Effect of Vascular Occlusion on Radiofrequency Ablation of the Liver: Results in a Porcine Model

OBJECTIVE. This study determined the effect of vascular occlusion on radiofrequency lesion shape, volume, and temperature in a porcine liver model.

SUBJECTS AND METHODS. Radiofrequency lesions (n = 33) were created in the livers of six domestic pigs in vivo using a multiprong radiofrequency electrode. Lesions were randomly assigned to one of four vascular occlusion groups: portal vein, hepatic artery, Pringle maneuver (both hepatic artery and portal vein), or no occlusion. Radiofrequency parameters were time, 7 min; power, 50 W; and target temperature, 100°C. Temperatures were measured 5, 10, and 15 mm from the electrode. After the animals were sacrificed, the lesions were excised. Lesion volume, diameter, and shape; maximum temperature; and time exposed to lethal temperatures (42–60°C) were determined.

RESULTS. Lesion volume was greatest with the Pringle maneuver lesions (12.6 ± 4.8 cm³), followed by occlusion of the portal vein (8.6 ± 3.8 cm³), occlusion of the hepatic artery (7.6 ± 2.9 cm³), and no occlusion (4.3 ± 1.0 cm³) (p < 0.05). Maximum lesion diameter was similar with the Pringle maneuver (3.3 ± 0.3 cm), the portal vein (3.3 ± 0.2 cm), and the hepatic artery (3.2 ± 0.2 cm) groups compared with no occlusion (2.6 ± 1.0 cm) (p < 0.05). Minimum lesion diameter ranged from 2.9 cm for Pringle maneuver lesions to 1.0 cm for lesions with no occlusion (p < 0.05). Vascular occlusion increased the time tissue was exposed to lethal temperatures (>42–60°C) and created more spherical lesions than no occlusion.

CONCLUSION. Vascular occlusion combined with radiofrequency ablation increases the volume of necrosis, creates a more spherical lesion, and increases the time tissue is exposed to lethal temperatures when compared with radiofrequency alone. Most of this vascular occlusion effect could be accomplished with hepatic artery occlusion alone.

Hepatocellular carcinoma is the most common solid-organ malignancy worldwide, with an expected annual incidence of 1 million [1]. In addition, the liver is the most common site of colorectal cancer metastases [2]. Optimal therapy for both primary and metastatic liver disease is complete resection with margins that have negative findings for tumor; however, only 5–15% of patients with newly diagnosed hepatocellular carcinoma, and fewer than 10–15% of patients with liver-only metastases, are candidates for curative resection [3].

Although recent advances in surgical technique and perioperative care have decreased morbidity and mortality related to liver resection, the postoperative complication rate remains as high as 42% in patients with cirrhosis [4]. This high risk coupled with the low number of resection candidates has increased the interest in focal ablation therapies for both primary and secondary liver tumors. Early clinical results of many ablative therapies, including radiofrequency ablation, percutaneous ethanol injection, laparoscopic or percutaneous cryotherapy, laser photoagulation, focused ultrasound ablation, and transcatheter arterial chemoembolization, have shown complete tumor destruction in some cases [5–11]. Radiofrequency ablation has the advantages of tissue coagulation at the probe insertion site (resulting in a low rate of bleeding during and after the procedure) and percutaneous application via a relatively small probe [5, 6].

One limitation of percutaneous radiofrequency ablation is the inability to consistently produce a large enough zone of necrosis to encompass hepatic tumors with an appropriate margin. Even when the tumor appears completely covered by the radiofrequency lesion, re-
currences occur; the inadequate initial treatment of tumors is manifested by a high local recurrence rate [12–15]. Several factors contribute to this high rate of local recurrence, but the most important seems to be related to hepatic and tumor vascularity [15, 16]. The inflow of relatively cool blood acts as a heat sink, limiting the size of the radiofrequency-induced lesion and contributing to the unpredictable lesion shape in the immediate vicinity of vessels. A recent clinical report [17] suggests that a Pringle maneuver (hepatic artery and portal vein occlusion) [18] during the ablation may decrease the incidence of local recurrence. The obvious disadvantage of this approach is that it requires a laparotomy that negates the advantage of minimal invasion of radiofrequency ablation. An additional laboratory study documents increased lesion size with vascular occlusion using a cooled-tip electrode [19]. However, early clinical results using the cooled-tip electrode are still associated with high rates of local recurrence after treatment [13].

The hepatic artery is readily occluded percutaneously, a procedure that can be accomplished during chemoembolization of liver tumors [20]. Although this method will not decrease hepatic and tumor blood flow to the same degree as a Pringle maneuver, compelling data support the concept of synergy between radiofrequency and hepatic arteriole occlusion [21, 22]. We hypothesize that the application of radiofrequency ablation in conjunction with hepatic arteriole occlusion will help overcome the detrimental effect of vascular inflow on radiofrequency lesion size, shape, and tissue temperature. If a spherical lesion of reasonable size can be obtained using the combination of radiofrequency and hepatic artery occlusion, percutaneous application of radiofrequency may yield results approaching those obtained at laparotomy using a Pringle maneuver.

Subjects and Methods

Animals and Surgery

Approval for this protocol was obtained from our institutional animal research committee, and all experimentation met the National Academy of Sciences policy on humane care and use of laboratory animals [23]. Six female domestic swine were used in this study (mean weight, 23.4 kg; range, 19.7–33.2 kg). The pigs were anesthetized with ti- betamine and zolazepam, 7 mg/kg (Telazol; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine 0.45 mg/kg intramuscularly (Rompun; Phoenix Pharmaceutical, St. Joseph, MO). Anesthesia was maintained with inhaled halothane gas, 1% until effective. After a 10% povidone–iodine solution was applied, the liver was exposed through a subcostal incision. The hepatic artery and the portal vein were isolated using umbilical tape, and vessels were occluded using an atraumatic vascular clamp or umbilical tape. Animals were euthanized after all procedures using IV Beuthanasia-D ([390 mg of pentobarbital sodium and 50 mg of phenytoin sodium per 100 mL] King Pharmaceuticals, Bristol, TN). Immediately after euthanasia, the radiofrequency lesions were excised and fixed in neutral buffered formaldehyde, paraffin embedded, sliced into 7-μm thick sections, and stained with H and E.

Radiofrequency Procedure

An electrosurgical device (model 30; RITA Medical Systems, Mountain View, CA) was used for all radiofrequency Medical procedures. The radiofrequency probe consists of a 15-gauge shaft through which four sharp prongs, each 0.021 inches in diameter (25 gauge), can be deployed. Fully extended, the prongs are in an “umbrella” configuration, with prongs at each 90° interval (Fig. 1). The last 1 cm of the probe tip and each prong constitutes the electrically active surface. For this study, the probe was inserted 1 cm into a randomly selected lobe of the porcine liver, and the prongs were deployed taking care to keep them in the liver parenchyma. Each radiofrequency lesion was randomly assigned to one of four groups: no occlusion (n = 9), hepatic arterial occlusion (n = 7), portal vein occlusion (n = 7), or a Pringle maneuver (n = 10), and the corresponding vascular occlusion was performed. The radiofrequency generator was set at 50 W of power and applied for 7 min with a target temperature of 100°C. When vascular occlusion was performed, blood flow was restored to the liver immediately after the cessation of radiofrequency energy. The number of ablations per animal ranged from four to seven, with 33 lesions created.

Temperature Monitoring

Thermosensors were placed 5, 10, and 15 mm from the radiofrequency probe at a depth of 0.5 cm. Temperatures were recorded using commercial temperature measurement software (Istrend; Dianachart, Denville, NJ) every 5 sec until the temperature at the 5-mm thermosensor returned to 42°C. Temperatures greater than 42°C are considered potentially lethal depending on the time of application [24], and temperatures greater than 60°C are associated with uniform tissue necrosis [25]. For the purposes of this study, results of tissue heating are presented for each of these target temperatures.

Lesion Volume Determination

Immediately after sacrifice, hepatectomies were performed and the livers were fixed in a 10% buffered formalin solution for a minimum of 24 hr. Livers were serially sectioned at approximately 3- to 4-mm intervals perpendicular to the probe shaft, and the slice thickness was measured. Slices were placed on an optical scanner (HP 4CT; Hewlett-Packard, Palo Alto, CA), and images were saved to image management software (Photoshop; Adobe, San Jose, CA). Analysis was performed on a Macintosh G3 computer (Apple Computer, Cupertino, CA) using the public domain program NIH Image (National Institutes of Health: http://rsb.info.nih.gov/NIH-image/). The area of necrosis on each slice was calculated by tracing the perimeter of the lesion, and volume was calculated by multiplying area by slice thickness. Volumes for each slice were added to obtain a total lesion volume.

Statistical Analysis

Lesion volume, temperature, time at temperatures greater than 42°C and greater than 60°C, and shape of radiofrequency lesions for each group were compared using a factorial analysis of variance. Values for p were calculated on the basis of Fisher’s probable least significant difference. For comparing lesion ranks, a Kruskal-Wallis one-way analysis of variance by ranks was performed, followed by Wilcoxon’s rank sum tests for pairwise comparisons. Statistical significance was defined as p value of less than 0.05.

Shape of Zone of Necrosis

Lesion shape was evaluated in two ways. Using the first method, a rough estimate of lesion “roundness” in two dimensions was obtained by computing the isoperimetric ratio [26] for each lesion at the most representative slice. This value was computed using the following formula:

\[ R = 4\pi A / l^2 \]

where R is the isoperimetric ratio, A is the area of the measured zone, and l is the perimeter of the lesion. The closer this value is to 1, the more circular the shape. Values for A and l were obtained using the computer program NIH Image. The mean value of R for each group was compared using a factorial analysis of variance.

The second method of evaluating lesion shape was a subjective consensus analysis by observers who were unaware of treatment modality. This analysis presumed the following pattern of growth of radiofrequency lesions: lesions first appear as discontinuous areas centered around each prong.
the zone of necrosis grows, it coalesces from a cruciform into a roughly circular or square shape. On the basis of this continuum, lesions were ranked using four basic shapes (Fig. 2): a discontinuous zone of necrosis, a continuous zone of necrosis in a roughly cruciform shape with deep concavities (>50% of radius) between limbs, a cruciform shape with shallow concavities (<50% of radius) between limbs, and a roughly circular shape. Seven ranks were used so that asymmetric lesions with components of two shapes could be categorized among the four basic shapes.

Results

Volume and Diameter of Necrosis After Radiofrequency Ablation

Vascular occlusion increased the volume of ablated tissue after radiofrequency ablation. The largest mean lesion volume was shown in the Pringle maneuver group, followed by portal vein occlusion, hepatic artery occlusion, and no occlusion. Pairwise comparisons between groups are summarized in Table 1. A significant difference in lesion volume was seen between the no occlusion group and all the vascular occlusion groups. The mean radiofrequency lesion volume in pigs pretreated with a Pringle maneuver was greater than the volume in any other group.

An overall statistically significant difference was also found between maximum lesion diameter among groups. Like lesion...
volume, pairwise comparisons showed significant differences between no occlusion and all vascular occlusion groups. The order of maximum diameter also followed the order for lesion volume (in descending order: Pringle maneuver, portal vein occlusion, hepatic artery occlusion, no occlusion). Minimum diameter was smallest for no occlusion, followed by hepatic artery, portal vein, and the Pringle maneuver (Table 1).

### Tissue Temperature

Maximum temperatures measured 30 sec after completion of radiofrequency ablation at 5, 10, and 15 mm were higher at locations closer to the probe (Table 2). Despite differences in mean temperatures, marked variability in individual temperature measurements resulted in large standard deviations, which prevented significant differences among groups regardless of the thermosensor location.

The amount of time tissue was exposed to minimum lethal temperatures (>42°C or 60°C) is summarized in Tables 3 and 4. Vascular occlusion (hepatic artery, portal vein, and Pringle maneuver) produced longer times at lethal temperatures than did no occlusion, although this finding was not quite statistically significant (for either 42°C or 60°C because of the large standard deviations within groups). No differences were detected among the three vascular occlusion groups.

### Radiofrequency Lesion Shape

Masked observers detected an overall significant difference between lesion shapes for the different groups (*p < 0.05, Kruskal-Wallis*). Table 5 shows the pairwise comparisons among groups. Although a nonparametric assumption was made for these data (and statistics were computed as such), mean values are presented for demonstrative purposes. No occlusion specimens showed a lower median rank than all vascular occlusion groups (hepatic artery, portal vein, Pringle maneuver) (*p < 0.05, Wilcoxon’s signed rank test). No occlusion lesions tended to be more complex in shape, and vascular occlusion lesions tended to be more spherical (Fig. 2). Patent vessels at the periphery of the radiofrequency lesion appeared to be the single most important cause of deformity in lesion shape (Fig. 3). Although the hepatic artery and portal vein occlusion groups had a higher median rank than groups treated with a Pringle maneuver, no significant difference was detected.

The isoperimetric ratio values confirmed the subjective impression of masked observers that greater degrees of vascular occlusion yielded rounder lesions. Mean values were the highest for the Pringle maneuver (0.74), followed by the portal vein (0.68), hepatic artery (0.64), and no occlusion (0.56) groups.

### Pathology

Sections through the radiofrequency lesion showed two distinct zones surrounded by normal liver (Fig. 4A). The zone more distant from the probe was red and measured 2–15 mm in thickness. Detailed examination of this zone revealed intense congestion, hemorrhage, detached Kupffer’s cells, dissolution of liver cell plates, and intact but edematous perilobular connective tissue (Fig. 4B). Sinusoidal red cells were intact. Hepatocytes were more eosinophilic and less basophilic than normal, nuclei were less vesicular, and many detached hepatocytes were spherical. Some viable hepatic tissue was present focally in this zone. The more central zone was a pale color because of the lysis of erythrocytes. In this central area RBC ghosts were congested, and

### Tables

<p>| Table 2: Lesion Temperatures at 5, 10, and 15 mm from Radiofrequency Probe 30 Sec After Ablation |</p>
<table>
<thead>
<tr>
<th>Vessel Occluded</th>
<th>Mean Temperature (±SD)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>63.2 ± 15.2</td>
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<tr>
<td>Hepatic artery</td>
<td>66.9 ± 17.9</td>
</tr>
<tr>
<td>Portal vein</td>
<td>66.6 ± 17.3</td>
</tr>
<tr>
<td>Hepatic artery and portal vein (Pringle maneuver)</td>
<td>70.6 ± 15.0</td>
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</tbody>
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<p>| Table 3: Length of Time Temperature Is Greater Than 42.0°C at 5, 10, and 15 mm from Radiofrequency Probe |</p>
<table>
<thead>
<tr>
<th>Vessel Occluded</th>
<th>Mean Time (sec ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>666.3 ± 154.5</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>868.7 ± 208.3</td>
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<tr>
<td>Portal vein</td>
<td>881.7 ± 221.5</td>
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<tr>
<td>Hepatic artery and portal vein (Pringle maneuver)</td>
<td>889.5 ± 243.5</td>
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<p>| Table 4: Length of Time Temperature Is Greater Than 60.0°C at 5, 10, and 15 mm from Radiofrequency Probe |</p>
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<th>Vessel Occluded</th>
<th>Mean Time (sec ±SD)</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>297.5 ± 256.9</td>
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<tr>
<td>Hepatic artery</td>
<td>434.1 ± 244.7</td>
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<tr>
<td>Portal vein</td>
<td>460.7 ± 219.8</td>
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<tr>
<td>Hepatic artery and portal vein (Pringle maneuver)</td>
<td>402 ± 220.2</td>
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<th>Table 5: Mean Lesion Shape</th>
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<tr>
<td>Procedure</td>
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<tr>
<td>None</td>
</tr>
<tr>
<td>Hepatic artery</td>
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<tr>
<td>Portal vein</td>
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<tr>
<td>Hepatic artery and portal vein (Pringle maneuver)</td>
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Note.—Data are results from a masked observer on a scale of 1 = discontinuous irregular lesion to 7 = circular lesion.

²p < 0.05 versus hepatic artery, versus Pringle maneuver, and versus portal vein.
³p < 0.05 versus no occlusion.
compressed parenchyma near the radiofrequency probe prongs were prominent (Fig. 4C). Disse’s spaces were widened, and hepatocytes were separated from the Kupffer’s cells. The hepatocytes themselves were vacuolated and the cell borders frayed. The cytoplasm was more eosinophilic and homogenous than that of normal liver, and the nuclei less vesicular. No intact hepatic tissue was detected in this inner pale zone. Radiofrequency lesions were indented by patent hepatic vessels, whereas thrombosed or occluded vessels did not have a noticeable effect.

Discussion

The ability of radiofrequency ablation to completely ablate a liver tumor is predicated on its ability to produce lethal temperatures in all areas of the tumor. Radiofrequency lesion size and shape both need to be adequate for successful ablation: a perfectly spherical lesion of inadequate volume will be just as likely to fail as a large lesion with an unpredictable or asymmetric shape. Thus, the goals of radiofrequency ablation are the creation of a zone of necrosis of adequate size to cover the tumor plus a 1.0-
cm margin [27, 28] and of roughly spherical shape to overlay typically spherical hepatic tumors. These goals have been only partially met with current radiofrequency technology when applied percutaneously; as a result, a high number of local recurrences have been reported in clinical series [12–14]. Recent work has shown a lower local recurrence rate when radiofrequency is applied intraoperatively in combination with a Pringle maneuver [17]. This disparity in results raises the question of the precise effects of blood flow on the radiofrequency lesion.

In this study, lesion size was examined using the total volume of necrotic tissue and lesion diameter. Both these factors depended on the status of vascular inflow. Thus, lesions without vascular occlusion were smallest, and those with a Pringle maneuver were largest. Because more blood enters the liver via portal circulation, portal vein ligation created larger lesions than hepatic artery ligation, as expected. Pharmacologic manipulation of hepatic blood flow in experimental models has also been shown to affect radiofrequency lesion size without vascular occlusion [27]. The marked differences in lesion size with and without vascular occlusion suggested by our study may explain a large part of the discrepancy between operative and percutaneous results. Larger lesions generated by the combination of radiofrequency and the Pringle maneuver may increase the chance that the entire lesion and margin are ablated, and thus decrease local recurrence rates.

New technology is being developed to create larger and rounder zones of ablation. The 50-W generator and four-prong electrode used for this study (model 30; RITA Medical Systems) have already been replaced with seven- and nine-prong devices and 150-W generators (models 70 and 90; RITA Medical Systems) that have the potential to create rounder and larger necrotic zones. Cooled-tip electrodes (which decrease charring and thus increase the amount of radiofrequency energy that can be deposited in tissue) have been available since 1997. These probes can create lesions up to 3–4 cm in diameter in normally perfused liver with a single probe. Even with the creation of a larger lesion by skilled hands using cooled-tip electrodes, a high local recurrence rate (34%) has been found [13]. More technical innovations are sure to follow that will increase the size of the lesion. To date, no studies have systematically compared the size and shape of zones of necrosis created with cooled-tip electrodes versus multiprong electrodes at identical power settings. For all the technologies, however, the effect of vascular flow at the lesion periphery will be an important factor that affects lesion size and shape. For this study, we were unable to use generators that create larger radiofrequency lesions because of the thin pig livers in our model. Large radiofrequency lesions come to the hepatic surface and become grossly distorted, which makes accurate calculations of lesion size, volume, and shape virtually impossible. Because ours was not a study of the overall efficacy of radiofrequency ablation, this limitation in technology was thought to be acceptable.

Although lesion size is critically important for determining the success of radiofrequency ablation, we believe that lesion shape is at least as important. In this study, lesion shape was highly dependent on vascular occlusion. Patent vessels created indentations on the radiofrequency lesion, resulting in asymmetric shapes, which is an undesirable result because most liver tumors are roughly spherical. The more spherical radiofrequency lesion seen with vascular occlusion is more easily overlaid on the tumor, decreasing the chances of an untreated tumor margin.

When complex shapes are created by radiofrequency, neither lesion volume nor maximum diameter adequately describes the size of tumor that can be ablated by a given size radiofrequency lesion. We believe that minimum diameter may be a better parameter for this purpose. If radiofrequency ablation were performed percutaneously without vascular occlusion, the minimum size of the ablated zone in this study would have been approximately 1.0 cm. However, with hepatic artery occlusion (which can also be performed percutaneously), the minimum diameter increased by 90%, to 1.9 cm. At the same time, hepatic artery occlusion caused the radiofrequency lesion to be more spherical. Further vascular occlusion (the Pringle maneuver) improved only incrementally after hepatic artery occlusion. Whether this minimum improvement justifies a laparotomy remains to be seen. Patients subjected to laparotomy may be best treated by cryoablation, which has a low local recurrence rate, allows multiple lesions to be simultaneously ablated in a shorter time than is possible with radiofrequency, can be precisely monitored with intraoperative sonography, has a low complication rate, and does not require vascular occlusion for most tumors [29–31].

The length of time that lethal temperatures could be seen in radiofrequency lesions increased with all forms of vascular occlusion. This result was expected because increased blood flow through and around radiofrequency lesions would be expected to cool the lesion at a faster rate. The increased time at high temperatures in lesions produced with vascular occlusion suggests an increased probability of uniform cell death throughout the radiofrequency lesion [21, 22, 24]. In the past, viable cancer cells have been found surrounded by necrotic tissue after radiofrequency ablation [12]. The more rapid cooling of the radiofrequency lesion without vascular occlusion may also have contributed to tumor cell survival and higher local recurrence rates in clinical series.

If radiofrequency is to be applied percutaneously, the combination of radiofrequency and hepatic artery occlusion appears to offer theoretic [21, 22] and practical (larger and more regular lesions) advantages when compared with radiofrequency alone. Hepatic artery occlusion can also be accomplished with selective chemoembolization before radiofrequency, which would have the added benefit of treating the tumor with both heat and high concentrations of chemotherapy. An additional benefit of chemoembolization before radiofrequency is that most tumors receive a disproportionate amount of blood from the hepatic artery circulation [32]. Occlusion of the hepatic artery would thus affect tumor circulation more than that of normal liver, which may lead to a degree of protection for normal liver when compared with tumors.

Our study had several limitations. We did not use a tumor model because an implantable model in large animals is not widely available. However, some data suggest that tumor is more susceptible to heat damage than normal tissue [33]. Radiofrequency lesion size and shape may be somewhat different for tumors than for normal liver because of the hepatic artery supply to most malignant hepatic tumors. As previously mentioned, occlusion of the hepatic artery may lead to a disproportionately larger lesion in tumors than in normal liver because of the dependence of most tumors on hepatic artery blood. This study also did not address the potential synergistic effect of radiofrequency combined with chemoembolization as compared with bland embolization. This effect would be best studied in a tumor model.

The results of this study and of clinical trials highlight the need for further clinical trials comparing the combination of hepatic artery occlusion (or chemoembolization) and radiofrequency ablation versus radiofrequency alone as a means of decreasing the high local recurrence rates that hamper the clinical application of radiofrequency.

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References