Entubulation of sciatic nerve of rats with poly-l lactide-co p-lactide

Tunelização do nervo ciático de ratos com “poly-l lactide-co p-lactide”

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ABSTRACT

Introduction: Axonal regeneration after a sectioning lesion is variable and depends on the environmental conditions of the two end points. An important enzymatic action and an immune response occur at this point. The Schwann cell at the end of the proximal segment starts a rapid mitotic division in an attempt to locate the target tissue for the severed neuron. The capacity for axonal regeneration after a sectioning lesion is variable and depends on the environmental conditions of the two end points.

Objective: To insulate the region of nerve regeneration from biological activities coming from foreign tissues, reducing the immune and enzymatic response and improving the healing conditions of the two end points.

Methods: The sciatic nerves of the right paw of twenty WISTAR EPM rats were sectioned, with a gap of 5 to 8mm. In ten animals a 20mm Poly-L Lactide-Co P-Lactide tube involved both sectioned extremities, without nerve suture. After 6 months the animals were sacrificed, the nerve was dissected and photographed. The distal muscles of the paw were sent to histological examination.

Results: All animals in which entubulation with Poly-L Lactide-Co P-Lactide was performed demonstrated an excellent nerve regeneration. Histological examination demonstrated complete muscle regeneration. In the animals not submitted to nerve entubulation no nerve regeneration was demonstrated and histological examination demonstrated severe muscle atrophy.

Conclusions: Poly-L Lactide-Co P-Lactide entubulation demonstrated satisfactory nerve regeneration without nerve suture after six months spam. Paw innervated muscles did not show any histological atrophic characteristics.

SUMÁRIO

Introdução: A capacidade de regeneração axonal após uma lesão por secção é variável e depende das condições ambientais que cercam os dois cotos terminais. Uma importante reação enzimática assim como uma resposta imunológica ocorre a este nível. As células de Schwann, ao final de segmento proximal, iniciam um rápido processo de divisão mitótica na tentativa de repor o tecido do neurônio lesado.

Objetivo: Isolar a região de regeneração do nervo secionado das reações biológicas originadas dos tecidos vizinhos , reduzir a resposta imunológica e enzimática, melhorando, assim, as condições biológicas dos dois cotos terminais.

Métodos: Os nervos ciáticos da pata direita de vinte ratos WISTAR EPM foram seccionados, com um espaço livre de 5 a 8mm. Em dez animais um tubo de 20mm, confeccionado com Poly-L Lactide-Co P-Lactide, envolveu ambas as extremidades secionadas (tunelização), não sendo efetuada sutura no nervo. Após 6 meses os animais foram sacrificados e o nervo dissecado e fotografado. Os músculos distais da pata foram enviados para exame histológico.

Resultados: Em todos animais em que a tunelização foi efetuada demonstraram uma excelente condição anatômica de regeneração nervosa. O exame histológico demonstrou completa regeneração muscular nesses animais. Animais em que...
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Conclusões: A tunelização com Poly-L Lactide-Co P-Lactide, promovendo o isolamento dos tecidos vizinhos, dos cotos terminais do nervo ciático, em ratos WISTAR EPM, permitiu satisfatória regeneração sem a necessidade de sutura do nervo, após seis meses. Os músculos da pata reinervada não demonstraram características histológicas de atrofia.

Palavras-chave: Tunelização de nervo, regeneração de nervo, cirurgia de nervo periférico.

INTRODUCTION

The high incidence of nervous system injuries, nerve regeneration and repair, has always been an important field dedicated to the discovery of new ways to recover nerve function after injury. Although the peripheral nervous system has the capability for regeneration[1,10,14], much research still needs to be done to optimize the environment for maximum regrowth potential[1,3,5,7,11]. Damaged soft tissues impair the process of nerve regeneration. Injury to the peripheral nervous system immediately elicits the migration of phagocytic cells, Schwann cells and macrophages to the lesion site in order to clear away such debris. Once the debris is cleared, the proximal end swells, experiences some retrograde degeneration, but it begins to sprout axons and the presence of growth cones can be detected. The proximal axons are able to regrow as long as the cell body is intact, and the site of lesion encounters in anatomical and physiological conditions so that a complete anastomosis can be achieved.3,4,5,7,11,18 Providing the ideal conditions, free of scars and foreign tissues to surrounding area of the two end points is the main target to favor the nerve repair. Therefore the undesirable failure that happens in some cases of nerve anastomosis can be minimized. We have proposed to evaluate the regenerative process without the negative effects that foreign tissues and also nerve suture exert in the two end points of peripheral nerve anastomosis. An a special bio absorbing product was used to construct a 20mm tube where both nerve extremities were inserted, remaining free of external influences, during the regenerative process.

METHODS

Twenty rats WISTAR EPM male, weighting 330g to 360g were studied. Animals were anesthetized with ZoletilR zoletamine 1mg/kg. The sciatic nerve of the right paw was dissected and served as proximal as possible to its origin (fig 1). Group A was the control group. Group B consisted of ten animals in which proximal and distal nerve extremities were introduced inside to both ends of a 20mm tube of POLY-L LACTIDE-CO P – LACTIDE. A gap of 5 to 8mm was left between the two end points (fig 2). The sectioned nerve was not sutured. Muscle and superficial tissues were sutured. The animals were observed for 6 months when they were sacrificed. Walking improvement was documented in moving picture every two weeks. Under microscopy the sciatic nerve was dissected and photographed (fig 3). The right paw was removed and immediately sent to histological examination. Twenty examples of rat gastrocnemius muscle were examined. All the material was fresh frozen in liquid nitrogen at – 160°C for histochemistry. The material was cut in Cryostat at -25°C obtaining 10 micra thick sections and stained for Adenosine Triphosphatase pH 4.6 and 9.4 (ATPase), NADH-Tetrazolium Reductase (NADH-TR), Unspecific Esterase, Alkaline Phosphatase, Modified Gomori-Engel Trichrome, Hematoxylin and Eosin, Oil Rd O and Crystal Violet.
RESULTS

Animal's locomotion was observed and recorded. A remarkable difference between both groups could be observed. Group A that has not had nerve entubulation demonstrated a persistent motor power deficit on the right paw. Animals of group B where nerve entubulation was carried out demonstrated a progressive improvement of motor power which stabilized around the 5th month. All animals of group B, at six months, demonstrated only a slight deficit distally localized corresponding to a 90% to 95% recovery. Nerve dissection demonstrated anatomical regeneration (fig 3).

In our control, NADH-TR was the more sensitive method for typing muscle fibers.

![Fig. 4 – Four muscles biopsied demonstrating reinervation pattern from different animals.](image)

Normal control showed type I fibers (dark) smaller than type II fibers (light). In the group A consisting of rats that underwent denervation, type I and type II fibers atrophy in small and large groups was observed as well as hypertrophy of type I and II fibers and disorganization of intermyofibrillar network suggesting neurogenic atrophy. In the group B were animals underwent Neurorrhesis and entubulation, muscle biopsy demonstrated reinervation in all animals (fig 4).

DISCUSSION

The entubulation conduit is a special tube manufactured from degradable biomaterial that guides the growing nerve fibers across the nerve gap into the distal cut end of the nerve. During regrowth occurs, the conduit is slowly reabsorbed by the body. The choice, based on physical and chemical properties, is critical in creating the most desirable environment for axon regeneration. The conduit should facilitate neurotropic and neurotrophic communication between the proximal and distal ends of the gap, block external inhibitory factors, and provide a physical guidance. There are some general characteristics already considered necessary for any conduit to be successful. Most importantly, the conduit should not elicit a significant inflammatory response or fibrosis upon implantation into host tissue. It should be biodegradable, which eliminates the need for surgical removal, but breakdown slowly enough to maintain a stable structure over long distances. In addition the conduit should be easily formed with controllable diameter and wall thickness, simple to implant, and easy to sterilize.

The bioabsorbable POLY-L LACTIDE-CO P – LACTIDE has demonstrated to have such characteristics. Six months after implant no inflammatory reaction could be detected. The membrane was totally absorbed and the integrity of the sciatic nerve could be verified and photographed. The histological exam demonstrated severe atrophy in control cases and complete muscle regeneration in all animals where entubulation of sciatic nerve was done. The lack of nerve suture did no demonstrate to be important considering the gap length. We could not be sure that it would have the same regeneration considering larger distances between both nerve ends.

Biomaterials engineering has been an important field of research over the last decade. New materials or interposition of stem cells in the gap of sectioned nerve in order to improve regeneration is still in its milestones but demonstrates a great possibility of success in a very close future. Nerve entubulation is already one of the good possibilities offered to surgeons to improve nerve repair surgical results.

CONCLUSIONS

After six months spam it was possible to demonstrate that entubulation with Poly-L Lactide-Co P-Lactide promoting the insulation from surrounding tissues of the two end points of sectioned sciatic nerve in rats WISTAR EPM was effective in promoting satisfactory nerve regeneration without nerve suture. The reinervation of the muscles of the right paw was able to permit that no atrophic characteristics could be demonstrated at immuno-histological examination.
REFERENCES


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