Experimental Studies

Hepatic Cryoablation: US Monitoring of Extent of Necrosis in Normal Pig Liver¹

PURPOSE: To determine the accuracy of ultrasonography (US) for prediction of hepatic tissue necrosis after cryoablation in normal pig liver.

MATERIALS AND METHODS: Five normal pig livers were treated with cryoablation monitored with US. After a single freeze cycle at 50% flow capacity, the widest diameter of the cryolesion was identified and marked with wire placement (22 wires in five lesions). Livers were removed 24 hours later, and wire tracks were marked with India Ink. Livers were sectioned, and the distance was measured between wire tracks and tissue necrosis caused by freezing.

RESULTS: The mean volume of areas of tissue necrosis was 11.6 cm³ ± 4.0, the mean diameter was 2.9 cm ± 1.0, and the mean maximum diameter was 2.9 cm ± 0.7. The mean distance between the edge of necrosis and the wire track was 1.1 mm ± 1.4. By excluding one outlier (6.5 mm), the mean distance from the ice ball to the necrotic area was 0.8 mm ± 0.8. Uniform necrosis of hepatic parenchyma within the cryolesion was confirmed.

CONCLUSION: US can be used to predict reliably the size of the necrotic area after hepatic cryoablation in normal pig liver. Knowledge of a small but consistent underestimation of tissue necrosis is important when planning cryoablation.

Several methods for preferential destruction of neoplastic liver tissue have been introduced in the past few years, including cryotherapy, radio-frequency ablation, focused ultrasound ablation, laser therapy, and percutaneous ethanol injection (1–6). Regardless of the technique that is used, accurate monitoring of the area targeted for tissue necrosis is of fundamental importance. A major drawback of noncryosurgical techniques is the limited ability to monitor with accuracy a developing area of tissue necrosis (2–6). Without reliable and accurate monitoring, it may not be possible to determine whether both tumor and an adequate margin have been ablated, which may lead to undertreatment and residual viable tumor, in some patients. Conversely, overtreatment may increase complications due to unnecessary damage to surrounding normal structures such as bile ducts.

Cryoablation (or cryosurgery) is a focal ablative technique that is widely accepted for destruction of primary and secondary liver tumors in conjunction with or in place of hepatic resection (1). In particular, cryoablation has shown promise for the treatment of metastases in multiple lobes, of tumors near or adjacent to major vascular structures, and in patients with limited hepatic reserve (7). The increasing popularity of hepatic cryosurgery has been prompted mainly by the ongoing improvement in ultrasonographic (US) technology for intraoperative use. Currently, intraoperative US is the standard of reference for imaging hepatic tumors and consistently demonstrates tumors as small as 3 mm in diameter (8–10). Intraoperative US is also used to guide the freezing process (11).

At US monitoring, the developing ice ball appears as a hyperechoic rim with posterior acoustic shadowing (12). This characteristic US appearance is produced by an impedance mismatch at the ice ball–liver interface, behind which US information cannot be obtained (13). This can cause difficulty in monitoring of the posterior border of the ice ball during surgery if an appropriate window is not available. Transition to the solid state occurs at approximately 0°C (14). Because reproducible necrosis of liver tissue by means of freezing

Author contributions:
necessitates temperatures of approximately \(-20^\circ\text{C}\) (14,15), however, the boundary between viable and nonviable tissue is not at the edge of the ice ball but rather somewhere within the ice ball. It is important to determine the exact location within the ice ball where reproductible tissue necrosis occurs, so that appropriate decisions about the extent of freezing can be made during surgery. The objective of this study was to quantify the distance from the ice ball edge, as depicted at US, within which reproductible tissue necrosis occurs in a pig liver model.

**MATERIALS AND METHODS**

Five normal swine (mean weight, 26.6 kg) were used in this study. Approval was obtained from the research animal use committee of our institution. General anesthesia was induced with an intramuscular injection of tiletamine hydrochloride and zolazepam hydrochloride (Telazol; Fort Dodge Laboratories, Fort Dodge, Iowa) and xylazine hydrochloride (Rompun; Bayer, Shawnee Mission, Kan.) and was subsequently maintained with inhaled halothane (Halocarbon Laboratories, River Edge, NJ). The skin was prepared with a 10% povidone-iodine solution, and a bilateral subcostal incision was performed for liver exposure.

After surgical exposure of the liver, a single cryoprobe with a 3.4-mm outer diameter (Endocare, Irvine, Calif.) was introduced into the liver parenchyma through an 18-F dilator-sheath combination (Onik-Cohen Percutaneous Access Kit; Cook, Spencer, Ind.). The intrahepatic position of the cryoprobe was confirmed by using both palpation and US. A single, 15-minute freeze cycle was then performed by using an argon gas-based cryosurgical system (Endocare). The purpose of the freeze cycle was to create a moderate-sized ice ball (approximately radius, 1-1.5 cm) and then to maintain that ice ball by setting a constant probe target temperature (50% of maximum flow rate).

At 14 minutes after initiating the freeze cycle, a 5.0-MHz electronic-vector neurosurgical burr-hole US transducer (Aloka Ultrasound, Wallingford, CT) was placed on the liver surface. This transducer has a detachable biopsy guide that allows passage of a needle perpendicular to the transducer crystal. Therefore, when the transducer is brought progressively closer to the ice ball, the initial intersection of the puncture guide and the ice ball represents a line tangential to the largest diameter of the ice ball (Fig 1). This location was marked by advancing an 18-gauge, Teflon-coated needle with a diamond-shaped tip (Onik-Cohen Percutaneous Access Set; Cook) into the liver parenchyma through the puncture guide, with direct US visualization. The needle was then exchanged for a 0.038-inch-diameter, 40-cm-long J-shaped guide wire with a stiff mandril. Wires were then cut flush with the liver surface and were left in situ. An active thaw cycle was performed until the cryoprobe was released from the tissue. The abdomen was closed in two layers, and the animal was allowed to recover.

After 24 hours, animals were anesthetized as already described, and a bolus of heparin (2,000 U) was administered intravenously. Animals then underwent repeated surgery with direct portal vein cannulation, followed by sacrifice with an intravenous overdose of pentobarbital sodium and phenytoin sodium (Reutha-nasia-D; Schering-Plough, Kenilworth, NJ). Immediately after sacrifice, the portal vein was infused with 10% neutral buffered formalin to maximize preservation of the cryolesion and the surrounding normal hepatic parenchyma. The liver was then removed en bloc.

Livers were fixed in formalin for at least 24 hours. The cut ends of the wires were located and trocars were advanced over them. The wires were removed, and India ink was injected through the trocars as they were withdrawn. Subsequently, the trocars were sectioned at 4-mm intervals along the transverse axis of the cryoprobe. This allowed the tracks of the wires, now stained with India ink, to be readily visualized on the liver sections (Fig 2).

Liver slices were examined to choose the slice that corresponded to the maximum diameter of the cryolesion. Tissue was mounted in paraffin blocks, sectioned at a thickness of 7 μm, and stained with hematoxylin-eosin. All histologic samples were then examined with light microscopy (model BX40; Olympus Optical, Tokyo, Japan), and the zone of necrosis caused by means of freezing was established. Each wire track was readily identified because of the India ink present in the lumen, and the distance from the zone of necrosis to the ink mark was measured with a standard micrometer available for the light microscope.

Wire distances were compared on a case-by-case basis with the Kruskal-Wallis test.

**RESULTS**

All animals survived treatment without hemorrhage or cracking of the liver surface. At reexamination of the animals after 24 hours, there was no evidence of intra-abdominal sepsis or hemorrhage. Some adhesions of the cryolesions to the surrounding structures were visualized.

The ice ball was visualized at US as a reflective interface with posterior acoustic shadowing. As predicted, no notable acoustic information was discernible within the ice ball (15). No difficulties were encountered during needle and wire placement, and final wire position was judged to represent the US-determined ice ball-liver interface. As thawing progressed, the hyperechoic rim of the ice ball receded. When the ice ball was completely thawed, the cryolesion was hyperechoic compared with normal liver.

At gross examination, the cryolesion consisted of a thin border of white tissue around a central hemorrhagic necrotic area. Histologic evaluation of the cryolesion demonstrated homogeneous coagulative necrosis of hepatic parenchyma with an abrupt demarcation between normal and necrotic tissue. At the cryolesion border, an infiltrate of neutrophils ex-
tended approximately 2.0 mm into the area of necrosis. There was no evidence of even minor gross or histologic damage outside the cryolesion, and no sections showed incomplete necrosis within the cryolesion after a single freeze.

The mean cryolesion volume (+1 standard deviation) at 50% freezing capacity, which was computed by using the formula for a prolate ellipse (length × width × height × 0.523, where length, width, and height are measured in centimeters), was 11.6 cm³ ± 4.0. The mean of the largest diameters was 2.9 cm ± 0.7; the overall mean diameter was 2.9 cm ± 1.0. These numbers are artificially low because three cryolesions became truncated in length as they reached the liver surface.

A total of 22 wires were placed around five cryolesions, for a mean of 4.4 wires per cryolesion (range, 3–6). Wire tracks were easily visualized because of the ink in the lumen (Fig 3). The mean distance from the edge of necrosis to the wire was 1.1 mm ± 1.4 (range, 0.0–6.5 mm). There was no coherent pattern that related lesion size to lesion-to-wire distance (P = .21). At eight (36.4%) of 22 points, there was precise placement of the wire at the border between normal and necrotic tissue, with no normal tissue between the wire track and the necrotic edge (Fig 4). Conversely, the necrotic area did not extend beyond the wires in any case. Figure 5 shows the distribution of distances between wire tracks and cryolesion borders.

**DISCUSSION**

To produce focal tissue ablation safely and effectively, several conditions must be met. First, the developing ablative lesion should be highly visible by using some form of imaging, preferably US for real-time monitoring. Second, the visible area must consistently correspond to the subsequent area of tissue necrosis. When either condition is not met, the result is an unpredictable area of tissue necrosis that carries a high risk of both under- and overtreatment.

For more than 10 years, cryoablation has been used with positive clinical results for destruction of liver tumors (16–18). Early on, it was recognized (13) that US may be nearly ideal for monitoring of the progression of the freezing process. It has been previously established (19) that the necrotic zone produced with cryoablation is roughly equal in size to the lesion seen at US; however, these measurements were not produced in relation to a fixed point. Therefore, a lesion could be created that is similar in size at both US and histologic examination yet not represent the same anatomic position. Shrinkage due to tissue fixation and critical-angle artifact at US further complicate the comparison (20). In our study, however, ice ball position at US was correlated with fixed anatomic landmarks (inserted wires), and our results suggest that the visible ice ball is an excellent indicator for the location of tissue necrosis. Eight of 22 (36.4%) points in our study demonstrated no difference between the visible edge of the ice ball and the edge of necrosis. Thirteen of the other 14 points were within 2.5 mm of the necrotic zone. Exclusion of the one unexplained distance (6.5 mm) yields a distance from the US-visualized ice ball to the necrotic area of only 0.8 mm ± 0.8. Knowledge of this slight but consistent overestimation of the necrotic zone at US is clinically useful, because a compensatory increase in ice ball size can provide adequate tumor coverage with appropriate margins.

This study has several limitations. A suitable tumor model in a large animal species would have been desirable to demonstrate similar parameters required for tissue destruction in tumor, as well as normal liver, but we are not aware of such a model. Even with an appropriate model, however, freezing of individual tumors will vary on the basis of the local thermal environment and cell type, and results may not be easily generalized to treatment outcomes in human malignancies. Additional sources of error include the 4-mm slice thickness of histopathologic specimens. Because the closest position of the wire to the necrotic zone was the measurement used in this study, thinner slices might have produced even smaller distances. Movement of the wires and
precision of needle and wire placement with use of the puncture guide are additional sources of potential error, but these were minimized by using stiff, diamond-tipped (rather than beveled), 18-gauge needles and stiff, J-shaped guide wires. We expect that errors in needle and wire placement, as well as refractive artifacts, were also minimized because of the perpendicular configuration of the needle guide in relation to the probe face. Another error could have been introduced if the ice ball was continually changing shape; this was minimized by maintaining a constant probe-tip temperature and by allowing the ice ball to equilibrate for 14 minutes before needle placement. The argon gas-based cryosurgical unit used in this study reaches target temperatures more quickly than liquid nitrogen–based systems. Our clinical experience with this technology leads us to believe that 14 minutes of freezing at 50% flow should yield an ice ball of stable size. Our results include no data point within the necrotic zone that would suggest that an ice ball had continued to enlarge after wire placement; this is further support for our hypothesis.

Other techniques for monitoring ice ball progression have been proposed, including use of thermosensors (20), impedance electrodes (21), magnetic resonance (MR) imaging (22), and visual monitoring of surface lesions (23). Thermosensors in particular can be quite useful when freezing takes place adjacent to an important, unprotected structure such as a major bile duct or when the geometry of probe placement makes US monitoring difficult. Unlike cryosurgery in the prostate gland, however, in which thermosensor monitoring is useful for protection of surrounding structures (20), a larger margin of error is acceptable when freezing most liver tumors. In addition, thermosensor monitoring, unlike US monitoring, is possible only at a limited number of points and may not accurately reflect the entire freezing process, particularly at the deep margin. MR imaging may eventually become the method of choice for monitoring of hepatic cryosurgery because of the ability to visualize the entire ice ball and surrounding structures and to predict temperature distribution within the cryolesion. Major redesign of current cryosurgical devices will be necessary, however, before such devices will be able to function efficiently within the magnetic field. The slow proliferation of open magnets designed for interventional radiology procedures will also limit widespread application of this technique in the near future.

Other focal ablative therapies that have been proposed for treatment for liver metastases include radio-frequency ablation, ethanol injection, focused ultrasound, and laser photocoagulation (2–6). Of these, radio-frequency ablation has been the most widely examined for use in the liver. A preliminary correlation of US and pathologic findings in small radio-frequency–treated lesions (1 × 2 cm) demonstrated concordance of imaging and tissue findings (24); however, clinical US results do not accurately reflect subsequent tissue necrosis (2). To date, clinical results after radio-frequency ablation have been disappointing, with residual viable tissue seen at computed tomography and MR imaging in five (38%) of 13 tumors in one series (2) and in 13 (42%) of 31 tumors in another (3). At surgical resection of four radio-frequency–treated lesions, residual viable tissue was seen in all four patients (2). The reason for these suboptimal results may be an inability to monitor lesion progression accurately with standard external US, which results in undertreatment of many lesions (24).

Intraoperative—rather than percutaneous—radio-frequency ablation combined with high-frequency linear-array US transducers could, in theory, improve results. Patients with colorectal metastases treated cryosurgically had a local recurrence rate of less than 10% in two representative, separate series (8,16) when adequate tumor coverage by the ice ball was confirmed at US.

Current disadvantages of cryoablation include relatively large cryoprobes (3–5 mm in outer diameter), occasional difficulty in visualization of the deep margin at US, and the requirement for surgical exposure of the liver, which increases costs and the possibility of patient morbidity.

Mixed results have been obtained in limited clinical trials of percutaneous laser thermotherapy with US or MR imaging guidance. This method of photocoagulation can destroy liver tissue, but an optimal monitoring technique has not yet been established. In one series (25), combined US and thermosensor monitoring resulted in a false prediction of adequate lesion coverage in two (12%) of 15 patients. In another series (5), laser photocoagulation with US monitoring was successful in treatment of 21 (38%) of 55 patients; the quality of intraoperative US became unsatisfactory when monitoring the coverage of lesions with a diameter of 3 cm or larger. MR imaging is under investigation (26,27) as a monitoring technique, as well, and has shown potential to overcome these limitations. Use of MR imaging for this purpose, however, is not possible with most current MR imagers.

**Practical application:** The stated goal (28) of liver resection is to remove a 1-cm-wide rim of normal tissue along with the hepatic tumor. In the absence of data to the contrary, this also should be the goal of cryoablation. Our results suggest that use of US results in underestimation of the area of tissue necrosis in normal liver by approximately 1 mm; therefore, consideration should be given to appropriately expanding the cryoablation margin to account for this shortfall.
Acknowledgments: The authors thank Margaret A. Rankin, BS, Mark A. Noble, and Alan H. Rappe, RTR, for assistance with animal care and Carie E. Poole for manuscript preparation.

References