Bipolar Electrocautery of Rodent Peripheral Nerve: A Model of Sunderland Type 3 Injury

Arash Moradzadeh1, Michael J. Brenner1, Elizabeth L. Whitlock2, Alice Y. Tong2, Janina P. Luciano2, Terence M. Myckatyn2, Daniel A. Hunter2, Susan E. Mackinnon1,2

1Department of Otolaryngology – Head & Neck Surgery, Washington University School of Medicine
2Division of Plastic and Reconstrucutive Surgery, Washington University School of Medicine

Abstract

Objective: Nerve injuries are a significant source of surgical morbidity. The prognosis after bipolar electrocautery of nerve tissue is uncertain, with management decisions based on limited clinical and experimental data. To better define such injuries, we compared bipolar electrocautery-induced nerve injury to crush nerve injury in a rodent sciatic nerve model. Subjects and Methods: Twenty-two rats received sciatic crush (a reproduceric Sunderland II) or bipolar electrocautery injury and were evaluated for functional, histomorphometric, and immunohistochemical recovery at 21 to 42 days. Axonal regeneration and endoneurial re-innervation were evaluated in double transgenic Thy1-CFP/S100-GFP mice. Results: Compared to crush injury, bipolar electrocautery injury caused greater disruption of myelin and neurofilament architecture at the injury site and decreased nerve fiber counts and percentage neural tissue distal to the injury (p<0.05). Complete functional recovery was seen after crush but not bipolar electrocautery injury. Serial live imaging demonstrated axonal regeneration at week 1 after crush injury and at week 3 after bipolar electrocautery. Qualitative assessment of motor endplate re-innervation at 42 days demonstrated complete neuromuscular endplate re-innervation in the crush group and only limited re-innervation in the bipolar electrocautery group. Conclusion: Bipolar electrocautery injury in a rodent model resulted in a Sunderland Type III injury characterized by gradual, incomplete recovery without intervention.

Introduction

Bipolar electrocautery has proven valuable in decreasing the risk of iatrogenic damage to peripheral nerve associated with unipolar cautery. Nonetheless, the surgeon will occasionally encounter a patient in whom misidentified neural tissue was inappropriately coagulated. Previous research suggests that bipolar electrocautery is more effective than monopolar electrocautery for a Sunderland Type IV injury (Hrutak LA, 1998), whereas a crush results in a more limited injury characterized by Wallerian degeneration and myelin and neurofilament (Sternberger Monoclonals - SMI312) antibodies, then stained with the * Cautery, as used here, refers to bipolar electrocautery injury of nerve. Objective: We now have the first animal model of a third degree nerve injury which will allow peripheral nerve research. Type 3 injuries have the widest variability in recovery and thus, further studies are necessary to elucidate the effect on function, ultimately with the goal of defining treatment paradigms for this uncommon but disabling injury.

Methods and Materials

Operative Procedures: Animals were anesthetized and the sciatic nerve exposed. Using a Bard System 5000 electrosurgical generator connected to a timer, nerves in the sciatic nerve were injured by application of 10W of current (in coagulation mode) through micro bipolar forceps for 1 second, approximately 5 mm proximal to the sciatic nerve injection in rats, and at a setting of 70W in mice. Nerves in the crush group were injured by firm application of the same micro bipolar forceps tips without any current for 30 seconds. Injured sites were marked with 10-0 nylon epineurial sutures.

Serial live imaging: Double transgenic Thy1-CFP/S100-GFP mice were used for serial live imaging of the axon and Schwann cells (SC) over time following nerve injury, and allows for neuroanatomical endplate staining to identify functional connectivity.

References


Figure 1. Sunderland classification of peripheral nerve injury. A: Sunderland Type I injury. B: Sunderland Type II injury. C: Sunderland Type III injury. D: Sunderland Type IV injury. Two additional Sunderland Type IVb injuries are characterized by Wallerian degeneration and myelin and neurofilament (Sternberger Monoclonals - SMI312) antibodies, then stained with the

Figure 2. Serial live imaging: Blue axons (Thy1-CFP), Green: Schwann cells (S100-GFP). Injury site is marked with a probe and distal to injury site, 20 x magnification. Qualitative assessment of motor endplate re-innervation at 42 days demonstrated complete neuromuscular endplate re-innervation in the crush group (Figure 4). In the casemouse, axons are not distal to the injury until week 3.

Figure 3. Whole-mount confocal images of transgenic mice demonstrate individual CFP-positive axons crossing the crush and cautery injury sites at 42 days. Arrow mark site of injury.

Figure 4. Neuromuscular junction (NMJ) staining in a double transgenic mouse. Axonal CFP (blue) reinnervated NMJs labeled by the yellow overlap between axonal-CFP staining (red) and S100-GFP staining (green). Spontaneous from non-reinnervated crush injury demonstrated reinnervation of nearly all NMJs for illustrative purposes. A: label confirming a nonreinnervated junction is shown here.

Figure 5. Walking track analysis. While crush (A) and bipolar electrocautery (B) both demonstrate some improvement, only bipolar electrocautery achieves full recovery at 3 weeks post-injury.

Figure 6. Immunohistochemical analysis of the injury site. Red: axon (neurofilament (Sternberger Monoclonals - SMI312) antibodies, then stained with the green: Schwann cells (S100-GFP). Injury site is marked with a probe and distal to injury site, 20 x magnification. Qualitative assessment of motor endplate re-innervation at 42 days demonstrated complete neuromuscular endplate re-innervation in the crush group (Figure 4). In the casemouse, axons are not distal to the injury until week 3.

Figure 7. Qualitative histomorphometric findings at 3 weeks. A: Crush injury site. B: Bipolar electrocautery injury site. C: Comparison of crush versus bipolar electrocautery injury. Injured sites were harvested and embedded in epoxy. 1 µm cross sections were stained with hematoxylin and eosin. The nerve crush model produces a more robust injury, with comparatively less regeneration and more damage-related debris evident. The nerve crush model demonstrates significant reduced nerve fiber count, nerve density, and myelinated neural tissue, all <0.05. Peripheral destruction was observed in nerve segments injured by bipolar electrocautery, but was not observed in segments injured by crush.

Figure 8. Quantitative histomorphometric findings at 3 weeks. A: Crush injury site. B: Bipolar electrocautery injury site. C: Comparison of crush versus bipolar electrocautery injury. Injured sites were harvested and embedded in epoxy. 1 µm cross sections were stained with hematoxylin and eosin. The nerve crush model produces a more robust injury, with comparatively less regeneration and more damage-related debris evident. The nerve crush model demonstrates significant reduced nerve fiber count, nerve density, and myelinated neural tissue, all <0.05. Peripheral destruction was observed in nerve segments injured by bipolar electrocautery, but was not observed in segments injured by crush.