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The safety and efficacy of bladder cryoablation in a beagle model by using a novel balloon cryoprobe

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ABSTRACT

The poor quality of initial transurethral resection (TUR) would leave residual tumor and compromise the prognosis. The common therapeutic option is to perform re-TUR, which adds burden to patients. We assumed that cryoablation could be applied as adjuvant therapy combined with TUR, thus lead to maxium tumor control. In our study, we investigated the safety and effects of focal bladder wall cryoablation in beagle model using a novel cryoablation balloon probe. Temperature was recorded throughout the freeze process in different parts of the bladder. The bladder was harvested immediately, 2 weeks or 3 months after surgery for histological evaluation. The results demonstrated cryoablation using our newly designed probe is safe and effective. Two-minute cryoablation could induce necrosis within 2-cm range in diameter to superficial muscle layer. The findings provided us reference before cryoablation could be applied in clinical practice.

Transurethral resection (TUR) remains golden standard to treat non-muscle invasive bladder cancer. Even in muscle invasive bladder cancer, TUR alone could be used as a method, or as a part of multi-modal therapy to preserve bladder [5,7]. Although standard TUR is required by guidelines, the incomplete resection still remains a great concern and could harm patients' prognosis [2]. Thus, new experimental bladder cancer treatment methods are emerging to overcome the problem.

Transurethral cryoablation is the minimal invasive approach and has the potential advantage of penetrating full-thickness bladder wall. Several animal studies have been reported the feasibility and safety on applying cryoablation in bladder through percutaneous or transurethral approach [3,4,6]. Despite the attempt in animals, concerns such as perforation or adjacent organ injury still exist. The probe in previous reports has several drawbacks. One of the biggest reason is the sharp shape, which was not appropriate for bladder [8]. Therefore, we developed a new cryoprobe (SS160-5006,Shenjiekang, Ningbo) that could be deployed through a standard cystoscopy. Thus, this study was taken to evaluate the safety and efficacy of the cryoprobe, and also to assess the morphological change of bladder following cryoablation.

The cryo-surgery was performed using a liquid nitrogen-based cryoablation unit (Shengjiekang, Ningbo, Zhejiang, China). This unit is composed of a control system and cryoprobe (SS160-5008, Shengjiekang). The cryoprobe, 50 cm in length, has a 6 mm-diameter balloon on the tip. The circulating nitrogen gas could be inflated into the balloon and provides the cold energy. The new design is soft on the tip and is supposed to produce effect to a larger area. We are able to regulate the liquid nitrogen release and control the freeze time through the control system (Fig. 1).

The Ethic Committee of Huashan Hospital approved our study. Fifteen healthy beagle dogs (male, 10–12 kg, aged 8–10 months) were obtained from Experiment Teaching Center, Shanghai Jiaotong University School of Agriculture and Biology. The beagles were divided into three groups: acute, sub-acute and chronic group, with each group five beagles.

After induction of general endotracheal anesthesia, dogs were placed in a supine position. A 5 cm midline incision was made on the lower abdomen. Bladder was identified and a 0.5 cm incision was made on the anterior wall. The cryoprobe was inserted through the opening to the posterior wall of bladder mucosa. In order to mimic the physiological condition, 50 ml saline was added to moderately fill the bladder. The opening on bladder was then

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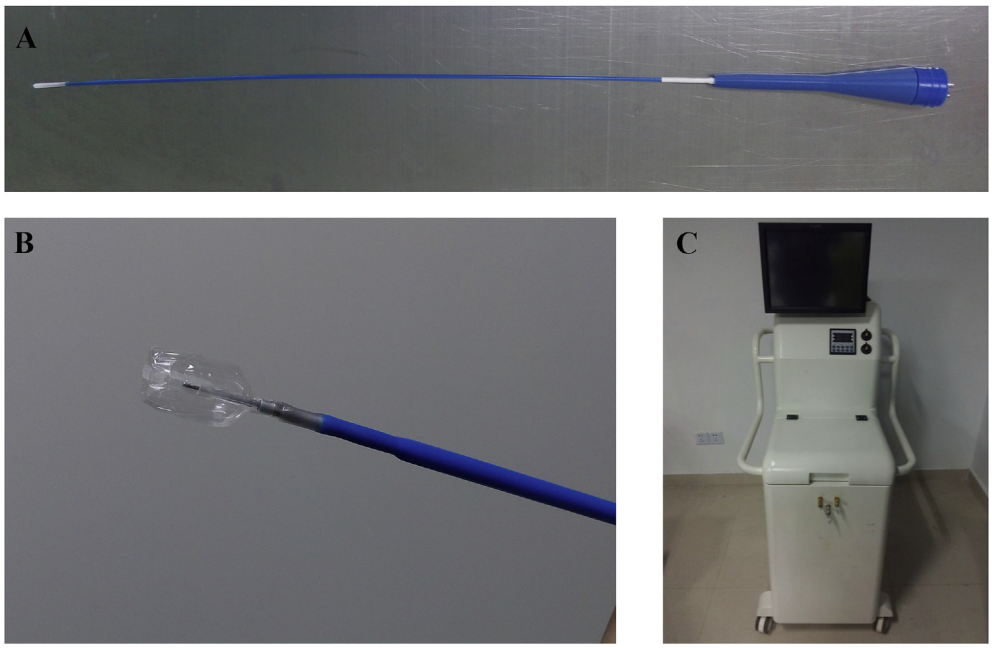

Fig. 1. (A) The picture of the cryoprobe. (B) The picture of the balloon after inflated. (C) The picture of the control system.

closed and ligated with probe using purse-string suture to maintain the bladder filling status. A 2-min cryoinjury was induced by abruptly freezing the mucosa side of the bladder wall. After thawing, the probe was removed out of the bladder.

During freeze, the temperature of different parts of bladder was measured using thermocouple thermometer. A thermometer probe was ligated with the cryoprobe to measure the temperature of freeze center in mucosa side. Other three probes were put in touch with the serosa side of the bladder. They were measuring the temperature opposite the freeze center, 1 cm radius from the center and freeze margin (Fig. 2B). The temperatures were recorded synchronously during the whole process of freeze. We measured the temperature of all fifteen models.

In our experiment, all cryosurgeries were performed successfully without any death or complications such as bladder perforation. The dogs that were planned to survive resumed eating and urination in 24 h after surgery and no wound infection or other bowel complications occurred, which demonstrate that cryoablation was safe and would not cause adjacent organ injury.

During surgery, the cryoprobe was placed inside to the posterior wall of bladder. The iceball was formed inside the bladder and had pale appearance outside the bladder. The iceball in 2 min has a diameter of 2.2 ± 0.3 cm. The temperature in different place of bladder was measured in sync with freeze and thaw. The temperature to time curve was draw according to the average temperature at different time points (Fig. 2A). The lowest temperature was −137.4 °C in 120 s. The temperature in freeze center could be divided into four phases 1) rapid cooling phase, from 0 to 20 s. 2) plateau phase, from 20 s to 120 s. 3) rapid warming phase, from 120 s to 160 s. 4) slow warming phase, after 160 s. On serosa side of the freeze center, the temperature change was similar to the mucosa side. However, on serosa side 1-cm away from freeze center, the lowest temperature was −42.4 °C. On the margin of the iceball, where the serosa appearance turned from rubby to pale, the temperature has just dropped a little from the normal.

Five dogs were sacrificed immediately after cryosurgery to study the acute effect. Five were sacrificed at 2 weeks and the rest five were sacrificed at 3 month to study the sub-acute and chronic effect

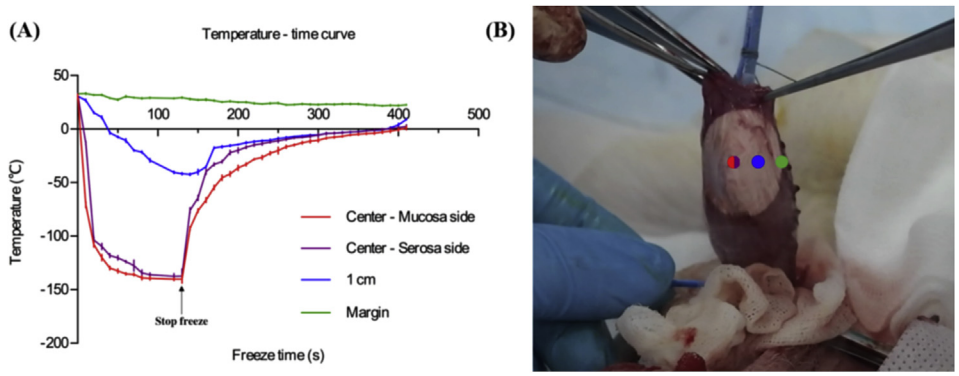

Fig. 2. (A) The average temperature change on different part of bladder during the whole process of freeze and thaw. The lowest temperature reached −137.4 °C in 120 s. **(B)** The appearance of bladder during cryoablation. The temperature measuring point was marked with different colors. Red was on mucosa side of freeze center. Purple was on serosa side of freeze center. Blue was on the serosa side 1 cm away from center. Green was on freeze margin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)