Microwave ablation in a hepatic porcine model: correlation of CT and histopathologic findings

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Abstract
Background. Thermal ablative techniques have gained increasing popularity in recent years as safe and effective options for patients with unresectable solid malignancies. Microwave ablation has emerged as a relatively new technique with the promise of larger and faster burns without some of the limitations of radiofrequency ablation (RFA). Here we study a new microwave ablation device in a living porcine model using gross, histologic, and radiographic analysis.

Materials and methods. The size and shape of ablated lesions were assessed using six pigs in a non-survival study. Liver tissue was ablated using 2, 4, and 8 min burns, in both peripheral and central locations, with and without vascular inflow occlusion. To characterize the post-ablation appearance, three additional pigs underwent several 4 min ablations each followed by serial computed tomography (CT) imaging at 7, 14, and 28 days postoperatively.

Results. The 2 and 4 min ablations resulted in lesions that were similar in size, 33.5 cm3 and 37.5 cm3, respectively. Ablations lasting 8 min produced lesions that were significantly larger, 92.0 cm3 on average. Proximity to hepatic vasculature and inflow occlusion did not significantly change lesion size or shape. In follow-up studies, CT imaging showed a gradual reduction in lesion volume over 28 days to 25–50% of the original volume.

Discussion. Microwave ablation with a novel device results in consistently sized and shaped lesions. Importantly, we did not observe any significant heat-sink effect using this device, a major difference from RFA techniques. This system offers a viable alternative for creating fast, large ablation volumes for treatment in liver cancer.

Key Words: microwave, thermal, ablation, liver, porcine, hepatocellular, metastatic, cancer

Introduction
In recent years there has been a growing interest in interstitial ablative approaches for the treatment of unresectable tumors in the liver and other solid organs. In addition to increasing the number of patients eligible for potentially curative therapy, local tissue ablation is often performed with lower morbidity than resection. Moreover, it can be employed using both percutaneous and laparoscopic minimally invasive approaches as well as with open surgery.

A variety of methods have been used to locally ablate tissue. Thermal ablation using radiofrequency ablation (RFA) is currently the most frequently used modality [1–3]. However, high local recurrence rates have been reported in patients treated with RFA, particularly for lesions > 3.0 cm in diameter [3–9]. RFA is also potentially limited by the heat-sink effect of nearby vessels resulting in incomplete tumor ablation [10–12]. The rate of heating during RFA can be slow compared with other techniques. In addition, the zone of active heating created by RFA is typically small, often requiring multiple overlapping zones to achieve a larger ablation lesion. As a result, RFA procedure times can be prohibitively long.

Microwave ablation, an alternative method of thermal ablation, offers many of the advantages of RFA and has several other theoretical advantages that may increase its effectiveness in tumor killing [13]. One limitation of traditional microwave ablation therapy has been the inability to treat large tumors without numerous overlapping ablations [14]. Recent advances in the engineering of the microwave antennas using ceramic probes have succeeded in improving the speed and size of the ablation [15]. The Microsulis Tissue Ablation (MTA) system (Microsulis Ltd, Edinburgh, UK) is a new microwave ablation device with the potential for larger, faster,
and potentially more accurate burns [15]. Most studies of this device report on short-term performance in ex vivo models. Therefore, we elected to study this device in a more realistic clinically relevant setting in an in vivo porcine model. A variety of clinically relevant parameters were evaluated, including the power, time, and ablation volume relationship; effects of ablation location and inflow occlusion; and short-term follow-up to characterize the post-ablation appearance on serial CT imaging.

Materials and methods

Microwave ablation

The Microsulis Tissue Ablation (MTA) system was used for all experiments. The MTA employs a 2.45 GHz, 100 W microwave device with an active 5.7 mm diameter radiator, powered by a magnetron generator. A microwave detector was used to ensure safe exposure of microwave energy to all personnel.

Animals

Swine weighing 40–60 lb were used in the study. All animals were treated in accordance with Johns Hopkins University Animal Care and Use Committee protocols.

Non-survival studies

Animals underwent general anesthesia and an open laparotomy was performed. The microwave ablation applicator probe was inserted into the liver parenchyma utilizing ultrasound guidance to optimize and characterize position relative to major vascular structures. To determine the effect of ablation time on the size and shape of lesions, ablations were performed using 100 W of power for 2, 4, or 8 min durations. These parameters were chosen on the basis of established heating curve data in liver for the MTA system [15].

One limitation of RFA techniques is the inability to achieve consistently shaped burns when near vasculature, known as the ‘heat-sink effect.’ To evaluate this in our study, we compared the shape and size of ablation lesions when the probe was placed peripherally in liver or centrally, in proximity to major vasculature. Central ablations were conducted with probe placement under ultrasound guidance within 1 cm of the origin of a major hepatic vein. To further characterize the effect of adjacent blood flow, both central and peripheral lesions were compared with and without temporary total in-flow occlusion. This was done with the application of a non-crushing clamp on the porta hepatis during the ablation period.

Hemodynamic monitoring was performed throughout each ablation, and the amount of bleeding upon withdrawal of the applicator probe was also recorded.

Pigs were euthanized immediately following ablation procedures.

Survival studies

The purpose of this series of experiments was to determine the change in size and shape of ablated lesions in living pigs over time and to correlate these changes with radiologic and histopathologic data. In three pigs, lesions were created in peripheral liver parenchyma without inflow occlusion. In each animal, two or three 100W 8 min ablations were performed. These pigs were recovered from surgery and underwent CT imaging at 7, 14, and 28 day intervals. Following the last imaging, animals were euthanized, livers were harvested, and ablated lesions were measured grossly as well as cross-sectioned and examined microscopically using vital staining.

Liver evaluation

The livers were harvested en bloc and sliced at 3 mm intervals. Lesion size was measured in the short axis and long axis in relation to the applicator probe. Lesion volume is calculated as \(\frac{4}{3}\pi \left(\frac{\text{short axis}}{2}\right)^2 \left(\frac{\text{long axis}}{2}\right)\). Portions of these liver samples were then fixed in 10% formalin, mounted in paraffin blocks, sliced at a thickness of 10 μm, and stained with hematoxylin and eosin (H&E). Slices were imaged on an optical scanner and saved as electronic files.

Ablation volume and shape determination

Radiologic studies were conducted by a radiologist (I.R.K.) blinded to the treatment group and time. Image processing was performed using a commercially available Advantage Windows workstation (General Electric Medical Systems, Milwaukee, WI, USA). Measurement of liver volume was performed by hand tracing the tumor contour on the axial portal venous phase images as described in prior studies [16,17]. Hand tracing was performed on every axial image to enhance accuracy in volumetric measurements. A three-dimensional model of the liver and tumor was generated.

Statistical analysis

The student’s t test was used for all analyses. A \(p<0.05\) level was used to determine statistical significance.

Results

Safety and performance studies

The 100 W ablations of varying duration (2, 4, and 8 min) resulted in consistent and grossly evident
ablations (Figure 1). Lesions were roughly ellipsoid in shape, becoming more spherical with longer ablation time. Table I summarizes the short-axis and long-axis diameters as well as the total ablation volume. Ablations lasting 2 min resulted in lesions that had an average volume of 33.5 cm³ (short-axis diameter = 3.7 cm). Ablations lasting 4 min were not statistically larger, with an average volume of 37.5 cm³ and average short-axis diameter of 3.8 cm. At 8 min, ablation lesions were significantly larger, with the short axis diameter averaging 5.3 cm and the average volume averaging 92.0 cm³.

Histologic assessment of the areas of ablation confirmed complete necrosis in the entire regions seen and measured on gross examination. Moreover, a sharp transition zone was identified between ablated tissue and normal appearing liver parenchyma correlating with the gross assessment (Figure 2).

There were no identifiable problems or complications during the liver ablation in the animals. They remained hemodynamically stable throughout the procedures and bleeding was not problematic either during ablation or upon removal of the probes.

Table I. Size of lesions by ablation duration.

<table>
<thead>
<tr>
<th>Ablation duration (min)</th>
<th>Short axis (cm)</th>
<th>Long axis (cm)</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.7±0.6</td>
<td>4.5±0.9</td>
<td>33.5±17.3</td>
</tr>
<tr>
<td>4</td>
<td>3.8±0.5</td>
<td>4.9±0.5</td>
<td>37.5±12.8</td>
</tr>
<tr>
<td>8</td>
<td>5.3±0.6*</td>
<td>6.4±1.1</td>
<td>92.0±6.5*</td>
</tr>
</tbody>
</table>

In these studies, all ablations were performed at 100 W in peripheral locations without inflow occlusion. Lesion lengths and volumes are given as mean ± one standard deviation. *p < 0.05.

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Impact of location and inflow occlusion on ablation characteristics

The results of the ablation location and inflow occlusion studies are shown in Table II. Applications of 100 W lasting 4 min were used for all ablations during this experiment. Lesions created in the peripheral liver without inflow occlusion were, on average, 3.8 cm x 4.9 cm and 37.5 cm³ in size. Application of inflow occlusion in comparable peripheral lesions resulted in increased size, averaging 4.4 cm x 6.1 cm and 64.5 cm³ in size. Ablation in perivascular central sites resulted in zones of comparable size and shape to peripheral sites, averaging 3.8 cm x 5.3 cm (volume 44.8 cm³). When inflow occlusion was applied to central ablation sites, lesion size averaged 4.5 cm x 6.6 cm (volume 68.9 cm³), not statistically different. Differences in the shape of ablation zones were difficult to quantify. In all cases, regardless of proximity to major vascular structures and application of inflow occlusion, no evident differences were observed in the subjective appearance of the shape, nor the relationship between short-axis and long-axis diameters. Specifically, parenchymal viability did not appear to be preserved immediately adjacent to major

Table II. Size of ablated lesions from vital structure and heat-sink study.

<table>
<thead>
<tr>
<th>Ablation site</th>
<th>Inflow occlusion</th>
<th>Short axis (cm)</th>
<th>Long axis (cm)</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral</td>
<td>No</td>
<td>3.8±0.5</td>
<td>4.9±0.5</td>
<td>37.5±12.8</td>
</tr>
<tr>
<td>Peripheral</td>
<td>Yes</td>
<td>4.4±0.9</td>
<td>6.1±0.1*</td>
<td>64.5±24.2</td>
</tr>
<tr>
<td>Central</td>
<td>No</td>
<td>3.8±1.2</td>
<td>5.3±0.8</td>
<td>44.8±26.3</td>
</tr>
<tr>
<td>Central</td>
<td>Yes</td>
<td>4.5±0.1</td>
<td>6.0±0.3</td>
<td>68.9±2.8</td>
</tr>
</tbody>
</table>

All ablations were performed at 100 W for 4 min each. Lesion lengths and volumes are given as mean ± one standard deviation. Volume is calculated as \( \frac{4}{3}\pi(\text{short axis})^2(\text{long axis}) \). n = 3 for each experiment.

*\( p < 0.05 \).
vascular structures and no shape alteration was observed near these structures.

Post-ablation CT of microwave lesions

CT appearance of regions of microwave ablation revealed areas of hypodensity and lack of perfusion (Figure 3). This appearance is similar to that seen with other methods of thermal ablation. Imaging of ablation regions at day 7 correlated with the initial ablation shape and volume of 4 min at 100 W found in our earlier studies. Serial CT imaging at day 14 and 28 revealed a consistent volume contraction over time (Figures 3 and 4, Table III). In seven of eight cases, the lesion volume on day 28 was 25–50% of that on day 7. In one case, the post-ablation site appeared larger on day 14, decreasing by day 28 to only 77% of the initial size. A Hounsfield unit analysis of this lesion suggested that this size increase was likely due to a bleed around this lesion.

Following the final CT imaging, the ablation sites were analyzed grossly for size and shape. Lesion sizes and volumes as measured by CT imaging and as measured by direct measurement of gross specimens were nearly identical (Table III, columns three and four). As seen in the initial studies, CT assessment and day 28 gross evaluation of the ablation sites did not appear to be affected by a heat-sink effect. Symmetric ellipsoid lesions were seen even in close proximity to hepatic vasculature. Vessels remain patent as evidenced by contrast enhancement.

Discussion

These data demonstrate that microwave ablation utilizing a single application of this high energy single probe device can result in reproducible and predictable large volume lesions in living liver tissue in little time. Lesion size can be increased by application of energy for longer duration, particularly beyond 4 min. A single 100 W probe application for 8 min resulted in lesions consistently >5 cm. This would typically represent the upper limit for most commercially available RF devices using considerably longer application times [18].

Based on these results, it appears that thermal ablation using this microwave device is unaffected by high blood flow in proximity its application. Specifically, ablation regions appeared to be of consistent size and symmetric shape, independent of the location near vascular structures. If validated in clinical studies, this feature may prove to be particularly advantageous over other ablation methods such as RFA. Several mechanisms have been postulated as to why microwave ablation devices resist heat-sink effects [19]. Unlike RFA, which relies largely on conductive dissipation of heat, microwave energy is delivered to a greater distance from the source, relying less on conductive heat transmission.

The gradual reduction in ablated lesion size over time was also notable. Lesions consistently underwent concentric contraction over the 4 week period of this study. Histologic analysis reveals that the necrotic liver tissue is gradually replaced by regenerative hepatic parenchyma. This is not unique to microwave ablation and has been well described in the literature with most other ablative techniques including RF, cryoablation, and HIFU (high intensity focused ultrasound) [20].

CT was a useful modality for monitoring change in lesion size/shape over time. On average, CT measurements differed from gross lesion measurement by only
Table III. Dimensions and volumes of lesions from CT imaging at postoperative days 7, 14, and 28.

| Parameter | Day 7 | Day 14 | Day 28 | Day 28
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>5.5 ± 0.7</td>
<td>4.9 ± 0.7</td>
<td>3.9 ± 0.8</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>3.3 ± 0.35</td>
<td>3.1 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>Volume (cm³)</td>
<td>31.5 ± 8.9</td>
<td>25.9 ± 10.3</td>
<td>11.5 ± 5.8</td>
<td>11.7 ± 6.2</td>
</tr>
</tbody>
</table>

Gross specimen size at day 28 is also shown for comparison. Numbers are given as means ± standard deviation. n = 8 for all experiments.

4 mm. Nevertheless, this level of accuracy may be insufficient for clinical purposes given that some neoplastic lesions that are the targets of ablative therapies are only 1 cm in size. MRI has often been used in the immediate and postoperative monitoring of hepatic ablation techniques and may prove beneficial in monitoring post-microwave ablative therapies [21].

Compared with RFA, microwave ablation has a wider zone of active heating. In some reports this can be up to 6 cm surrounding the antenna [15, 22–24]. This may allow more uniform tumor killing both within a targeted area and next to vasculature. Unlike RF energy, microwave energy does not appear to be limited by charring and tissue desiccation [14]. In addition, the time of ablation may be faster, multiple simultaneous probes can be used, and no return electrodes are necessary that can lead to inadvertent skin burns [14, 25–27]. Finally, the size and shape of the zone of ablation may be more consistent and less dependent on heat sinks from vascular structures in proximity [25, 28, 29].

Several limitations are notable in this study. First, while the length of ablation time was varied in these experiments, a constant ablation power (100 W) was used. More recent microwave devices in development can deliver power of 150 W or greater. Other power settings may result in different ablation characteristics. Second, non-tumor-bearing tissue was used in these studies. Further studies will help to better elucidate the effects of this new microwave ablation probe and its safety in preclinical and clinical settings.

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References


