Irreversible Electroporation of the Femoral Neurovascular Bundle: Imaging and Histologic Evaluation in a Swine Model

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ABSTRACT

Purpose: To evaluate imaging, histologic changes, and safety of irreversible electroporation (IRE) on the femoral neurovascular bundle in a swine model.

Materials and Methods: The study was approved by the institutional animal ethics committee. IRE was performed on the right femoral neurovascular bundle of 9 swine, which were subsequently sacrificed at 24 hours (n = 4, acute group), 7 days (n = 4, subacute group), or 21 days (n = 1, delayed group). Clinical observation, computed tomography (CT), and pathologic examination were carried out.

Results: After the procedure, 7 of 9 subjects were able to stand and walk, and the remaining 2 subjects could eventually do so within 1 week. The femoral vessels were patent on CT and gross examination. There was microscopic evidence of venous thrombosis in 75% of the subacute group. Except for mild perineural inflammation observed in 1 subject in the subacute group, the femoral nerves were intact on gross and histologic examination. Significant damage to the surrounding muscle and soft tissue was identified on CT and histology, manifesting as necrosis, hematoma, and inflammation.

Conclusions: The ablative effect of IRE on muscle and soft tissue manifested as necrosis, hemorrhage, and inflammation. Histologic changes were observed in the perineural tissue and veins in a few subjects. The clinical implication of such changes and safety of clinical use of IRE for lesions encasing the neurovascular bundle in humans are yet to be determined.

ABBREVIATION

IRE = irreversible electroporation

Irreversible electroporation (IRE) is a new ablation technique increasingly used in tumor ablation. The application of high-voltage electric impulses is thought to increase the permeability of cell membrane. With increasing voltage, this process switches from a reversible to an irreversible phenomenon, leading to loss of cell homeostasis and eventually cell death (1). An early study by Onik et al (2) found no evidence of nerve damage in the neurovascular bundle of canine prostates ablated by IRE. However, a subsequent investigation dedicated to the study of nerves by Schoellnast et al (3) demonstrated acute axonal injury in the sciatic nerves of 9 swine, and Li et al (4) identified similar findings in the sciatic nerves of 30 rats. Nevertheless, a more recent study by Tam et al (5) found nerve damage in only a minority of the exit nerve roots in 8 swine lumbar vertebral bodies treated by IRE. Different methodologies were employed by these studies, with heterogeneous nerve size, perineural tissue composition, electrode orientation, and electric parameters. These differences may account for the discrepancy of results.

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The safety of IRE in the proximity of nerves appears to be unresolved. Experiments conducted in organs of important clinical interest, such as limbs and spine, would potentially aid in simulating clinical scenarios. The effect of IRE on arteries was investigated previously in studies by Maor et al (6–8), which demonstrated loss of vascular smooth muscle cells with intact extracellular matrix, elastic fiber, and repopulation of endothelial cells. The connective tissue scaffold is preserved, which maintains vascular patency and allows regeneration. The high vessel patency rate observed in clinical series supported these findings (9–11). However, the imaging features of IRE-treated neurovascular bundle and surrounding soft tissue have not yet been characterized. This study used a swine model to evaluate (*a*) the imaging and histologic changes of IRE-treated femoral neurovascular bundle and (*b*) the safety of IRE on the neurovascular bundle.

MATERIALS AND METHODS

Study Design

This study was approved by the institutional animal ethics committee. To simulate clinical application in human subjects better and to accommodate for the size of devices, the study was conducted using Yorkshire swine (35–60kg). Nine swine were divided into three groups. In the acute group, four swine were sacrificed 24 hours after the IRE procedure to study acute complications and early histologic changes. In the subacute group, four swine were sacrificed 7 days after the IRE procedure to study complications after necrosis of treated tissue and integrity of the structure of study targets. In the delayed group, one swine was sacrificed 3 weeks after the IRE procedure to observe the recovery of tissue and fibrotic change of structures. The primary endpoints were radiologic and histologic changes of the femoral neurovascular bundle. A secondary endpoint was the safety of IRE from the clinical (presence and duration of limping and procedure-related mortality), radiologic (computed tomography [CT] findings of hematoma, vascular thrombosis, and enhancement), and histologic (muscular, vascular, and neural tissue changes or injury) perspectives.

Sedation and Anesthesia

General anesthesia, monitoring after the procedure, and euthanasia were conducted by the veterinary team and animal care staff of the Laboratory Animal Services Centre (D.K.R.) with more than 18 years of experience in laboratory animal–related research and his veterinary team and animal care staff. After induction with intramuscular ketamine (18 mg/kg), xylazine (1 mg/kg), and buprenorphine (0.02 mg/kg), the subject was intubated and then positioned in a supine position. General anesthesia was maintained via intravenous infusion of a mixture of propofol (∼6 mg/kg/h) and ketamine (∼6 mg/kg/h) with neuromuscular blockade using vercuronium (bolus 0.11 mg/kg/h). After the IRE procedure, analgesia was provided using buprenorphine 0.02 mg/kg twice a day for 5 days. An antibiotic (marbofloxacin 2.5 mg/kg/d) was also given for 5 days. Subjects were observed for limping of gait and movement of the right hind leg daily after the procedure until sacrifice. The day on which the subject could bear weight and walk (with or without limping) was also recorded. Euthanasia was achieved by administration of ~60 mg/kg of intravenous pentobarbital sodium.

IRE Ablation

Irreversible electroporation was performed by three interventional radiologists (S.C.H.Y., J.W.Y.H., C.M.C.) with more than 10 years of experience in interventional radiology. IRE was delivered using NanoKnife System (AngioDynamics, Queensbury, New York) via two monopolar electrodes (15 cm long). The right inguinal region was used to conduct the experiment. Under ultrasound guidance, two electrodes were inserted perpendicular to the neurovascular bundle, one on each side. The neurovascular bundle was positioned at the midpoint of the exposed part of the electrodes (Fig 1). Considering the parameters used in previous studies (2,3,12,13) and to simulate the clinical setting, voltage potential was set at 1,500 V/cm (voltage, 2,000–3,000 volts; electrode spacing, 1.4–2.2 cm), with an exposed

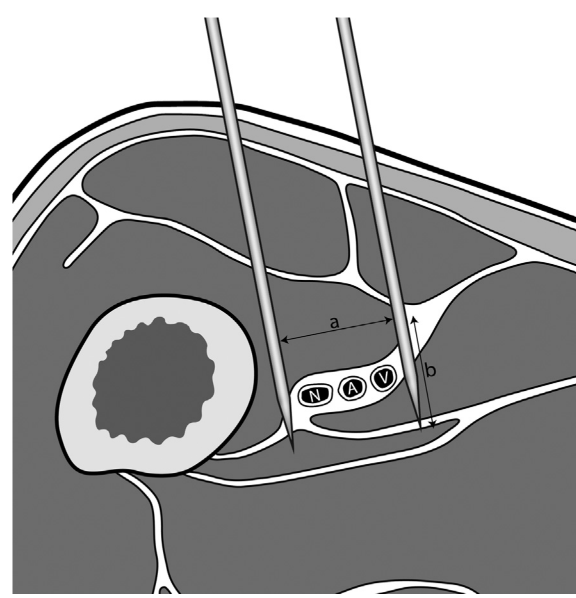

Figure 1. Schematic line drawing illustrating the relationship of the electrodes with respect to the right femoral neurovascular bundle. The parallel electrodes were inserted perpendicular to the long axis of the neurovascular bundle. Distance (*a*) denotes the electrode spacing (1.4–2.2 cm). Distance (*b*) denotes the length of the exposed tip (2–3 cm). The neurovascular bundle was positioned at the midpoint of the exposed tips. N = femoral nerve, A = femoral artery, V = femoral vein.