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Acupuncture Needle-Assisted Nerve Fenestration: Reducing Iatrogenic Neuropathic Outcomes

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Abstract

Introduction: Partial nerve injuries frequently result in neuropathic pain that is treatment refractory and the mechanisms of which are poorly understood. Although several nerve injury models have been investigated in detail, one clinically relevant model that has received little attention is nerve fenestration, or perineurial windowing. Utilized in end-to-side nerve repair techniques and a potentially important component of penetrating and iatrogenic nerve injury, nerve fenestration is usually associated with appreciable nerve injury. The objective of this study was to develop a less injurious method of Sciatic nerve fenestration (SNF).

Methods: Male rats were studied using an approved protocol including videography, paraffin-sectioning and plastic-thin sectioning techniques. Acupuncture and standard cutting needles were used to incise perineurium longitudinally; incisions ranged from 1.5 to 9 mm in length and were carried out using microsurgical instrumentation.

Results: Breaching the epineurium with long incisions (8-9 mm), microsurgical stabilization, and using acupuncture needles to pierce and underskirt the perineurium resulted in full-thickness incisions. Histological analysis confirmed markedly reduced axonal injury.

Discussion: Revised SNF technique using acupuncture needles reduces direct nerve damage; a finding with implications for clinical practice and pre-clinical research. We predict that minimization of axonal injury during fenestration will reduce post-operative pain.

Keywords: Nerve Injury; Acupuncture; Wallerian degeneration; Nerve repair; Neuropathic pain; Bystander effect; Chronic pain

Introduction

Partial nerve injuries frequently result in neuropathic pain that is treatment refractory and the mechanisms of which are poorly understood. Although several nerve injury models have been investigated in detail, one clinically relevant model that has received little attention is nerve fenestration, or perineurial windowing. Controlled opening of the perineurium, known as perineurial windowing or nerve fenestration, is utilized in nerve repair [1,2] and has potential application as a nerve injury model [3]. Perineurial compromise is a component of all penetrating nerve injuries. In particular, iatrogenic nerve injury, e.g., acute angle needle injuries during intravenous or central line insertion, may result in nerve fenestration. Few studies have examined optimal approaches to perineurial windowing [4,5]. The purpose of this study was to reduce the substantial nerve injury usually associated with nerve fenestration.

Clinically, perineurial windowing is utilized in end-to-side and reverse end-to-side neurorrhaphy [5,6]. End-to-side neurorraphy involves grafting a donor and damaged nerve together through a perineurial window [5]. Of note, endogenous growth factors produced after axonal injury may enhance regeneration and minimization of axonal injury during surgical fenestration may or may not be beneficial [2,7].

In the pre-clinical context, e.g., models of painful nerve injury there are compelling motivations to develop techniques that breach perineurium without inducing axonal injury. For example, publications describe the injection of neuroactive substances into endoneurium, proposing to isolate the effects of axonal injury from the effects of the substance itself [8]. While potentially expedient, the degree to which the injected nerve is uninjured is controversial. Recent studies show that needle insertion into the nerve induces trauma [9] and can induce behaviors consistent with chronic pain [10]. Ongoing interest in models of direct and indirect nerve injury effects [11,12] led us to examine perineurial windowing, in particular sciatic nerve fenestration (SNF) in more detail. We report a novel method for SNF that involves 1) much longer incisions than usual and 2) utilizes acupuncture needles, markedly reducing axonal injury. While SNF produces endoneurial edema and other changes, these are of separate importance for neuropathic pain models [12-14] the methodological advance reported here merits further study.

Materials and Methods

An ACUC approved protocol was used to study male rats (225-300 g): 12 Sprague-Dawley, 40 Lewis, and 3 Wistar rats (all Harlan). Standard 21-30 gauge regular-bevel (cutting) needles, 1- 1.5 inches, (Becton-Dickinson, NJ); J-Type 30 mm acupuncture needles (Seirin-America, Weymouth, MA): 0.12 mm (Chinese gauge #44), 0.14 mm (#42), 0.16 mm (#40), 0.18 mm, (#38), 0.20 mm (#36), 0.25 mm (#32); and L-type (coil handle) 30 mm acupuncture needles (Seirin-America) were used: 0.20 mm (#36), 0.25 mm (#32), 0.30 mm (#30). Needles with microscopically visible defects were discarded [15].

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Fenestration method

Under halothane anesthesia skin and muscle were reflected to expose sciatic nerve in mid-thigh. Proximal division of peroneal and tibial nerves excluded animals from further study [16]. The sciatic nerve was freed and a nerve tie consisting of a 2 mm x 20 cm strip of purple nitrile glove (Kimberly-Clark), was used to maintain tension, and align needle and nerve, while advancing the needle proximally to underskirt the perineurium under microscopic visualization. Depth below the perineurium was gauged by opacification by endoneurial elements intervening between needle and normally translucent perineurium. After advancing the desired distance, the needle tip was pushed through the perineurium, the end of the needle was stabilized with forceps and the needle was bowed upwards underneath the perineurium. A sharp scalpel was the grazed along the needle from proximal to distal, freeing needle from nerve and incising perineurium. It was observed in the first stages of this study that the use of standard cutting needles was associated with ease in piercing of the epineurium; however advancing the needle immediately underneath the perineurium was more difficult, due to the relative stiffness of the open-bore needle. Indeed, utilization of these needles was associated with substantial Wallerian degeneration in portions of the distal nerve segment clearly related to the fenestration itself as described below.

The training required to carry out this technique was as little as two sessions for operators skilled in animal surgery. A certain degree of manual agility was required and not all trainees were successful in accomplishing fenestration without appreciable trauma to the nerve. The success of the minimal-trauma nerve fenestration was confirmed with direct microscopic observation, confirmation of those present for surgery and histological assessment. For the purposes of this report, the work of a single operator (B.M.) is presented. Following surgery and recovery from inhalational anesthetic, the animals appeared to recover well and ambulated freely in the cage.

Histological methods

Nerves were harvested one to six weeks after surgery and prepared as described [17]. Nerves were harvested at earlier time periods in order to demonstrate active Wallerian degeneration; nerves were harvested at later time periods in order to appraise the lasting damage to the nerve as a result of the procedure. Control nerves were also harvested following sham procedures and nerve transection (positive control). The details regarding the timing of when nerves were harvested are included in Figure 1. In brief, perfusion with PBS and 4% paraformaldehyde in Sorenson's buffer (SBS) preceded dissection and post-fixation in 4% paraformaldehyde/3% gluteraldehyde. Nerves were processed for plastic embedding, sectioned using thick plastic section technique, and stained with Toluidine blue. An Olympus BH3 microscope with a DP73 camera and CellSense (Olympus) was used to capture images adjusted for white balance, contrast and brightness but otherwise unprocessed.

Results

Longer nerve fenestration reduces endoneurial bulging through the perineurial window

As reported by Spencer et al. [3] the short SNF (1.5-3 mm) induced pronounced bulging of endoneurial contents, Figure 1A and 1B [18]. With longer SNF (8-9 mm), endoneurial bulging was reduced. Instead, the nerve splayed open and mildly protruded, Figure 1C-1E. The development of endoneurial bulging occurred with short nerve fenestrations regardless of needle type, e.g. comparison of Figure 1A and 1B. This appeared to be related to the intrinsic elasticity of the perineurium and the gross dimensions of the nerve in counterpoise to the positive pressure of endoneurial contents. In the context of the short perineurial window, e.g. less than 3 mm, the edges of the incised perineurium formed a constricting ring around the base of the extruding nerve. With the longer fenestration, the edges of incised perineurium separated further apart, effectively accommodating the contents of the nerve under positive endoneurial pressure. The extent of Wallerian degeneration distal to the short fenestration was sunstantial, and notably greater than that noted following long fenestration, shown Figure 1L and 1M.

Acupuncture needles reduce direct injury to axons

Fenestration with standard cutting needles produced a considerable amount of axonal injury, compared to control, and transected nerve Figure 1, panels G-I. Acupuncture needles induced markedly less axonal injury while completely opening the perineurium, Figure 1J and 1K. A trial of various types of acupuncture needles revealed that J-type needles, 0.20 mm, were optimal. As detailed in Table 1, the 0.20 mm Seirin J-type needle was found to be superior to the other needles tried for this application. The 0.20 mm needle exhibits an optimal balance of stiffness and springiness allowing advancement the needle forward without excessive bending, but still retaining the potential for bending or bowing when additional pressure is applied. J-type needles of greater or lesser caliber where found to be too stiff or too pliable respectively. The Seirin L-type needle has a metal coil handle and is held to exhibit electrostatic properties that are desirable in certain settings. In several attempts to use the L-type needle for nerve fenestration, it was observed that the contact of the needle with the nerve caused a distinctive myoclonic jerk. This twitching phenomenon was observed reproducibly with the L-type needle and was felt by us to represent an undesirable activation of muscle efferent fibers by stored electrical charge. Finally, non-Seirin brand J-types 0.20 mm needles were utilized in selected animals. These needles did not provide the tactile smoothness of the Seirin brand needles and this was felt to be indicative of increased risk for damage to the endoneurial contents.

Following SNF, some edema was observed histologically as previously reported [2]. Nonetheless, distal Wallerian degeneration was minimal, Figure 1L and 1M. The gross appearance of the nerve at dissection was remarkably similar to the appearance at the time of surgery with minimal fibrosis developing around the nerve itself. No constricting bands were observed and in our experience, infection of the surgical site is an infrequent occurrence (<1%). Photomicrography of standard cutting and acupuncture needles showed that acupuncture needles are much smaller, smoother, and without machined cutting surfaces which likely contribute to the overall reduction in nerve injury with acupuncture needles, Figure 1N-1P

Discussion

This report describes a new approach to nerve fenestration markedly reducing associated axonal injury. This approach is important in that it introduces a new form of minimal nerve injury that will be useful in studies of neuropathic pain; pain associated with nerve repair procedures, pain associated with degenerating nerve explants and nerve repair studies. This method incorporates microsurgical techniques, the use of acupuncture needles, and extension of perineurial fenestration length. Each of these components is important: microsurgical technique, especially customized nerve ties, controls the relative angle of needle entry and provides back-pressure as the needle is insinuated into the endoneurium. Extension of the SNF to 8-9 mm is critical for reducing focal pressure on the endoneurial contents at the fenestration

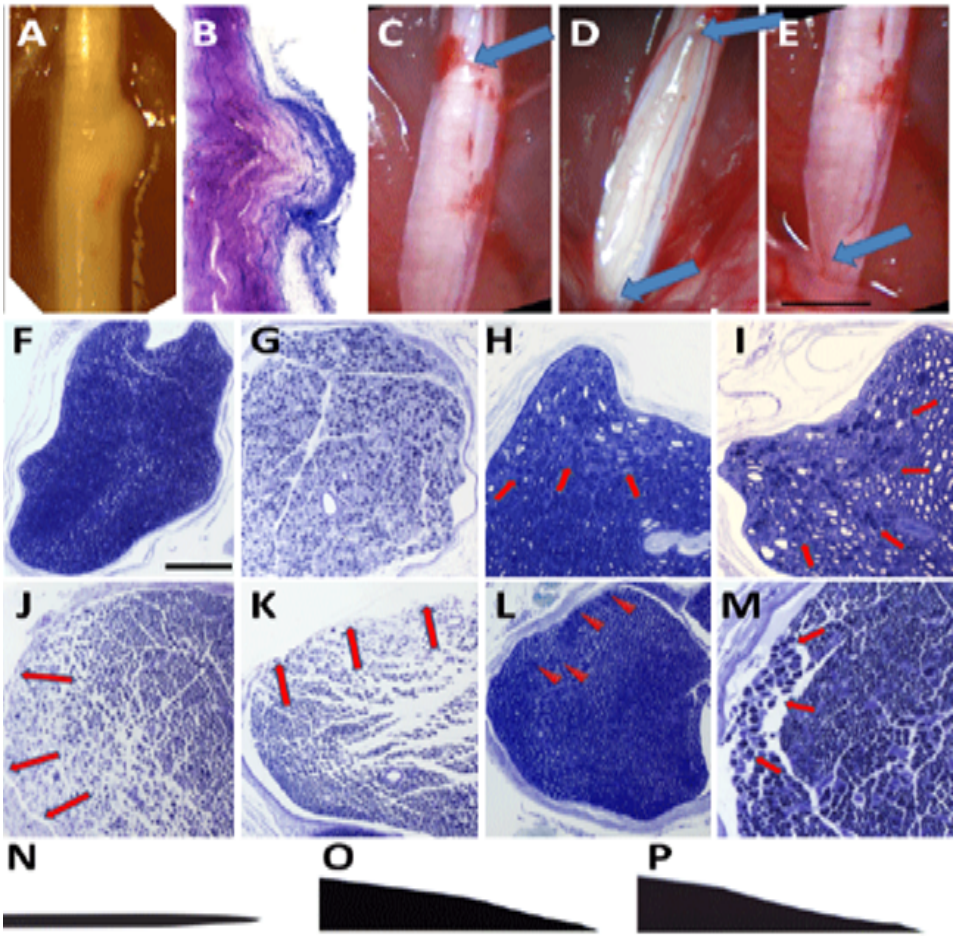

Figure 1: Consequences of short and long sciatic nerve fenestration A) Short fenestration, post-mortem photograph taken 1 week after 1.5 mm fenestration of the sciatic nerve with a Seirin J-type 0.2 mm needle. Prominent bulging of endoneurial contents is observed. B) Short fenestration, histological section of a immersion fixed nerve cut with freezing-sliding technique and stained with 1.0% toluidine blue. One week post-operatively, prominent bulging of endoneurial contents is evident, axonal transection as well as Wallerian degeneration is observed in this nerve fenestrated with a standard-beveled cutting needle. C-E) Intraoperative appearance of the sciatic nerve at the time of long sciatic nerve fenestration. Although a wide separation of the incised edges of perineurium is observed, there is little bulging of the endoneurial contents around the edges of the opening in the nerve. The proximal and distal ends of the fenestrations are shown by arrows. Bar equals 3 mm. F-M) Histological appearance of nerves under control and experimental conditions, plastic embedded thin sections stained with toluidine blue. F) Intact nerve from unoperated control animal. G) Nerve distal to complete transection illustrating profuse Wallerian degeneration and edema, as characteristically observed 7 days after lesioning (positive control). H and I) Examples of plastic sections 1.5 mm distal to the fenestrations, 7 days post-operative, in which fenestration was performed using an 18 gauge and 21 gauge standard-bevel cutting needle, respectively. The nerves shows substantial numbers of axons undergoing Wallerian degeneration, areas of axonal degeneration are delineated by medium red arrows. J and K) Examples of plastic sections at the level of the nerve fenestration, 7 days post-operative, Illustrating complete opening of the perineurium, demarcated by long red arrows, Seirin J-type 0.2 mm needle. L and M) Examples of plastic sections distal to the level of the nerve fenestration with a Seirin J-type 0.2 mm needle. There are sporadic myelin ovoids, red arrow heads, panel L, and occasional evidence of nerve edema, small red arrows, panel M. Scale Bar equals 0.5 mm, F, G, J, K, and L; 0.25 mm H and I; 0.1 mm M. N-P) Photomicrographs of selected needles: N) 0.20 mm J-series acupuncture needle, details in text. O) 21 gauge cutting needle, P) 18 gauge cutting needle. Bar (shown in F) equals 2.5 mm.

rim. This likely reflects the limited intrinsic compliance of perineurium which is such that short openings produce a taut rim of perineurium through which endoneurial contents extrude due to the high relative pressure between endoneurial and extraneurial space.

The longer opening increases the effective compliance of the perineurium and decreases local forces at the rim. Concordantly, lengthening the incision did not result in increased Wallerian degeneration distal to SNF. The use of acupuncture needles to penetrate the perineurium and establish the incision was novel, to our knowledge, and essential for minimizing axonal injury. As we and others have observed [9], nerve penetration by normally-beveled cutting needles, is associated with considerable direct axonal injury.

Besides the potential clinical application in the context of end-to-side nerve repair, we view this as a new model of partial nerve injury that merits additional study. Animal models of neuropathic nerve injury have advanced the understanding of the transition from acute to chronic pain. The majority of these models involve complex injuries to nerve [19,20]. Because multiple degenerative changes following nerve injury, including proximal neuroma formation, distal Wallerian degeneration, target organ denervation [21], blood-nerve barrier compromise, and macrophage influx and activation, it has been difficult to ascribe distinct mechanistic significance to specific injury components. We are in the midst of investigating whether this may be a new model of neuropathic injury and whether this minimal injury model may be associated with pain behavior in various model systems. We believe that this technique of nerve fenestration which provides access to the endoneurium may have other applications.

There is increasing interest in the role of macrophages in peripheral nerve as mediators of neuropathic pain. Our fenestration technique clearly results in an increase in macrophages in the affected nerve (unpublished observations) and may be of relevance to this evolving area of study. Finally, this technique may have implications for study of nerve repair and the development of nerve conduits. This is perhaps the most compelling reason to study SNF with minimal axonal injury. Finally, this technique may have implications for study of nerve regeneration and the development of nerve conduits, further study is merited.

Potential limitations of this model system include the technical demands of model induction, the degree of inter-procedural variability and the extent to which changes induced by the model are relevant to the study of pain. Model induction does require access to a surgical microscope and the time required to induce the model ranges from 20-40 minutes depending on the experience of the operator and the anatomical features of each animal. There are situations where the location of the sciatic nerve in the thigh is variable with some animals exhibiting localization of the nerve more rostrally and other more caudally. This has a significant impact on the conduct of the procedure as it is critically important to expose and access a significant length of nerve in the thigh. As the procedure is performed using an anterior approach, a caudally positioned nerve can be difficult to access and require more procedural time. Nonetheless, we have found that is it possible to performed several surgeries in one sitting, enough that it is possible to prepare a cohort of animals for behavioral testing in a single surgical session of several hours' durations. An advantage of this model is that there is a significant 'indirect' injury to the nerve in the form of compromised blood-nerve barrier, and yet model induction does not require an 'invasive' surgery with osteotomy or other potentially painfully invasive components. A second potential limitation of this model system includes potential inter-procedural variability. As noted above, there is some degree of variability of placement of the nerve in the thigh, this has an impact on the procedure and sometimes necessitates additional time spent exposing the nerve. Other factors include repeat utilization of the acupuncture needle. In our experience, we find that no more than 3 animals can be prepared using a single needle. Although we have not investigated the reasons why, it is clear to our staff that with repeated use, the acupuncture needle requires that more pressure be applied to penetrate the perineurium, a phenomenon we view as undesirable. In fact, most acupuncture needles are designed for single-use and the cost of these needles is nominal. The procedure is transferrable: It has been possible to train several persons in the induction of this model and we see reproducible results in terms of histological and behavioral outcomes (data not shown). On occasion, there is a concern that the fenestration is incomplete or that the perineurium is not completely transected, in these animals, if surgical efforts to rectify the problem are not satisfactory, that is to say carried out with only minimal additional manipulation of the nerve; the animal is excluded from further study. A third potential limitation of the model system includes the relevance of the induced injury and its importance for further study. We view this model as important both in terms of potential clinical application and for pre-clinical nerve injury and basic nerve repair research. The potential direct clinical application has to do with the use of this technique to end-to-side nerve repair and is discussed elsewhere in this report. More importantly, we find that this model induces changes in the nerve which are often concomitant with other forms of nerve injury but which have, until now, not been studied in isolation. As a result, it is not known whether nerve edema and violation of the blood nerve barrier, in isolation, are likely to induce pain behavior. It is not known, to what extent, these injuries will result in the up-regulation of pain signaling molecules in the relevant structures and it is not known whether the induction of nerve edema and compromise of the blood-nerve barrier will result in a predilection towards pain in particular settings. All of these questions are of fundamental interest with the aim of improved understanding of nerve function in normal and pathological states.

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