LABORATORY INVESTIGATION

Combination Radiofrequency Ablation and Local Injection of the Immunostimulant Bacillus Calmette–Guérin Induces Antitumor Immunity in the Lung and at a Distant VX2 Tumor in a Rabbit Model

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ABSTRACT

Purpose: To evaluate whether the combination of radiofrequency (RF) ablation and local injection of the immunostimulant *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) induces systemic antitumor immunity.

Materials and Methods: Japanese White rabbits with lung and auricle VX2 tumors were randomized into three groups: control (n = 8; no treatment), RF ablation only (n = 8; RF ablation to the lung tumor), and RF ablation with local BCG injection into the lung tumor (n = 8). Treatments were performed 1 week after tumor implantation. Survival was evaluated with Kaplan–Meier method and log-rank test. Weekly mean volume and specific growth rate (SGR) of auricle tumors were calculated, and comparisons were made by Mann–Whitney test.

Results: Median survival of control, RF-only, and RF/BCG groups were 23, 41.5, and 103.5 days, respectively. Survival was significantly prolonged in the RF-only and RF/BCG groups compared with the control group ($P = .034$ and $P = .003$, respectively), but no significant difference was found between the RF-only and RF/BCG groups ($P = .279$). Only in the RF/BCG group was mean auricle tumor volume decreased 5 weeks after implantation. No significant difference in SGR was found between the control and RF-only groups ($P = .959$), but SGR in the RF/BCG group was significantly lower than in the control group ($P = .005$).

Conclusions: The combination of RF ablation and local injection of BCG resulted in distant tumor suppression compared with the control group, whereas RF ablation alone did not produce this effect. Therefore, the combination of RF ablation and local injection of BCG may induce systemic antitumor immunity.

ABBREVIATIONS

BCG = bacillus Calmette–Guérin, DC = dendritic cell, RF = radiofrequency, SGR = specific growth rate

Previous studies have shown that survival times are longer in patients with resected lung tumor in which infiltrating lymphocytes were confirmed. This finding is indicative of enhanced antitumor immunity control of systemic tumors (1,2). In previous studies, a combination of transcatheter hepatic arterial embolization and the infusion of matured dendritic cells (DCs) in patients with hepatocellular carcinoma has prolonged recurrence-free survival (3). A clinical trial of the intratumoral administration of DCs combined with systemic chemotherapy for esophageal cancer has also been conducted (4).

Recent studies suggest that radiofrequency (RF) ablation enhances systemic antitumor immunity (5). RF ablation causes thermal coagulative necrosis with multiple

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tumor antigens (6), which are captured by antigen-presenting cells such as DCs. These DCs induce tumor-specific T lymphocytes (7–9). However, antitumor immunity induced by RF ablation is weak (9), and it is not well established that systemic tumors can be controlled by RF ablation.

Enhanced antitumor immunity may be able to control systemic tumors. In previous experiments, the combination of RF ablation and local injection of OK-432 induced a systemic antitumor immunity and prolonged survival time (10) because OK-432 stimulates the maturation of DCs (10). *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) is used to treat bladder cancer as an antineoplastic agent, and enhances immunity by promoting maturation of immature DCs (11).

We hypothesized that the systemic antitumor immunity induced by RF ablation would be enhanced by maturation of immature DCs via local injection of BCG. This hypothesis was investigated by performing RF ablation and local injection of BCG in animal models of lung and auricle tumors.

MATERIALS AND METHODS

Experiments were approved by the facility's animal care committee. We used female Japanese White rabbits (body weight, 2.0–2.5 kg). **Figure 1** shows the flowchart of the study design. General anesthesia was induced by intramuscular injection of a 1-mL solution containing 40 mg (0.8 mL) ketamine and 4 mg (0.2 mL) xylazine before procedures, and this solution was intravenously injected during RF ablation as necessary. All procedures were performed by one operator (S.H., board-certified radiologist with 7 y of clinical and experimental experience in interventional radiology) and two assessors (T.O. and A.Y., board-certified radiologists with more than 10 y of clinical and experimental experience in interventional radiology).

Tumor Implantation

Isolation of the VX2 tumors and implantation into rabbits was performed as previously reported (10). Briefly, a single-cell suspension (2.5×10^6 cells per milliliter) of VX2 tumor was prepared, and 0.2 mL of this suspension was injected via a coaxial 20-gauge needle through an 18-gauge needle that was inserted into the lower lobe of the left lung. In addition, 0.2 mL of this suspension was injected hypodermically into the left auricle with a 20-gauge needle. A noncontrast lung computed tomography (CT) scan was performed, and the size of the auricle tumor was measured with calipers at 1 week after implantation (week 1). Establishment of the lung tumor was confirmed as a nodule 3 mm or larger in diameter on CT. The auricle tumor volume was calculated by the sphere formula: 3.14 × length × width × thickness/6. Establishment of the auricle tumor was considered as a tumor 10 mm^3 or larger in volume. Rabbits with both lung and auricle tumors were used as the experimental model.

Study Groups

Implantation of VX2 tumors in rabbits was performed until 24 experimental animals were obtained. These

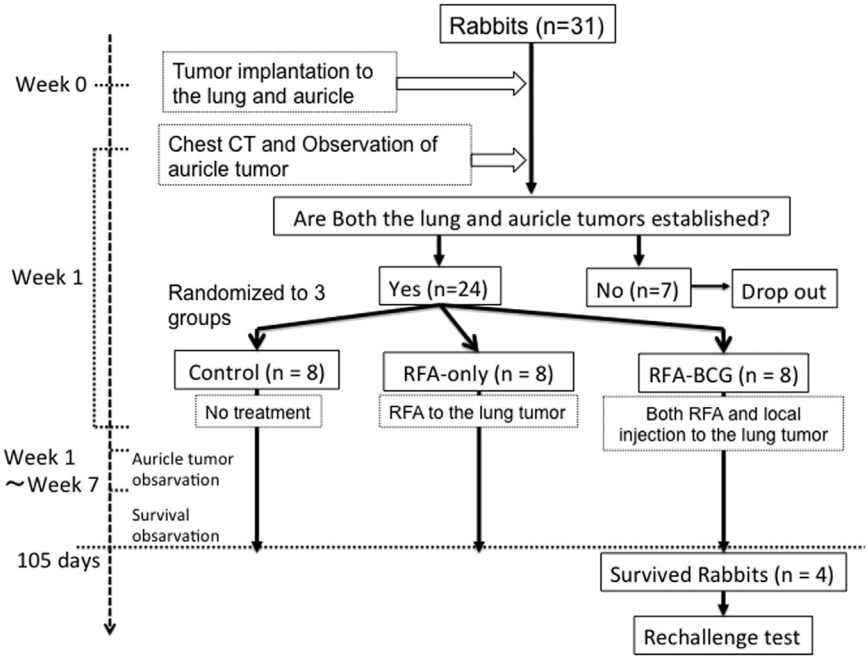

Figure 1. Experimental flowchart. Week 0: implantation of VX2 tumor was performed. Week 1: confirmation of lung and auricle tumor was performed, and animals were randomized into one of three groups (control, RF ablation only, or RF ablation with local BCG injection). Weeks 1–7: auricle tumors were monitored. Survival periods were observed for a maximum period of 105 days. After the observation period, surviving rabbits in the RF/BCG group underwent the rechallenge test.