LABORATORY INVESTIGATION

Comparison of Double-Freeze versus Modified Triple-Freeze Pulmonary Cryoablation and Hemorrhage Volume Using Different Probe Sizes in an In Vivo Porcine Lung

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ABSTRACT

Purpose: To determine size of ablation zone and pulmonary hemorrhage in double-freeze (DF) vs modified triple-freeze (mTF) cryoablation protocols with different probe sizes in porcine lung.

Materials and Methods: In 10 healthy adult pigs, 20 pulmonary cryoablations were performed using either a 2.4-mm or a 1.7-mm probe. Either conventional DF or mTF protocol was used. Serial noncontrast CT scans were performed during ablations. Ablation iceball and hemorrhage volumes were measured and compared between protocols and probe sizes.

Results: With 1.7-mm probe, greater peak iceball volume was observed with DF compared with mTF, although difference was not statistically significant (16.1 mL ± 1.9 vs 8.8 mL ± 3.6, $P = .07$). With 2.4-mm probe, DF and mTF produced similar peak iceball volumes (14.0 mL ± 2.8 vs 14.6 mL ± 2.7, $P = .88$). Midcycle hemorrhage was significantly larger with DF with the 1.7-mm probe (94.3 mL ± 22.2 vs 19.6 mL ± 2.1, $P = .02$) and with both sizes combined (93.2 mL ± 17.5 vs. 50.9 mL ± 12.6, $P = .048$). Rate of hemorrhage increase was significantly higher in DF (10.4 mL/min vs 5.1 mL/min, $P = .003$). End-cycle hemorrhage was visibly larger in DF compared with mTF across probe sizes, although differences were not statistically significant ($P = .14$ for 1.7 mm probe, $P = .18$ for 2.4 mm probe, and $P = .07$ for both probes combined). Rate of increase in hemorrhage during the last thaw period was not statistically different between DF and mTF (3.0 mL/min vs 2.8 mL/min, $P = .992$).

Conclusions: mTF reduced rate of midcycle hemorrhage compared with DF. With mTF, midcycle hemorrhage was significantly smaller with 1.7-mm probe; although noticeably smaller with 2.4-mm probe, statistical significance was not achieved. Iceball size was not significantly different across both protocols and probe types.

ABBREVIATIONS

ARC = Animal Research Committee, DF = double-freeze, F = freezing, mTF = modified triple-freeze, PT = passive thawing

Image-guided cryoablation has become an accepted treatment option to manage early-stage primary lung carcinoma and lung metastases in select patients (1,2). It is also an option for patients with multiple early-stage lung cancers in an effort to preserve lung function. Cryoablation relies on applying freezing temperatures (< −40°C) and inducing tissue injury through several mechanisms, including protein denaturation, cell rupture secondary to osmotic shifts, and microvascular thrombosis (3). Traditionally, a double-freeze (DF) (freeze-thaw-freeze-thaw) cycle has been used for tissue cryoablation (4); however, many studies have shown that different tissues react differently to the application of

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ablative energies (3,5), and parameters such as duration, lethal isotherm, and number of freeze-thaw cycles can affect the extent of tissue ablation (3,6–8). Given the small amounts of water per unit volume and low thermal conductivity of the aerated lung parenchyma, optimal ablation parameters in the lung are different from those in solid organs. Several studies have investigated this issue in porcine models (7–9). Hinshaw et al (8) reported that using 3 freeze-thaw cycles led to a larger ablation zone with expanded cytotoxic isotherms. This phenomenon has been ascribed to increased thermal conductivity of lung parenchyma owing to filling of the alveolar spaces with fluids after the first thaw (7). In addition, the triple-freeze protocol results in earlier visualization of iceball formation on computed tomography (CT) images compared with the DF protocol (8). Considering the difficulty in visualizing iceball formation in lung parenchyma on CT images until the initial thaw (10), using this modified protocol may allow for earlier monitoring of the ablation zone with respect to the tumor margins.

Previous studies have focused primarily on optimizing the ablation zone and the ablative effect on tissue, whereas pulmonary hemorrhage from cryoablation has not been extensively evaluated. Pulmonary hemorrhage often becomes clinically evident during the first thaw cycle and may progressively increase during the cryoablation (4,11). Clinically, this may account for hemoptysis, which can occur during and after lung cryoablations (12). Excessive pulmonary hemorrhage may limit safe completion of the desired cryoablation regimen. This study compared both the size of iceball formation and the extent of pulmonary hemorrhage between the conventional DF and the modified triple-freeze (mTF) cryoablation protocols. In addition, 2 different cryoablation probes were used in each protocol to explore the potential effect of probe size on ablation zone and pulmonary hemorrhage. The specific aims of this study were (*a*) to determine the ablation zone of DF versus mTF pulmonary cryoablation protocols with different probe sizes and (*b*) to determine the pulmonary hemorrhage induced by 2 protocols with different probe sizes.

MATERIALS AND METHODS

Cryoablation of Porcine Pulmonary Tissue

This study was approved by the institutional Animal Research Committee (ARC). Ten healthy adult Yorkshire pigs were used in this study (2 females and 8 males; age range, 88–149 days; weight range, 24.5–51.8 kg) (**Table 1**). Surgical anesthesia was induced in compliance with ARC policies. For initial sedation, the animal was administered 1–4 mg/kg of tiletamine (Telazol; Zoetis Inc, Parsippany, New Jersey) intramuscularly. The animal was then intubated. Anesthesia was maintained by inhalation of 1%–2.5% isoflurane in oxygen at a flow rate of 2 L/min. Blood pressure and electrocardiogram tracings were monitored continuously. Once the pig was stabilized, it was placed in a CT gantry in prone position. Planning noncontrast CT images were obtained through the chest. Two clinically available sizes of percutaneous cryoprobes, 2.4 mm (PCS-24; Endocare Inc, Irvine, CA) and 1.7 mm (PCS-17; Endocare Inc) in diameter, were used. Cryoprobes were inserted in the target lobe in a planned pathway and positioned with a posterior and medial trajectory. The probes were inserted as deep in the lung parenchyma as possible to achieve the full ablative potential, without causing injury to vital structures or major vessels, and at least 1 cm away from the lobar vessels. Manipulation of probes was limited to avoid pulmonary hemorrhage from probe placement. Passive thaw was achieved by turning off the gas flow and allowing for the cryoablation zone to melt passively. Active thaw was achieved by running preheated helium gas through the probe.

Table 1. Demographics of Yorkshire Pigs Used in Study

Pig	Body weight (kg)	Sex	Age at Procedure (d)
1	39.0	Female	126
2	25.4	Male	90
3	43.6	Female	149
4	51.8	Male	149
5	24.5	Male	114
6	24.5	Male	119
7	25.0	Male	119
8	32.2	Male	88
9	29.0	Male	121
10	33.1	Male	121

Two different cryoablation protocols were used. The conventional DF protocol included 10 minutes of freezing, 8 minutes of passive thawing, and another 10 minutes of freezing (10F-8PT-10F). The mTF protocol included 3 minutes of freezing, 3 minutes of passive thawing, 7 minutes of freezing, 3 minutes of passive thawing, and 10 minutes of freezing (3F-3PT-7F-3PT-10F). The mTF protocol used in this study was similar to a protocol introduced by Hinshaw et al (8) but with the second thaw cycle limited to 3 minutes. This choice was based on anecdotal clinical observations that hemorrhage primarily occurs during the thaw periods, and limiting length of the thaw periods may reduce the hemorrhage size. In both DF and mTF, the total freeze time was 20 minutes, and the longest freeze period was 10 minutes. In both protocols, an additional 10 minutes of active thawing was added at the end to observe the effects of thawing in the lung (**Fig 1**).

Four simultaneous ablations were performed in the first 2 pigs, and 2 simultaneous ablations were performed in the remaining 8 pigs (**Table 2**). An unexpected intraprocedural death occurred in pig 2 after the first freeze cycle. This was communicated to the ARC, and as part of the correction and prevention, the study was converted to 2 simultaneous ablations in the remaining 8 pigs. Pig 2 was eventually excluded from analysis owing to incomplete data. The cause of death was described as cardiopulmonary arrest with pulmonary hemorrhage. In accordance with local ARC