LABORATORY INVESTIGATION

Stress Test of Contrast-Enhanced US with Phenylephrine in a Rabbit VX2 Liver Tumor Model: Differentiating Benign Periablational Enhancement from Residual Tumor after Radiofrequency Ablation

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ABSTRACT

Purpose: To differentiate benign periablational enhancement (BPE) from residual tumor after radiofrequency (RF) ablation by using a stress contrast-enhanced ultrasonography (US) test with phenylephrine in a rabbit VX2 liver tumor model.

Materials and Methods: VX2 tumors were implanted in the livers of 40 rabbits for two experiments. In experiment one, liver tumors from 32 animals were completely ablated. On days 2, 7, 14, and 21 after RF ablation, eight animals were randomly chosen for contrast-enhanced US before and after phenylephrine administration, and the microvessel density (MVD) of BPE at these four time points was assessed. In experiment two, liver tumors from eight animals were partly ablated, and each animal underwent contrast-enhanced US before and after phenylephrine administration on day 7 after RF ablation. Perfusion parameters were observed, including maximum intensity (IMAX), rise time (ie, time between 10% and 90% of IMAX), time to peak, mean transit time, and area under the curve (AUC), along with the profile of time–intensity curves (TICs) in BPE and residual tumor in response to phenylephrine.

Results: Among the four time points after ablation, the IMAX and AUC before phenylephrine administration and the MVD of BPE were greatest on day 7 ($P < .05$). The profile of TICs and the corresponding perfusion parameters in residual tumor did not change significantly in response to phenylephrine. However, those from BPE changed significantly ($P < .05$).

Conclusions: Contrast-enhanced US with phenylephrine stress may be helpful in differentiating BPE from residual tumor after RF ablation in hepatocellular carcinoma.

ABBREVIATIONS

AUC = area under curve, BPE = benign periablational enhancement, HCC = hepatocellular carcinoma, IMAX = maximum intensity, mTT = mean transit time, MVD = microvessel density, RF = radiofrequency, ROI = region of interest, RT = rise time, TIC = time–intensity curve, TTP = time to peak

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Radiofrequency (RF) ablation, a minimally invasive procedure, has become the most frequently used alternative treatment for unresectable liver tumors (1). However, local recurrence continues to be a main cause of treatment failure (2).

The accuracy of early assessment of completeness of RF ablation with follow-up contrast-enhanced computed tomography (CT) or magnetic resonance (MR) imaging is inevitably limited because of postablation changes (3). Benign periablational enhancement (BPE) (4), an ablation-induced inflammatory peripheral rim that can be seen immediately after ablation and can last as long as 6 months, can obscure viable residual tumor.

A previous study (5) showed that tumor vessels do not react to vasoactive drugs because of immaturity and the lack of normal smooth muscle and pericyte structure. Theoretically, the blood perfusion in BPE and viable residual tumor in response to vasoactive drugs would considerably differ from each other. A pharmacoangiography procedure was used to differentiate malignant from benign lesions (6). In 2009, Wu et al (7) assessed RF ablation by using CT perfusion with phenylephrine in a rat subcutaneous tumor model, and found that phenylephrine markedly decreased blood flow in BPE but showed little effect on the untreated viable tumor. We are aware of no reports of postablation assessment of hepatocellular carcinoma (HCC) with pharmacologic modulation.

Previous studies demonstrated that contrast-enhanced ultrasonography (US) can provide results comparable to those of contrast-enhanced CT/MR imaging in HCC detection and treatment assessment (8,9). In the present study, we established complete and partial RF ablation models in rabbit VX2 liver tumors and used a stress contrast–enhanced US test with phenylephrine to differentiate BPE from residual tumor after RF ablation.

MATERIALS AND METHODS

Experimental Design

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the animal care committee of the authors' institution.

A total of 40 male New Zealand White rabbits weighing 2.5–3.0 kg were used in the study. VX2 tumors were implanted in the left lobe of livers of the 40 rabbits. Tumor size was measured by conventional US from day 7 after tumor inoculation. When the tumors were approximately 1 cm in diameter, five groups of animals underwent RF ablation. Two experiments were performed in this study. In experiment 1, the liver tumors from 32 animals were completely ablated. On days 2, 7, 14, and 21 after ablation, eight animals were randomly chosen for contrast-enhanced US before and after phenylephrine administration. In experiment 2, the implanted tumors from eight animals were partly ablated, and each animal was scanned with contrast-enhanced US before and after phenylephrine administration on day 7 after ablation. All animals were euthanized immediately after the contrast-enhanced US scan after phenylephrine administration. Corresponding tumor slices in orientation to the contrast-enhanced US image data were obtained and evaluated histologically. The profile of time–intensity curves (TICs) and the corresponding perfusion parameters, including the maximum intensity (IMAX), rise time (RT; ie, time between 10% and 90% of IMAX), time to peak (TTP), mean transit time (mTT, ie, time from the start to the peak and then to the time when the intensity had decreased by half), and area under curve (AUC), of the BPE and viable residual tumor in response to phenylephrine were evaluated.

Tumor Inoculation

The animals were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg body weight), wrapped to maintain body temperature throughout the procedure, and placed in a warm container afterward until fully awake. VX2 tumor is a highly malignant carcinoma derived from a virus-induced papilloma of rabbits. The tumor can be implanted in the liver of rabbits and show some biologic similarities to human HCC, and the procedure has been described in previous studies (10,11). The VX2 strain was maintained by successive transplantation into the hind limbs of carrier rabbits. First, subcutaneous VX2 tumor was harvested and cut into fragments 1 mm in diameter in Hanks solution. Second, the left lobe of liver of the recipient rabbit was exposed through a subxiphoid abdominal incision. Third, an incision 10 mm deep into the left middle liver lobe was made with a pair of ophthalmic elbowed forceps, and the tumor fragment was placed into the incision. Then, a small piece of gelatin sponge (2 × 2 mm) was embedded into the wound for hemostasis, and the abdominal wall was sutured. All animals received buprenorphine (0.03 mg/kg body weight) for pain management for 2 days during recovery.

Complete and Partial RF Ablation Model

Tumor size was measured by grayscale US from day 7 after tumor inoculation. The animals received RF ablation treatment when the diameter of tumors was 0.80–1.31 cm at approximately 12 days after inoculation. RF ablation was performed under US guidance with a 460-kHz RF generator (Rita Medical Systems, Mountain View, California) and a 16-gauge probe and 15-cm-long monopolar needle electrode (Rita Medical Systems). The left lobe of the liver with the implanted tumor was exposed through a subxiphoid abdominal incision and padded with sterile gauze. In the complete ablation model, the electrode was inserted into the tumor center 2.0 cm deep, and tumors were treated with the output power at 30–40 W for 3 minutes at a temperature of 80°C ± 5. In the partial ablation model, only the peripheral portion of the tumor was ablated, along with surrounding normal tissue, leaving part of the tumor untreated. The electrode was inserted into the periphery of the tumor 2.0 cm deep, and tumors were treated at a temperature of 80°C ± 5 for 2 minutes at 30–40 W. These two ablation protocols had been proven to produce complete and partial tumor ablation models, respectively, in our preliminary experiments.