The Safety of Irreversible Electroporation on Nerves Adjacent to Treated Tumors

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OBJECTIVES: **To evaluate the safety of irreversible electroporation (IRE) on the sciatic nerve after IRE ablation of adjacent tumor.**

METHODS: **In this study, VX2 tumors were implanted adjacent to the sciatic nerves in 26 New Zealand white rabbits. The rabbits were divided into 3 groups 10 days after implantation, with 2 treatment groups of 10 rabbits each and a control group of 6 rabbits. In 1 of the treatment groups (IRE-S group), the rabbits underwent ablation with a single IRE application, and in the other group (IRE-D group) the rabbits underwent 2 IRE applications. IRE ablation was performed under ultrasonographic guidance. The 26 rabbits were euthanized as follows: half of the animals in each group on the seventh day and the rest on the 28th day after IRE ablation. The sciatic nerves were removed for histopathologic evaluation immediately after euthanasia. Sections from selected specimens were stained with hematoxylin and eosin and Masson's trichrome method for collagen; immunohistochemistry was performed for S100 and neurofilaments (markers for Schwann cells and axons, respectively). Clinical, radiologic, laboratory, and pathologic findings were analyzed.**

RESULTS: **The nerves from the IRE-S and IRE-D groups showed preserved endoneurial architecture and the presence of numerous small-caliber axons along with Schwann cell hyperplasia, consistent with axonal regeneration. The tumor lesions were completely necrosed. A fibrous scar was observed in the adjacent muscle tissue, confirming ablation at the site. Nerve damaged also showed in the control group for tumor advanced and no signs of repair; the tumor showed rapid progression.**

CONCLUSION: **The nerves adjacent to the tumor may undergo severe damage after IRE ablation, but their function and structure can return to normal in a short time. IRE ablation may be a feasible treatment option for tumors situated adjacent to nerves.**

INTRODUCTION

Irreversible electroporation (IRE) is a new nonthermal ablation technique, which uses short pulses (70–90 μs) of high-voltage stimulation (maximum 3000 V) to induce cell membrane porosity and acts by generating innumerable, permanent, nanoscale damage in the cell membranes, resulting in cell death.[1] The advantage of IRE over other local ablation techniques is that it selectively destroys cells, leaving surrounding extracellular matrix structures intact. Therefore, the anatomic framework that gives shape and strength to vulnerable structures, such as the bile ducts, blood vessels, and ureters, is preserved during IRE.[2] This allows for the safe ablation of tumors situated near these vulnerable structures. There have been some studies on the effect of IRE on nerves. So far, the effect of IRE ablation on nerves has mainly been studied using rat and pig models.[3,4] In 2011, Schoellnast et al.[4] reported that there is preservation of the

Key words
- Ablation
- Irreversible electroporation
- Nerve
- Safety
- Tumor

Abbreviations and Acronyms
CRP: C-reactive protein
CT: Computed tomography
H&E: Hematoxylin and eosin
IRE: Irreversible electroporation
LY: Lymphocyte count
MT: Masson's trichrome
NSE: Neuron-specific enolase
WBC: White blood cell

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endoneurial architecture after IRE of the sciatic nerve in swine, and that proliferation of Schwann cells can be seen 2 weeks later. Two years later, they reported that the preservation of Schwann cells may enable axonal regeneration 2 months after IRE ablation of nerves.[5] The preservation of the extracellular matrix and the proliferation of Schwann cells, which organize themselves into columns (the bands of Büngner), can provide guidance to regrowing axons.[6] The possibility of regeneration of nerves after IRE ablation makes this a useful technique for the treatment of tumors adjacent to nerves because permanent nerve damage can be avoided.

To simulate the clinical situation, we established a tumor adjacent to the sciatic nerve in a rabbit model, and studied the effect of IRE ablation of the tumor on the nerve.

METHODS AND MATERIALS

Experimental Animals

The study was approved by the Research Animal Care and Use Committee of Guangzhou Fuda Cancer Hospital. Twenty-six male New Zealand white rabbits, weighing 2.0 to 2.5 kg each, were provided by the Animal Experimental Center of Jinan University. The animals were randomly assigned to 3 groups: 10 rabbits were assigned to receive a single IRE ablation (the IRE-S group) and 10 to receive double IRE ablation (the IRE-D group); 6 rabbits were assigned to the control group.

The VX2 strain was maintained by successive transplantation into the hind limbs of carrier rabbits (which were purchased from Guangzhou Jennio Biological Technology Co., Ltd., Guangdong, China.). For implantation of the VX2 tumor between the sciatic nerve and muscle, the tumor was first removed from the carrier rabbit and minced in 0.9% saline solution. The recipient rabbits were anesthetized with intramuscular injection of ketamine (44 mg/kg body weight). Then, incision was made in the right hip of rabbits, and the sciatic nerve was located and exposed. The minced tumor tissue, 0.5 cm in diameter, was embedded 10 mm deep to the sciatic nerve, between the nerve and muscle, and the wound was sutured. All rabbits were given normal feed for 10 days. Computed tomography (CT; SOMATOM Definition 64 AS; Siemens Medical Solutions, Forchheim, Germany), performed 10 days later, revealed tumors of up to 1.6 cm in diameter.

IRE Ablation

IRE ablation of the tumors under general anesthesia was performed in 20 rabbits 10 days after tumor implantation. The rabbits were placed in the supine position, and ultrasonography (Philips iU22; Philips Healthcare, Nederland, Eindhoven, Netherlands) was performed before ablation to visualize the VX2 tumors. General anesthesia was induced with intramuscular ketamine (44 mg/kg body weight) and maintained with 1.5% to 2% isoflurane. The rabbits were ventilated with 100% oxygen, a tidal volume of 30 mL/kg, and a respiratory rate of 30/min. Pancuronium (0.12 mg/kg body weight) was injected intramuscularly to block the muscle contractions that may occur during IRE. The ablation parameters (1500 V/cm between the electrodes, 70-μs pulses, and 90 pulses per ablation) were determined using the preplanning software distributed by the electrode manufacturer and ensured that the electric field would cover the entire tumor. For the IRE-S group, a single treatment session consisting of 1 IRE ablation session was performed. Two 19-g single monopolar electrodes (with 1.5-cm exposure length) were inserted within 5 mm of the implanted tumor edge. The IRE-S group was treated first to optimize the IRE ablation parameters and technique. For the IRE-D group, 2 consecutive sessions of IRE ablations were performed using the above-mentioned parameters. The electrode was placed in 2 overlapping areas in succession to treat the entire tumor (Figure 1). All IRE ablations were performed using the same NanoKnife IRE system (AngioDynamics, Latham, New York, USA) and monopolar electrodes. The ablation zones were monitored and measured in real time with ultrasonography during the procedure. After the probes were removed, the muscle relaxation was reversed with intramuscular neostigmine and atropine (both 5 μg/kg body weight). Autonomous respiration was gradually restored, and the animals were allowed to awaken naturally. They were given appropriate postoperative care. Enrofloxacin (Baytril; 5 mg/kg), a fluoroquinolone antibiotic, was injected intramuscularly for 3 days postoperatively. Each animal also received meloxicam (0.4 mg/kg) orally once a day for pain, starting immediately after recovery and continuing for 3 days postoperatively. Silver sulfadiazine cream (Guangdong Hengjian Pharmaceutical Co., Ltd, Guangdong, China) was applied on the abrasions to facilitate healing. CT was repeated 24 hours after the procedure and then before euthanasia at 7 days or 28 days after IRE ablation.

Contrast-Enhanced CT of Rabbit Tumor

Contrast-enhanced CT images were obtained for all 26 animals before ablation and then at 24 hours, 7 days, and 28 days after ablation. After the initial unenhanced images of the tumors were obtained, iopromide 1.5 mL/kg (Bayer Schering Pharma, Guangzhou, China) was power injected through the ear vein at a rate of 0.4 mL/s, and images were acquired in the arterial dominant phase 15 seconds after injection and in the portal dominant phase 45 seconds after injection. The following CT parameters were used: 110.0 kV, 37.0 mA, 5-mm collimation, and 0.8 seconds. When enhanced CT showed imaging changes in the tumor area, the size of the lesion was identified and measured.

Functional Assessment

Functional recovery was assessed independently immediately after the procedure and then at 24 hours, 4 days, 7 days, 14 days, and 28 days after ablation. Functional assessment was done using a modified Tarlov scale and the semiquantitative toe-spreading reflex test. According to the modified 5-step Tarlov scale, grade 0 indicates complete paraplegia of hind limbs; grade 1, barely detectable movement of hind limbs in response to a hind limb pinch; grade 2, spontaneous movement at all hind limb joints but inability to bear weight or walk; grade 3, able to bear weight and to walk with an abnormal gait on a 1.8-cm-wide ledge; and grade 4, able to walk with normal gait on a ledge.[7] An arbitrary 4-step scale was applied to the toe-spreading index test, as follows: degree I, barely visible spreading of any of the second through the fourth toes; degree II, readily discernible, though slight, spreading of the second through the fourth toes; degree III, unequivocal spreading of the second through the fourth toes (though less forceful than