Ultrasound validation of mathematically modeled irreversible electroporation ablation areas

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Objective. *Currently, the prediction of the dimensions of irreversible electroporation (IRE) ablation is modeled using algorithms derived from mathematical and ex vivo models. These algorithms have not been validated using in vivo studies. The aim of this study was to assess the correlation between the mathematical prediction model to and ultrasonographic and histopathologic findings for in vivo ablations in a porcine model.*

Methods. *IRE ablations were performed on porcine liver and spleen with probe spacings ranging from 0.6 to 2.6 cm. Pre and 2-hour postablation ultrasound (US) images were recorded and validated with confirmation by histopathology. Three dimensions of the regions of ablation were recorded, and ablation volumes were calculated and correlated with theoretic mathematical models for each given probe spacing.*

Results. *In vivo axial and anterior-posterior (AP) distances of ablation were greater than predicted for nearly all probe spacings ($P < .05$). Ablation volumes were significantly less than predicted for the all probe spacings when modeled using both a cylinder and an ellipsoid. Geometrically, mathematically derived regions of ablation demonstrated more central tapering ("necking") and diminished volumes compared to their in vivo counterparts. The relationships between probe spacing and AP dimensions of ablation were less linear ($r^2 = 0.57$) than the relationships observed via ultrasonography.*

Conclusion. *The current mathematical models predict regions of ablation observed in vivo poorly. They underestimate dimensions of ablation and, by extension, the volumes of ablation. Further work should be done to improve models for ablative planning, and physicians should recognize the limitations of existing models when planning ablative treatments. (Surgery 2016;159:1032-40.)*

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Currently, multiple methods of thermal ablation are used to treat locally a variety of malignancies of the liver, lung, and kidney. Irreversible electroporation (IRE) has emerged as a non-thermal alternative, and thus has a theoretic advantage over radiofrequency/microwave/cyroablation/high-frequency ultrasound ablation by being able to treat a similar volume of tissue with minimal damage to surrounding tissues.[1-9] Since that time, various studies have demonstrated the safety and efficacy of IRE in a number of different settings, with the most promising results in locally advanced pancreatic cancer, hepatocellular carcinoma, liver metastases from various sources, and prostate cancer.[1,2,4-8,10-14] Currently, hepatic and pancreatic lesions represent those neoplasms targeted most frequently with IRE owing to the greatest demonstrated efficacy in treating neoplasms in these 2 organs.

For each of these malignancies, standardized protocols have been developed regarding voltage across electrodes as well as number and duration of pulses. Lesions are identified routinely, and electrode placement is performed by bracketing the target lesion using intraoperative ultrasonography or computed tomography (CT) guidance. Electrode placement is determined based on the desired region of ablation. These regions to be ablated have been calculated using mathematical models developed by AngioDynamics.[15] Given the heterogeneity of tissues, this model uses a test pulse between probes after placement to account for variables, such as tissue conductivity, resistivity, and density. It is important to note that IRE works by inducing pores in the cellular phospholipid bilayer via an electric current. Like other ablative

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modalities, IRE is influenced by tissue density, conductivity, and water content like other ablative modalities, but importantly IRE is not influenced by the heat sink effect. Furthermore, whereas other ablative modalities induce cell death via necrosis, IRE induces apoptotic cell death.

Studies have examined postablation histologic and ultrasonographic findings both immediately postablation and over time showing correlation between cell death via apoptosis and change in tissue characteristics.[1,16] Further investigations have imaged the areas of ablation using MRI as well as CT and have characterized postablation changes using these modalities.[1,17,18] Investigators have correlated histopathology with modeled predictions of ablation as well as preablation and postablation imaging characteristics on CT.[17,19-21] These studies have suggested that peripheral apoptotic effects are less permissive than those closer to the electrodes and that models may underestimate the actual volume of tissue effected by a given electroporation treatment.

Despite the extensive analysis of ablation zones via histopathology as well as various imaging modalities, no studies have used in vivo ultrasonography and histopathologic confirmation to validate the regions of ablation calculated via mathematical algorithm that determines electrode spacing intraprocedurally. In this study, we aim to validate the mathematical modeling predicted electroporation volume with in vivo histopathologic findings. In doing so, we assessed the accuracy of preexisting mathematical models of ablation.

METHODS AND MATERIALS

Animals. This study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and the protocol was approved by the Animal Care and Use Committee of the University of Louisville. The University of Louisville animal care and use program is fully accredited by the American Association for the Accreditation of Laboratory Animal Care, International. Five female, all-white Yorkshire × Landrace swine (*Sus scrofa*) from Oak Hill Genetics (Ewing, IL) were obtained at 8–10 months of age, weighing 85–90 kg at the time of arrival. During quarantine and acclimation, animals were group housed in pens with elevated fiberglass slatted floors providing a minimum 20 ft^2 per animal in a temperature (22.0–22.0°C) and humidity (30–70%) controlled room on a 12:12 hour light:dark cycle. Pens were cleaned daily, and animals were fed 5084 Laboratory Porcine Diet Grower (LabDiet, PMI Nutrition International, Richmond, IN) twice daily in amounts recommended by the manufacturer. Animals were provided filtered tap water ad libidum from arrival through the end of the study. Animals were free of the following pathogens: *Actinobacacillus pleuropneumoniae*, *Mycoplasma pneumonia*, porcine reproductive and respiratory syndrome, atrophic rhinitis, Pneudorabies virus, and acute malignant hypothermia. Animals were free of internal and external parasites. Before any experimental manipulations were initiated, animals were allowed to acclimate for ≥7 days. Veterinary staff assessed the animals before each procedure for general health, vital signs, oral intake, and urine and fecal output. Baseline complete blood cell counts and serum biochemistries were performed before operation.

Technique of ablation. Transdermal fentanyl patches (50 μg/h, Ortho-McNeil-Janssen Pharmaceuticals Inc., Raritan, NJ) were applied onto the skin of the shoulders of the animals the evening before operation. The animals were fasted for 12 hours, but access to water was not restricted. The animals were anesthetized with subcutaneous 4.4 mg/kg telazol (Fort Dodge Animal Health, Overland Park, KS), 2.2 mg/kg ketamine (Fort Dodge Animal Health), and 2.2 mg/kg xylazine (Butler Animal Health, Dublin, OH). The animals were then intubated and maintained on 1–3% Isoflurane (Butler Animal Health) and 100% oxygen. Artificial Tears ophthalmic ointment (Butler Animal Health), was applied to the surface of both eyes. Body temperature was maintained with a warm air circulating blanket (Bair Hugger, Arizant Healthcare, Eden Prairie, MN). A 20-gauge intravenous catheter was placed in the marginal ear vein, and 10 mL/kg/h Normosol-R (Hospira, Lake Forest, IL) was administered to maintain blood pressure. Each animal received 2 mg/kg carprofen IV (Pfizer Animal Health, New York, NY) and 0.04 mg/kg buprenorphine IV (Butler Animal Health) for analgesia before operation. The animals were placed on mechanical ventilation, and heart rate, body temperature, SpO_2, reflexes, and blood pressure were monitored continuously throughout the anesthetic procedure to maintain the animals in a surgical plane of anesthesia. To control muscle twitches during IRE, 30 mg succinylcholine (Butler Animal Health) was administered intravenously and was readministered as needed during the procedure based on muscle twitches of the animal. Each animal was placed in dorsal recumbency. A 7-French polysulfone vascular access port (Access Technologies, Skokie,