Microneural Anastomosis with Fibrin Glue: An Experimental Study

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Summary

An experimental study was designed to compare the histological analysis of nerve anastomosis with 10-0 microsurgical sutures and fibrin adhesive. Wistar albino rats’ sciatic nerves were transected and repaired either with fibrin adhesive-Beriplast P (M/s Centeon – Cadila Health Care) or with 10-0 monofilament microsutures. Histological assessment was performed at 10, 20, 30, 60 and 90 days after surgery. Functional recovery of the sciatic nerves started at two months and was near normal by three months. Separation of the stumps did not occur in any of the glued nerves. Histological evaluation showed no appreciable difference in the outcome of nerve regeneration after microsurgical repair using sutures or fibrin tissue adhesive. However, inflammation and granuloma formation were appreciated at the suture site, which presented a focal hindrance to myelin and axonal regeneration. Fibrin glueing is attractive for clinical purposes, since it is simpler and less time consuming than suturing.

Key words: Nerve repair, Microsuture neuroanastomosis, Fibrin glue, Adhesive, Axonal regeneration, Wallerian degeneration.

Introduction

Reuniting severed nerves by using fibrin glue instead of traumatizing sutures dates back to the experimental work of Young and Medawar and Tarlov et al in the early 1940s. However, the method remained purely experimental until the 1970s, when Matras et al1 developed it further. Experimental2-5 and clinical6 studies on this subject have been published recently.

Our study was performed to compare the healing and regeneration of peripheral nerves in rats using fibrin glue and microsutures by histological assessment. The present study is the only one of its kind to document focal hindrance presented by suture granulomas to the regeneration of myelin and axons.

Material and Methods

A total of 40 male Wistar albino rats, weighing between 250 gm and 300 gm, were used for this study. Animals were anesthetized with intraperitoneal sodium pentothal in a dose of 40 mg/kg. Dorsal aspect
of right hip and thigh was shaved and prepared and animals were fixed to a wax tray. The sciatic nerve was exposed, dissected and transected using No.11 blade under operating microscope. In 20 animals, cut ends of the nerve were repaired with three epineural microsutures using 10-0 monofilament nylon placed at 120° under 25 times magnification. In the remaining 20 rats, the transected nerves were placed on a small piece of latex glove material and glued with fibrin glue -Beriplast P (M/s Centeon - Cadila Health Care).

Beriplast P was reconstituted. Fibrinogen concentrate (vial 1) containing 65 mg of fibrinogen, 40 U of factor XIII and 5 mg of human albumin was dissolved in Aprotinin solution (vial 2) containing 1000 K.I.U/ml to form the sealant solution. Thrombin (vial 3) containing 4.9 mg of dry thrombin was dissolved in 1 ml of calcium chloride solution (vial 4). Subsequently, two drops of the fibrinogen sealant solution followed by the thrombin solution were added by the assistant over the aligned nerve stumps held by the surgeon with two Dumont forceps at 25 times magnification. Care was taken that a maximum contact area between nerve ends was obtained and the stumps, were accurately coapted. The sealant was applied as a cuff and not to the section surface. Stabilization of the nerve ends was maintained for 2 minutes. The latex material was removed after 5 minutes. Wounds were washed with saline and skin closed with 2-0 silk sutures. All animals were given standard diet and housed in similar conditions. They were followed up for a range of 10 days to 90 days.

Histological assessment was made at 10, 20, 30, 60 and 90 days after the operation. Four nerve pairs were available for comparison on each occasion. For this purpose, the repaired nerves were exposed and 25 mm segments were excised including a portion of the proximal as well as distal stumps with the junction at the center. Five micron sections were cut and stained with hematoxylin and eosin (H&E) stain. Assessment of myelin was done with Klüver-Barrera stain (Luxol Fast Blue with Periodic Acid Schiff protein counterstain). Immunohistochemical staining was done for neurofilament protein using Streptavidin Avidin Biotin (LSAB) conjugate immunoperoxidase method. Histological assessment of degeneration, loss and regeneration of myelin as well as axons was performed. Other features such as occurrence of inflammation and suture granulomas were noted.

Results

Recovery of the sciatic nerve function started at two months after anastomosis with either fibrin glue or microsuture repair and was near normal by three months. Separation of the stumps or adhesion to the surrounding structures did not occur in any of the repaired nerves when seen under operating microscope.

Histological examinations of glued and sutured nerves at 10, 20, 30, 60 and 90 days after operation showed no appreciable difference in healing or outcome of nerve regeneration between glued and sutured nerves. On the tenth day after surgery, little fibrin was seen in the glued nerves. The proximal stumps showed thickening of the axons while the distal stumps showed marked axon and myelin degeneration. There was evidence of myelin ballooning, fragmentation and loss, accompanied by products of myelin degradation. There also occurred prominent axonal degeneration and loss (Figs. 1a, and b). By the 20th day, the changes became more prominent. Some Schwann cell proliferation was also observed. At the end of the first month after anastomosis with suture or glue, Schwann cell proliferation was very prominent in the distal stumps as well as in the transected sites in both groups. Inflammation and suture granulomas were observed in cases with microsuture repair, which presented a focal obstacle for myelin and axonal regeneration (Fig. 2). At two months after surgery, there was evidence of prominent regenerating myelin at the junction along with regenerating axons (Fig. 3). They were seen penetrating into the distal stump. Axons were not uniformly arranged in both groups. At the end of three months after surgery, distal segments had regenerated myelin and axons (Figs. 4a and b). The junction had been bridged by numerous axons some of which ran parallel, whereas the others were haphazard.

Discussion

Nerve repair is a process of cellular repair. Amputated nerve cells regain their axoplasmic flow by sending out new processes to compensate for their transected parts. The number of neurons do not increase but the repair of each cell takes place in an environment of intense cellular proliferation. When an axon is cut, the corresponding nerve cell undergoes characteristic structural and functional changes. The typical response, first observed by Nissl (1892), includes an increase in cell body volume, displacement of nucleus to the periphery and disappearance of basophilic material from the cytoplasm.7

When a nerve trunk is injured or transected, significant changes take place in normal morphology.
and tissue organization in both the stumps. In proximal stump, axons degenerate for some distance upward leaving the corresponding endoneural tube behind as an empty cylinder. Wallerian degeneration ensues, consisting of lysis of the distal axon followed by fragmentation of the myelin sheaths with formation of myelin ovoids. There is invasion of the Schwann cell basal lamina by macrophages which phagocytose and remove the remaining debris. Parent Schwann cells proliferate within the parent basal lamina forming columns of cells, known as the bands of Bungner, which reside in the distal degenerated nerves available for reinnervation, from the proximal stump.

Within first few days after transection, myelinated axons in the proximal stump produce a great number of axonal sprouts and thin myelin sheaths which advance distally. The success and failure of peripheral nerve repair is determined by the density of axons which regenerate through the interstump zone into the
Distal stump and by the population of these axons that reach their correct targets (original Schwann cell basal lamina) and make connections. In peripheral nerve microsurgery, accurate adaptation and apositioning of the different fascicles is important. Epineurial, perineurial and interfascicular techniques have been advocated. Microsurgical magnification and more precise knowledge of anatomy have improved the results. Perineural suturing gives better alignment of the fascicles and approximation of the perineurium. Studies in the rats have demonstrated that the number of reconnected axons do not differ after perineural or epineural nerve repair but the accuracy of reconnection is higher in perineural repair. Suture placement has been thought to cause a hindrance to the sprouting axons and compress the blood supply to the fascicles, thereby impairing the regeneration of the transected nerve ends after repair. Moreover, formation of suture granuloma obstructs myelin and axonal regeneration. These factors led to the development of various tissue sealants for the purpose of atraumatic tissue repair. Clinical studies have been undertaken to establish its efficacy in peripheral nerves, brachial plexus and facial nerve repair. Hypothetically, fibrin glue repair technique itself may have its own disadvantages, such as penetration of the adhesive through the suture line and pronounced connective tissue reaction induced by glue, causing nerve compression. However, Palazzi demonstrated that fibrin glue is a sealant and not a nerve barrier. There is no appreciable clot retraction because the sealant does not contain thrombocytes.

The present study is the first of its kind to demonstrate inflammation and suture granuloma formation after microsuture repair, which results in focal hindrance to the regeneration of myelin and axons. Moreover, fibrin glueing is simple and less time consuming. The method of fibrin adhesive repair may lead to a considerable shortening of the operating time, especially in brachial plexus surgery, peripheral nerve repairs, facial nerve anastomosis after excision of cerebellopontine (CP) angle tumors, and occulomotor, trochlear and abducens nerve repairs after surgery of cavernous sinus lesions.

**Conclusion**

Our experimental study demonstrated similar healing or outcome of nerve regeneration between glue and microsuture nerve repair. However, suture granuloma presented a local hindrance for uniform axonal/myelin regeneration. Fibrin glueing is an attractive alternative, since it is simpler, less time consuming, less expensive in case of neurotization with multiple cable grafts and God-sent in case of nerve repairs at inaccessible sites like the CP angle and the cavernous sinus.

**References**