BTX Applications

Tech-Trends
Volume 4, Series 1

Irreversible electroporation of rat sciatic nerve; determining the long-term effects on nerves

The Effects of Irreversible Electroporation (IRE) on Nerves

Introduction

Irreversible electroporation (IRE) is a technique used to locally target tumors with electrical pulses which cause permanent tissue damage leading to tumor death. The technique is often termed "tissue ablation." IRE involves the application of "microsecond electrical pulses which are applied across the cell to generate a destabilizing electric potential, causing formation of permanent nanoscale defects in the cell membrane. The permanent permeability of the cell membrane leads to changes in cell homeostasis and cell death [8,9].[1]"
There are alternative methods of tissue ablation consisting of chemical (ethanol or acetic acid) ablation, and thermal therapies, such as radiofrequency, laser, microwave, ultrasound, and cryoablation however, these techniques present mild to severe complications including "nonuniform destruction, protection of tumor by the heat sink effect next to large vessels, destruction of tissue collagen with associated destruction of normal structures [10].[1]" The effect of IRE is potentially less detrimental than other tissue ablation techniques, since the nature of applying an electrical field allows for local targeting. IRE has been tested in humans for various cancers and treatment analysis has determined that the procedure is safe and offers potential advantages over current thermal ablative techniques such that IRE ablation can achieve tumor cell death, yet preserve the surrounding cells and tissues from damage.
Li, W. et al., studied the effect of IRE on the sciatic nerve in the recent publication "The Effects of Irreversible Electroporation (IRE) on Nerves." Using the **BTX ECM 2001 Electroporation system**, and the **7 mm Tweezertrode**, IRE was administered to the sciatic nerve in rats and was compared to a control group for nerve damage; data was collected for 10 weeks post IRE. Electrophysiological, histological, and functional results showed that the nerve treated with IRE can attain full recovery after 7 weeks.

Methods

Animals and grouping

Young adult female Sprague-Dawley rats (n= 60) weighing 200 g to 220 g were randomized into 2 groups with 30 animals each: group I(IRE) and group II (Control).

Surgical procedures and irreversible electroporation

The animals were anaesthetized by an intraperitoneal injection of sodium pentobarbital solution (10 mg/mL, 40 mg/kg body weight) and the hair on the right femur was removed. Under aseptic conditions the right sciatic nerve was exposed by making a skin incision and splitting the underlying muscles in the right lateral thigh. At least 15-mm long segment of sciatic nerve was ready for electroporation. A specialty electrode (**BTX Tweezertrode 7 mm electrode**) was applied directly on the sciatic nerve (Fig. 6). The distance between the electrodes measured with a caliper was approximately 1.0 mm. A sequence of 10 direct current square pulses of 380 V (generating an approximate electrical field of 3800 V/cm), each 100 ms long, was applied between the electrodes using the **BTX pulse generator ECM 2001**. In each animal of group I(IRE,) the procedure was applied to produce a treated length of about 10 mm. In each animal of group II (Control), the electrodes were only applied directly on the sciatic nerve for the same time. Keeping the distance of the electrodes approximately 1.0 mm.

Injury level was marked by 9/0 nonabsorbable sutures. At the end of the operation, the muscle and skin were sutured. All surgeries were performed by the same surgeon. Tests were performed immediately after the surgery and at 3 days, 1 week, 3, 5, 7 and 10 weeks following surgery.

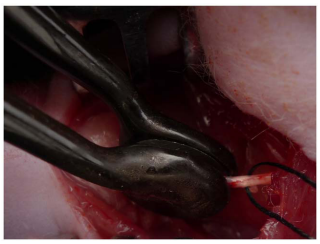

Fig.6 The IRE device clamping the exposed right sciatic nerve.

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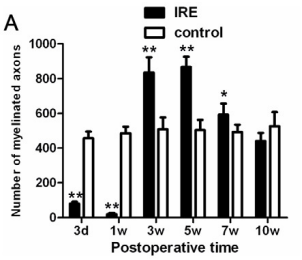
Results

Results from this study demonstrated complete neuronal recovery. Total nerve regeneration post- IRE damage demonstrated that neural cell survival is maintained. Furthermore, "muscle innervated by the sciatic nerve were observed prominently contracting in all animals treated with IRE.[1]" Muscles returned to normal function after 7 weeks, and all of animals from the study survived. Additionally, Schwann cells were observed in recovered tissue and the normal myelin sheath structures were restored in the ablative segment. Though IRE was applied directly to the nerve, minimal endothelial damage in small vessels (of the nerve) was observed in the early phase. After 3 weeks, the damaged vessels were restored to their normal state.

An electric field of 3800 V/cm and 10 pulses were determined to be the optimal ablative protocol. Considering that a substantial length of sciatic nerve was treated with direct, high electrical field IRE pulses, the nerve was able to demonstrate a full functional recovery. Preservation of nerves (involving malignant tumors) with respect to the application of IRE pulses, demonstrates that IRE could be a promising treatment for tumors that impact nerves.



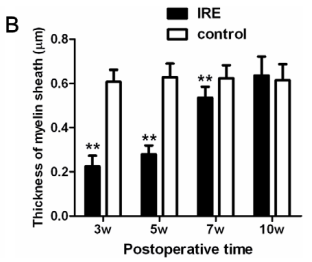


Fig. 3 (a) The statistical analysis of the number of myelinated axons. (b) The statistical analysis of thickness of myelin sheath.

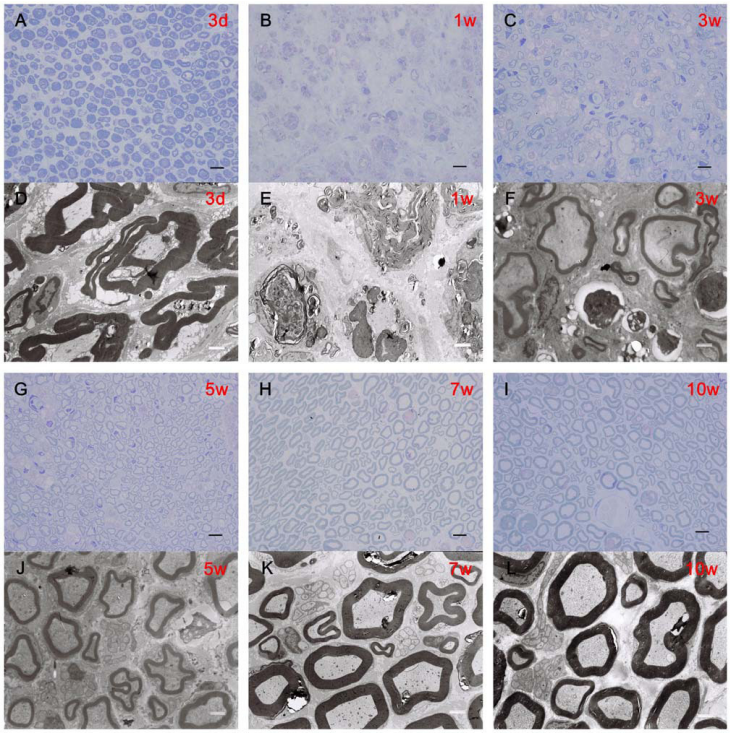


Fig.2 Remyelination of sciatic nerves. (a–c, g–i) Toluidine blue staining. Light micrographs of transverse semi-thin sections at the injury sites of IRE at 3 days, 1 week, 3, 5, 7 and 10 weeks after injury. (d–f, j–l) Transmission electron micrographs(TEMs). Ultra-thin sections at the injury sites of IRE at 3 days, 1 week, 3, 5, 7 and 10 weeks after injury were observed under TEM. Scale bars: A–C, G–I, 10 μm. D–F, J–L, 1 μm.

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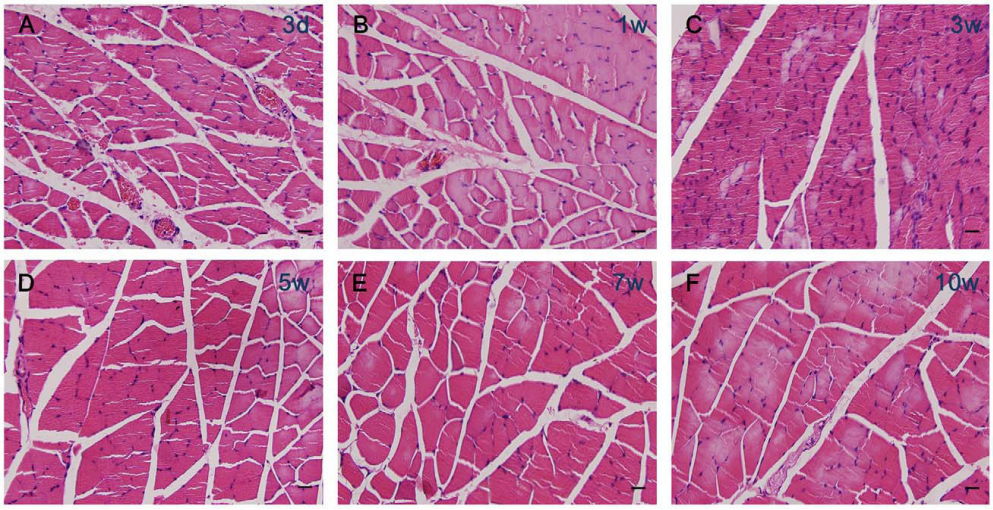

Fig. 4 Histological analysis of target muscle. (a–f) Hematoxylin and eosin staining. Light micrographs of transverse sectioned gastrocnemius muscle on the IRE side at 3 days, 1 week, 3, 5, 7 and 10 weeks after injury. Scale bars: 10 μm.

Conclusion

At present, thermal ablation is the most widely employed tissue ablation technique. However, thermal ablation creates "coagulative necrosis and leaves necrotic tissue, which are toxic to the organs [10].[1]" Furthermore, it has been shown that nerve regeneration in thermal ablative zones is not possible, whereas results from this study show that total nerve regeneration after IRE is possible. IRE is defined by unique characteristics which allow for nerve regeneration, these include:

(1) Structures which are mainly formed by proteins are not damaged by IRE ablation [10].
(2) IRE treatment does not affect the basal lamina or endoneurial integrity but the axon is damaged.
(3) Injuries are similar to second-degree injury (Sunderland) or axonotmesis. The endoneurium and perineurium remain intact and therefore facilitate nerve regeneration.
(4) Regenerating axons follow their normal course and arrive at their original target, so the prospect of recovery is excellent in such injuries [19].

After 7 weeks, affected muscles recovered normal functions supporting that IRE is a safe ablation method for clinical application.

[1]The Effects of Irreversible Electroporation (IRE) on Nerves
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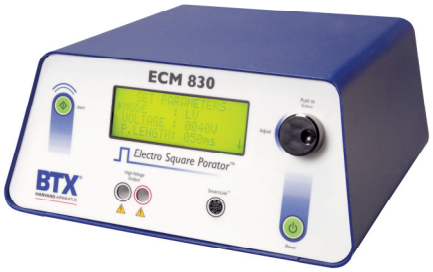

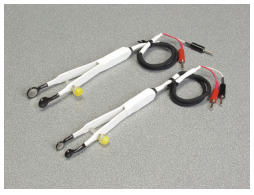

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