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Purpose: We investigated a newly developed bipolar and multipolar RF ablation system with an internally cooled electrode and resistance controlled power output in a standardized model of perfused ex vivo kidney tissue.

Materials and Methods: RF energy was applied at different power levels (20, 30 and 60 W) for 1, 3, 5 and 9 minutes. Each treatment parameter was repeated 5 times. For the 20/30 W levels a bipolar electrode with an active conducting part of 20/30 mm was selected. At 60 W 2 bipolar electrodes with an active conducting part (30 mm each) were connected. Lesion volumes and shapes were calculated by measuring the maximum vertical, long axis and short axis diameters of the macroscopic lesion.

Results: Lesion volume increased significantly with the treatment time and generator power applied (p < 0.0001). Lesion size in multipolar ablated zones was larger than that in bipolar ablated zones. A reliable dose-effect relationship existed between the generator power/applied treatment time and ablated tissue lesion size. All lesions were elliptical.

Conclusions: Bipolar and multipolar RF ablation with an internally cooled electrode and tissue resistance control represent an interesting advance in RF technology. The development of lesion size and volume is predictable, while a uniform lesion shape can be achieved in perfused ex vivo kidney tissue. Further in vivo trials are required to test whether complete and reliable tumor tissue ablation is possible with this system.

Key Words: kidney; surgical procedures, minimally invasive; carcinoma, renal cell; catheter ablation; swine

RF A is an attractive, heat energy based ablation modality for small renal masses under investigation. It is only needle invasive, has hemostatic potential and is relatively low cost. Clinical reports using monopolar RFA to treat small renal masses are being published in increasing numbers with promising results. However, there have also been reports of treatment failures.1–3

To our knowledge only monopolar RF devices have been used for kidney tissue ablation in clinical settings to date. RF current flows from the active electrode, which is inserted into the tumor, via the patient body to the grounding electrode, which is placed at the patient skin. Reported complications of monopolar RFA are skin burns at the grounding pad location due to high power densities guided through the body of the patient.4 Collateral damage caused by uncontrolled electrical current paths5 and concerns about the reliability of tumor cell destruction.6 A bipolar electrode could be an alternative to overcome these disadvantages by placing the 2 electrodes on 1 applicator shaft, separated by an insulator without the need for any grounding pads. Although it is already used in clinical practice, variables affecting the coagulative effect of RFA have not yet been completely evaluated. Systematic investigations are rare, given the large variability in ablation protocols. Therefore, under standardized conditions we investigated a newly developed bipolar and multipolar RF system with an internally cooled electrode and resistance controlled power output for kidney tissue ablation.

MATERIALS AND METHODS

Ex vivo tissue model of perfused porcine kidneys. We used the model of the isolated, perfused, ex vivo porcine kidney, which has been described and evaluated for other minimally invasive ablation technologies, eg interstitial laser, high intensity focused ultrasound.6,7 Kidneys with a mean weight of 262 gm (range 190 to 371) were removed from pigs within 5 minutes of commercial slaughtering and immediately perfused with cold (4°C) sodium chloride solution (0.9%) through a 10Fr catheter placed in the renal artery. After the effluent from the renal vein ran clear, the organs were stored at 4°C. The experiments were started no later than 4 hours after slaughtering. During the trials we used a roller pump to continuously perfuse the kidneys with sodium chloride solution (0.9%) at 37°C. Perfusion pressure was set to 110 to 130 cm H2O, as measured by a water column, resulting in a perfusion rate of 60 to 100 ml per minute. RF electrodes were inserted in the center of normal renal parenchyma in the mid region, and the upper and lower pole with a lesion distance of at least 3 cm and 3 to 5 lesions per kidney, perpendicular to the surface of the kid-
ney under direct vision. The kidney was placed in a plastic basin containing sodium chloride solution (0.9%) controlled at 37°C.

RF system. We used a newly developed bipolar and multipolar RF system (CelonLabPOWER, Celon AG, Teltow, Germany), which produces a maximum power of 250 W (470 kHz) and which is feedback controlled by measuring tissue resistance. The rigid bipolar applicator (CelonProSurge, Celon AG) has 2 single electrodes that are axially placed on one shaft (diameter 1.8 mm) separated by an insulator (fig. 1). With one bipolar applicator connected (2 electrodes), the unit is in bipolar operating mode and the current flows between the two electrodes (fig. 1). If 2 bipolar applicators are connected (4 electrodes), the unit is in multipolar mode. In this case, all the possible electrode pairs are activated automatically one after the other for a specific period of time, that is 3 seconds (fig. 2). The RF output is divided between the individual probes according to the tissue resistance present under microprocessor control. The electrodes are cooled internally by a peristaltic pump (delivery rate 30 ml per minute, cold (4°C) sodium chloride solution).

Ablation protocol. Power was delivered for 1, 3, 5 and 9 minutes to investigate the influence of treatment time on lesion size. To investigate the influence of the power level on lesion size different power levels were selected. The manufacturer recommends selecting the power level according to the active conducting part (sum of the length of the electrodes, insulator and tip) of the applicator using the formula, 1 W per 1 mm active conducting part. Different applicators are commercially available with active conducting parts of 20, 30 and 40 mm. Due to the anatomy of the porcine kidney conducting parts of 20 and 30 mm could be used. Therefore, for an applicator with an active conducting part of 20 mm a power level of 20 W was selected and for a conducting part of 30 mm it was 30 W. Furthermore, 2 applicators with an active conducting part of 30 mm each (mutual radial distance 13.5 mm) were connected and a power level of 60 W was selected. Each parameter setting was repeated 5 times. Ablation cycles were performed using digital real-time monitoring with treatment parameters recorded.

Lesion measurement. The ablation zone was cut along the longitudinal plane, passing through the axis of 1/2 probes and then cut transversely (transverse plane) into slices. Maximum lesion diameters in mm were measured, namely the vertical diameter (DV) along the needle axis, the long axis diameter (DL) perpendicular to it and the short axis diameter (DS) in the transverse plane. Lesion volumes in mm³ were calculated by assuming the volume (V) of a prolate ellipsoid using the formula, \(V = \frac{4\pi}{3} \times (DV \times DL \times DS)/8\). Lesion shape was assessed by the ratio of DV and the average of DL and DS, in accordance with Pereira et al.⁴

Histology. Representative samples were immediately frozen, sectioned at 5 μm in a cryostat and stained for tissue oxidative enzymes using NADH to assess cellular viability. Positive reactivity was defined as robust staining of cells that maintained normal morphology and had normal-appearing nucleoli.⁸ Additionally, representative samples were also stained with routine hematoxylin and eosin.

Statistical analysis. Lesion diameter and volume are presented as the mean ± SD. Statistical analysis was performed using the statistical software package SAS, 8.2 release (SAS Institute, Cary, North Carolina). Differences in lesion size and volume were analyzed using 1 and 2-way ANOVA and the Student t test. The 1-sample t test was performed to compare whether lesion shape was different than 1 (spherical shape). Statistical significance was considered at p < 0.05.

RESULTS

Lesion morphology. The macroscopic ablation zone appeared as a firm, white-yellow lesion with a sharp demarcation to untreated tissue. Untreated tissue had a normal anatomical morphology in hematoxylin and eosin stained.
specimens. Hematoxylin and eosin staining of the treatment zone revealed a variable appearance, including preserved renal parenchymal architecture, zones of cauterization with substantial defects and cellular necrosis, focal tissue disruption, cytoplasmic vacuolization, distortion of cell shape, viable-appearing nuclei and decreased eosinophilia (fig. 3, A). NADH stained sections showed a sharp demarcation between positive stained viable cells outside of the ablation zone and no cellular viability (unstained areas) in the treatment zone, which was visible grossly and microscopically (fig. 3, B). Gross lesion size corresponded to the microscopic ablation area on NADH stained sections.

**Lesion size.** Bipolar Mode (see table): In the case of single bipolar electrodes (20/30 W) statistical analysis showed that long and short axis diameters increased significantly with treatment time (1 vs 3, vs 5 and vs 9 minutes, p < 0.0001). The vertical diameter did not increase significantly with treatment time but except for 20 W/9 minutes it was significantly larger than the long and short axis diameters (p < 0.001). A higher power level created larger lesion diameters only for the vertical diameter (p < 0.001). Long and short axis diameters were comparable in size and showed no statistically significant differences at all treatment times and power levels.

Multipolar Mode (see table): With a treatment time of 1 to 9 minutes all lesion diameters increased significantly (p < 0.001). The vertical and long axis diameters were comparable in size after 5 and 9 minutes of treatment time (no statistical difference) and they were significantly larger than the short axis diameter at all treatment times (p < 0.001).

Comparative Studies at Identical Treatment Times: The vertical diameter of multipolar ablated lesions was significantly larger than that of 20 W bipolar ablated lesions (p < 0.0005), although it was the same as that of 30 W bipolar ablated lesions. At all treatment times the long axis diameter of multipolar ablated lesions was significantly larger than that of 20 and 30 W bipolar ablated lesions (p < 0.001). At treatment times of 5 and 9 minutes the short axis diameter of multipolar ablated lesions was larger than that of 20 and 30 W bipolar ablated lesions (p < 0.01 and < 0.05, respectively).

**Lesion volume and shape** (fig. 4). Longer treatment times created larger lesions. Statistical analysis showed that lesion volume increased significantly with treatment time (1 vs 3, vs 5 and vs 9 minutes) at all selected power levels (20, 30, and 60 W). The volume of lesions increased significantly with treatment time (1 vs 3, vs 5 and vs 9 minutes) at all selected power levels (20, 30, and 60 W). The volume of lesions increased significantly with treatment time (1 vs 3, vs 5 and vs 9 minutes) at all selected power levels (20, 30, and 60 W).

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**Table. Treatment parameters and lesion diameters**

<table>
<thead>
<tr>
<th>Power (W)</th>
<th>Mean Applied Energy ± SD (kJ)</th>
<th>Mean Lesion Diameter ± SD (mm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Vertical</td>
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<tr>
<td>20:</td>
<td></td>
<td></td>
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<tr>
<td>1 min</td>
<td>1.24 ± 0.36</td>
<td>18.5 ± 2.76</td>
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<tr>
<td>3 mins</td>
<td>3.18 ± 0.42</td>
<td>20.75 ± 1.83</td>
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<tr>
<td>5 mins</td>
<td>4.87 ± 0.54</td>
<td>21.58 ± 2.05</td>
</tr>
<tr>
<td>9 mins</td>
<td>8.41 ± 1.05</td>
<td>20.88 ± 1.52</td>
</tr>
<tr>
<td>30:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>1.84 ± 0.21</td>
<td>26.2 ± 2.03</td>
</tr>
<tr>
<td>3 mins</td>
<td>4.80 ± 0.85</td>
<td>28.50 ± 2.37</td>
</tr>
<tr>
<td>5 mins</td>
<td>7.38 ± 1.03</td>
<td>27.80 ± 2.99</td>
</tr>
<tr>
<td>9 mins</td>
<td>11.52 ± 2.42</td>
<td>27.88 ± 3.31</td>
</tr>
<tr>
<td>60 (2 × 30):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>2.42 ± 0.21</td>
<td>24.83 ± 2.67</td>
</tr>
<tr>
<td>3 mins</td>
<td>6.69 ± 0.90</td>
<td>28.66 ± 1.49</td>
</tr>
<tr>
<td>5 mins</td>
<td>10.17 ± 2.36</td>
<td>27.33 ± 3.34</td>
</tr>
<tr>
<td>9 mins</td>
<td>19.41 ± 1.07</td>
<td>30.40 ± 1.85</td>
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the current density between electrodes. However, charring necrosis more efficiently than monopolar RFA by increasing heat transmission, in that current density decreases as the inverse of the fourth power of the distance square of the distance from the electrode, while heating the tissue in several directions skip lesions with remainders. The system controls the current only travels through the tissue between the electrodes and not through the body to a return plate. There are currently few experimental studies of prototype bipolar RFA systems available, which concentrate mainly on liver tissue. In the context of kidney tissue ablation Nakada et al investigated an expandable bipolar device with 2 separate electrodes in a small series of ex vivo and in vivo porcine kidneys (acute study). They noted the advantages of bipolar ablation in terms of lesion size (larger lesions), consistency and targeting. Ex vivo studies in liver tissue have demonstrated that bipolar RFA creates coagulation necrosis more efficiently than monopolar RFA by increasing the current density between electrodes. However, charring and desiccation of tissue at the electrode tip of the bipolar electrode can be more intense than is the case with monopolar electrodes. This problem may be avoided by infusing saline in the tissue or cooling the electrode internally. Our results demonstrate that a dose-effect relationship exists between generator power/applied treatment time and the target volume of the ablated tissue. Lesion size and volume can be accurately controlled by treatment time and generator power. These results may be helpful for improving our understanding of how best to control and optimize RF ablation for clinical practice, in which a priori prediction of RF parameter settings is necessary for optimal ablation of a given tumor size or volume. However, further investigations in this area are necessary.

To our knowledge the presented RF system is the first and in fact the only commercially available bipolar RF device with the electrodes integrated into 1 needle. Previous reports of bipolar RFA used 2 separate electrodes placed apart from each other in the tissue. The system controls power output during treatment by measuring tissue resistance instead of tissue temperature or tissue impedance. The advantage of measuring resistance is that the length of the cables and type of electrode have no impact on the measurement value. It is possible to show the effective power introduced into the tissue, which is responsible for the therapeutic effect.

Multiple applicators can be useful when targeting large or asymmetrical tumors, or tumors near blood vessels. Currently available multiprobe RF devices offer monopolar electrodes with simultaneous or sequential power application. The Celon device has a different ablation technique, involving computer controlled switching between electrodes and independent power control for each electrode. By crossing the tissue in several directions skip lesions with remaining viable cells can probably be avoided.

Complete and reliable cell death in the treatment zone is a major concern of RFA. Our histological findings on hematoxylin and eosin specimens of the ablation area are in accordance with the observations of others who performed RF ablation in healthy in vivo kidneys. Most importantly we observed that changes on hematoxylin and eosin staining, eg preserved renal architecture, cannot be relied on to determine cellular viability. Our NADH staining showed negative reactivity of cells in the lesions, demonstrating that the cells were not viable. In contrast, in hematoxylin and eosin specimens of ablated renal tumors Matlaga and Michaels et al observed no areas of preserved renal architecture, but rather areas of viability on corresponding NADH stained specimens.

A previous study has shown skip lesions between expandable monopolar RF electrodes. An explanation for this may be the decrease in current density that occurs at a distance from the energy source in the monopolar RF mode, making the periphery of the lesion particularly prone to vascular cooling. Haemmerich et al reported that bipolar RF ablation creates coagulation zones that are significantly closer to blood vessels compared to monopolar RF ablation. They concluded that this may decrease the survival of tumor cells next to blood vessels and decrease recurrence rates. Their explanation of the findings is that bipolar electrodes allow the lesion to be framed with higher current densities between the electrodes.

Some limitations with regard to our study must be mentioned at this point. All ablations were performed in healthy, ex vivo, saline perfused kidneys and not in vivo, blood per-
fused renal cell carcinoma tumor tissue. It is yet unclear whether the results obtained in ex vivo tissue identically reflect the results in human renal tumors because of different tissue textures, tissue impedance, blood flow and cell biology. However, at identical ablation parameters using a temperature based RF device lesion sizes are comparable when obtained in ex vivo\(^2\) and in vivo\(^8\) porcine tissue.

We compared lesion formation at 1 fixed interelectrode space. Due to the relatively small size of porcine kidneys we selected applicators only up to an active conducting part of 30 mm, which limited lesion dimensions. Despite these disadvantages our model provides a reliable basis for comparing different ablation algorithms under standardized conditions.

CONCLUSIONS

The practical experience gained from our experiments with this new bipolar and multipolar RF device equipped with its internally cooled electrode and tissue resistance control is promising. A reliable dose-effect relationship exists between generator power/applied treatment time, and lesion size and volume of ablated tissue, while a uniform lesion shape can be achieved in perfused ex vivo kidney tissue. Bipolar and multipolar RFA represents an interesting advance in RF technology. Further in vivo trials are required to confirm our results and test whether complete and reliable tumor tissue ablation is possible.\(^11,17,18\)

Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
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<td>RCC</td>
<td>renal cell carcinoma</td>
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<td>RF</td>
<td>radio frequency</td>
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<tr>
<td>RFA</td>
<td>RF ablation</td>
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REFERENCES