bronchial lesions (3).

Recently percutaneous cryosurgery is a minimally invasive technique that is used for the treatment of primary and secondary tumors of a variety of solid organs, including lung cancer (4-6). Minimally invasive percutaneous ablative therapeutic procedures for treating lung cancers are currently being studied as treatment alternatives (7). Because of the high incidence of lung cancer and the poor outcome of advanced cases who receive traditional therapies (8), percutaneous cryoablation of the lungs has been a great development.

In vitro and *in vivo* experiments paved the way for cryosurgical clinical investigations and for the development of different cryosurgical approaches for treating lung cancer and pulmonary metastases (3, 6, 9). The process of freezing and the mechanism of damage during cryosurgery have been widely described in previous reports (10, 11).

However, unlike other solid organs, lungs contain air which prevents conduction of low temperatures and interferes with the creation of an ice ball. Lungs are also close to the heart and major vessels, and pulmonary cryosurgery will endanger these important structures.

Currently no data is available on experimental imaging and time exposures of percutaneous cryosurgical response in normal and pathological lung tissue when exposed to low temperatures. Neither the *in vivo* sensitivity of the pulmonary parenchyma to different time exposures, nor the histological changes after low temperature exposure, *i.e.,* percutaneous pulmonary cryosurgery, *in vivo*, have as yet been reported in medical literature.

For the first time, this report describes an imaging and pulmonary parenchyma damage following the percutaneous freezing in a porcine model of normal lungs and provides a suggestion for optimizing the technique.

Material and Methods

Experimental Animals

Six certified healthy Tibetan miniature pigs provided by the Animal Experimental Center of South Medical University, weighing on average 25 kg (21-30 kg). For 2 weeks prior to the experiment, the pigs were observed to ensure a normal behavior. An approval of the study was obtained from the hospital's IRB.

Cryoablation Technique

The cryoablation equipment was the Joule-Thomson effect-based cryosurgical system (EndoCare, CA, USA). Pressurized argon gas is used for freezing (up to −150°C), and helium gas is used for thawing (up to 60°C).

The pigs were fixed in a right recumbent position, and preoperative skin preparation and disinfectors were applied. An intramuscular injection with 3 ml of Sumianxin was given to each pig for anesthetic induction. General anesthesia was maintained by endotracheal intubation with inhalation of 1.5-2% isoflurane with oxygen flow maintained at about 1.5 L/min. Three 1.7 mm cryoprobes were percutaneously inserted into the left lung lobe under CT guidance, one in the upper and two in the lower lobe, so as to be well away from each other. Cryoablation of the left lung consisted of 2 cycles of freezing, 10 minutes for each cycle, followed by 5 minutes of thawing. The argon gas output power was set to 100%; the temperature reading of the cryoprobe tip displayed -140 ± 5°C. Ten minutes later, the flow of argon was stopped and helium gas was input into the frozen zone to raise the temperature to 25 ± 5°C. This active thawing was maintained for 5 minutes, and then a second cycle of freeze-thaw was carried out. The freezing process was guided with CT monitoring. After cryoablation of the left lung, the pigs were fixed in a left recumbent position, and three cryoprobes were inserted into the corresponding position of the right lung lobe. In comparison to the left lung cryoablation, the right lungs underwent 3 cycles of freeze/thaw, the first and second cycles consisted of an active 5-minute freezing followed by an active 5-minute thaw, and the third cycle of 10-minute freezing and an active 5-minute thaw. The temperature of freeze-thaw in the right lung was the same as the left lung. During the experiment, cardiopulmonary function was monitored. After removing the probe, the surgical hole was filled with thrombin gelatin sponge. An intramuscular injection of 5 mg of furosemide, 5 mg of dexamethasone, and 160 IU of Penicillin was administered 15 minutes after finishing the cryoablation procedure.

Evaluation

One pig died 4 hours after cryosurgery and an autopsy was carried out. The other 5 were given an appropriate diet and were kept under close observation, including behavior and cardiac-breath function, until they were to be put down. Two pigs were euthanized using an intravenous injection of 100 mg/kg of pentobarbitone on post-freeze day 3, and the remaining 3 pigs were euthanized in the same way on post-freeze day 7. Both lungs were removed and fixed in 10% formalin for pathologic study. The lung samples were taken 4 hours, 3 days and 7 days after the operation, respectively.

For ease of description, the freezing area on CT imaging during the cryoablation and the lung sample taken after the cryoablation are called "ice ball" and "cryolesion", respectively. The size of the cryolesion was evaluated macroscopically on fixed specimen sections and the diameter of the section was

calculated. Microscopic analysis was carried out on the center, periphery and 1-cm outside edge of the cryolesion, as well as the surrounding areas of the lung that seemed unaffected, for H&E staining. Six samples from each pig were analyzed with 20 magnification fields per area.

All continuous variables are expressed as mean ± SD. The SPSS statistical package version 13.0 (SPSS Inc., Chicago, IL) was used to analyze the results. The paired t-test was used to compare continuous variables between and within groups, respectively. A P value of < 0.05 was considered statistically significant.

Results

General Condition

One pig presented acute symptoms including bradycardia and hypothermia 30 minutes after cryosurgery, and died 4 hours after the freezing, and the other 5 pigs experienced a weak condition for 4-6 hours and then exhibited relatively normal behavior and regularly took food. During the first 12 hours after the freezing, the animals had tachycardia on an electrocardiogram and tachypnea, but then the heart rates and breathing rates gradually appeared normal. In all animals, there was no cardiac involvement and no significant damage of lung parenchyma apart from the freezing areas on autopsy.

Ice Ball on CT imagings During the Cryoablation

On the CT the ice ball and cryoprobe were shown as high and higher density shadows relative to the lung parenchyma, respectively, with a relatively vague margin. With the time proceeded, the margin of the ice ball gradually became clearer and the ball expanded in size. Also, ice balls became enlarged in relation to cycles of freeze-thaw. Between the first to 5th minute after the freezing, the ice ball maximum diameter grew from 2.4 cm to 3.2 cm, from 2.9 cm to 3.2 cm and from 3.5 cm to 3.3 cm during the first, second and third cycle of freeze-thaw, respectively (Figure 1).

Cryolesion on Lung Samples in Comparison to Ice Ball on CT Imagings

The lung samples of pigs were taken 4 hours, 3 days and 7 days after cryosurgery. Macroscopically, the cryolesions appeared black with a well-defined circumscription from surrounding tissue (Figure 2). The maximum diameter of right lung cryolesions with 3 freeze-thaw cycles was larger than that of the left lung cryolesions with 2 cycles ($P < 0.001$). Moreover, the cryolesions in all the samples taken 4 hours, 3 days, or 7 days after the cryoablation were larger than those of ice balls shown by CT during the freezing ($P < 0.001$) (Table I).

Microscopically Necrotic Area in Comparison to Macroscopically Cryolesion

Microscopically uniform hemorrhage and necrosis were seen both in the central and at the periphery zones of cryolesion of all the samples, apart from those taken at postoperative 4 hours showing few necroses at the edge of cryolesion. In other words, histologically necrotic area was enlarged over time after freezing up to the 3rd day when microscopically necrotic areas were equal to the cryolesion area (Figure 3).

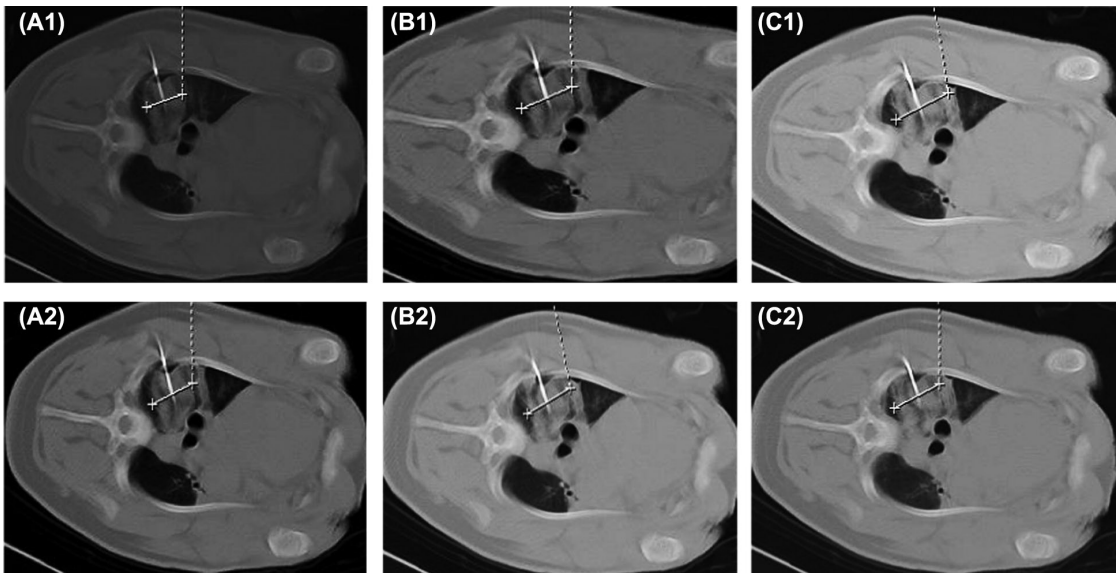

Figure 1: The lung CT imaging after cryoablation. The ice ball grew gradually in relation to the increase in time and cycles. Pictures show the ice balls at the 1st and 5th minute after freezing, respectively. (**A1**) and (**A2**): the first cycle; (**B1**) and (**B2**): the second cycle; (**C1**) and (**C2**): the third cycle.

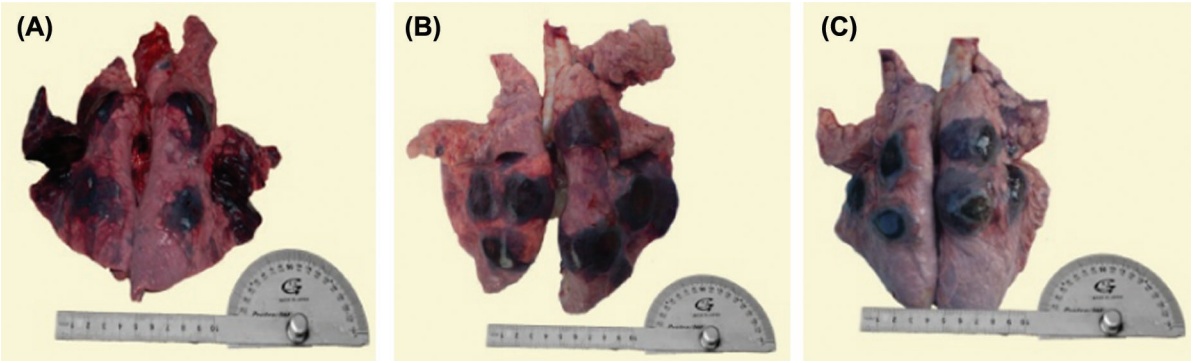

Figure 2: The lung samples taken at different post-cryoablative time. (**A**) 4 hours after cryoablation; (**B**) 3 days after cryoablation; (**C**) 7 days after cryoablation.

Discussion

Image-guided percutaneous thermal ablation is increasingly being used as an alternative for patients with local lung cancers not amenable to surgery. Radiofrequency ablation of lung neoplasms has found widespread use, and is currently the most commonly utilized thermal ablation method (12, 13). Radiofrequency (RF) ablation has been shown to be effective and safe in the treatment of various stages of non small cell lung cancer and pulmonary metastases limited to the lung (12). Preliminary studies suggest that cryoablation has potential for this application (5, 15). Cryoablation may be preferable to RF ablation for tumors adjacent to mediastinal structures since cryoprobes can be placed close to the mediastinal and hilar vessels to intensify the freezing and to avoid perfusion-mediated heating without fear of vessel damage, because the collagenous architecture of the vessel wall is preserved (14). Moreover, unlike RF ablation, cryoablation allows intraprocedural monitoring. The ice ball can be seen on all cross-sectional imaging modalities including ultrasonography (US), CT, and MRI to maximize the chance of treating the tumor completely and to avoid complications. However, the feasibility and efficacy of this technique for lung tumors have not been established.

We have carried out CT guided percutaneous lung cryoablation on 6 normal pigs, of which 5 had a smooth post-operative process until they were euthanized, and one pig developed an acute reaction after cryoablation and died 4 hours later. All animals showed a visualized ice ball on CT during the cryoablation, clear cryolesion of lung samples on autopsy and histological cryonecrosis. The preliminary data show that image-guided percutaneous cryoablation of the lungs is feasible and minimally with satisfactory local ablation for lungs.

Table I
The maximum diameter of the ice ball and cryolesion (cm, mean ± SD).

	n	Ice ball	Cryolesion	*P*
Right lung	18	2.7 ± 0.3	3.7 ± 0.7	<0.001
Left lung	18	2.3 ± 0.3	3.1 ± 0.6	<0.001
Both lungs	36	2.5 ± 0.4	3.4 ± 0.7	<0.001

This experimental study focuses on technical issues of percutaneous pulmonary cryoablation. Our study shows that (1) The ice ball shown on the CT is gradually expanded over time proceeded; (2) Three cycles of freeze-thaw yield a larger ice ball compared with two cycles; (3) The size of cryolesion macroscopically is larger than that of the ice ball showed on CT imaging, suggesting that cryolesion after finishing cryoablation continues to grow; and (4) On the 3rd and 7th day after cryoablation, all the cryolesions, including periphery areas, appear histologically to be necrotic.

Clinically, the efficacy of cryoablation is dependent upon several parameters, including the end temperature, number of freeze-thaw cycles and range of the freezing. From our study, pulmonary cryoablation seems to be rather special compared with cryoablation of a solid organ, such as the liver and kidney, in the technology.

According to cryoablation for solid organs, after one cycle of freeze-thaw, most specimens are necrotic, and some are still viable. In contrast, there are no specimens that have viable cells following two cycles. A third freeze-thaw cycle cannot further increase the efficiency of cryoablation (14-16). However, lung cryosurgery is exceptional. Lungs are air-containing organs. Because the air prevents conduction of low temperatures and there is not enough water in the parenchyma, when the cryoprobe is inserted into the normal lung parenchyma, initial freezing can make a small ice ball only. However, after thawing, the massive intra-alveolar hemorrhage excludes the air and results in a larger ice ball that forms in the following cycle of freeze-thaw (17). Therefore, for cryosurgery of lung cancer, 3 freeze-thaw cycles should be performed. Kawamura *et al*. (18) using 3 freeze-thaw cycles to make an ice ball of 2.5 to 3.0 cm in diameter, treated 35 tumors in 20 patients with pulmonary metastases, achieving one-year survival of 89.4% according to the Kaplan-Meier method.

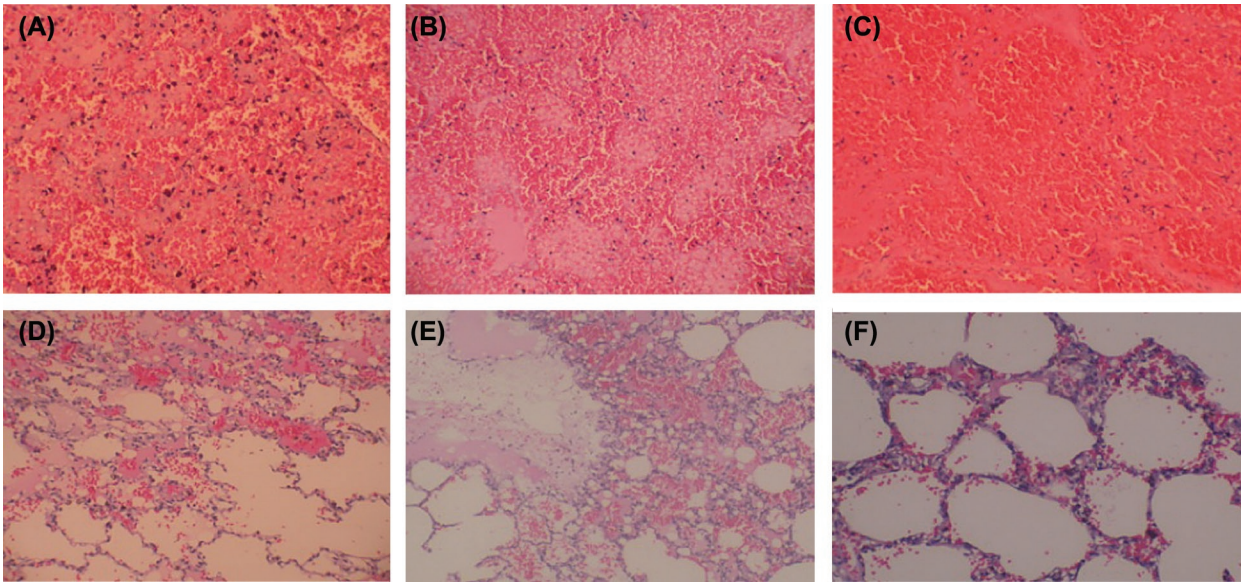

Figure 3: The pathological features of lung cryolesion. H&E staining, magnification 20×. After cryoablation, the central region of lung cryolesion taken 4 hours, 3 days and 7 days postoperatively was showing hemorrhage and necrosis (**A**-**C**), but at the periphery of the cryolesion the necrosis was seen only in lung samples taken 3 days and 7 days after cryoablation (**D**), while lung samples taken 4 hours after cryoablation only showed vasodilatation, edema and hemorrhage without necrosis at the periphery of the freezing area (**E**). At 1 cm outside edge of cryolesion, alveolar structure was still maintained and part alveolar septum capillaries congested with little hemorrhage (**F**).

In this experiment, cryoablation for the left lung was designed as two cycles of a 10-minute freezing, whereas the experiment on the right lung involved two cycles of a 5-minute freezing and a third cycle of a 10-minite freezing. It seems that the cryoablation outcome largely depends upon the number of cycles, not the freezing time if the time is over 5 minutes. This is a topic to be further studied.

Temperatures lower than −40°C are considered necessary to cause the irreversible necrosis of the target cells. According to liver freezing, the diameter of the zone of −40°C or less was smaller than ice ball diameters (19), *i.e.*, at the peripheral zone (freeze margin) cell destruction may initially be incomplete (20, 21). Therefore, it is suggested that the treated tumor is frozen with an additional 1 cm or more of margin ('safety margin') of normal tissue. However, for pulmonary cryoablation, the entire ice ball, including its peripheral zone, showed complete necrosis seen in the lung sample taken on the 3rd day after cryoablation. The fact indicates that the pulmonary alveolus tissue seems more susceptible to freezing compared to solid organs, and therefore, there is more severe cell destruction even if at the peripheral zone in which the temperature is not lower than the critical point to induce cell necrosis. Clinically, for cryoablation of a lung tumor there may not be a need to have a "1 cm safety margin", hence reducing the destruction of normal lung parenchyma, avoiding injury to the adjacent organs and tissue and minimizing the post-procedural adverse effects.

Our study has an essential defect in that the cryoablation was performed on normal lungs and not on a lung tumor which is solid. Further experimentation on a lung cancer model is needed to solve the problem. Our clinical experience on cryoablation of centrally located lung cancer showed that some cases, in whom cryoablative range has not covered the so-called safety margin because of the tumor being close to the important structures such as the heart or aorta, have achieved complete or sub-complete response and longer-term disease-free survival (22-24). This may show the particularity of pulmonary cryoablation.

In conclusion, the experimental study suggests that the image-guided percutaneous cryoablation of lungs is a feasible method. With the refinement of the technology, it is believed that the miniinvasive method could be established as a part of the complex oncological management in patients with primary lung malignancies or secondary pulmonary malignant lesions.

Acknowledgement

This work was supported by the national building foundation for Key Clinical Program (oncology) of the China Ministry of Health. We also thank Miss Chunmei Deng and Chunjuan Deng for their technical support, and Dr. Jing Wang and Dr. Lihua He for data management.

This material is an original report, funded by project of "Synthetic study of crysurgery for lung cancer" (2010-T-25, 7/2010-6/2012), Science and Technology Bureau, Haizhu District, Guangzhou, China. It has not been previously

published or submitted elsewhere for publication and will not be sent to another journal until a decision is made concerning publication by Technol Cancer Res Treat. There is none personal conflicts of interest of any of the authors.

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Received: June 9, 2011; Revised: January 8, 2012; Accepted: January 10, 2012