ISSN- 0102-9010

INFLUENCE OF HIGHLY PURIFIED PREPARATIONS OF HYALURONIC ACID ON PERIPHERAL NERVE REGENERATION *IN VIVO*

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ABSTRACT

The tubulization repair technique is a useful model for studying peripheral nerve regeneration since it provides quantifiable parameters for assessing the effects of exogenously applied substances on nerve repair. In this study, we observed that the local administration of hyaluronic acid (HA) in a tubular prosthesis at the time of implantation significantly improved the repair process, and that this effect was dependent on the viscosity of the HA preparation. The sciatic nerve of C57BL/6J mice was transected and the proximal and distal nerve stumps were sutured into a polyethylene tube (PT, 0.76 mm i.d.) to bridge a nerve gap of 4 mm. The tubes were implanted either empty, or filled with a low-viscosity (MW = 450 - 1000 kDa) or high-viscosity (MW = 7000 kDa) commercial preparation of HA. After 4 weeks, the PT with the regenerating nerve cables were processed for histological analysis and the total number of myelinated axons was counted using a computer-controlled system. Low-viscosity HA significantly increased peripheral axon regeneration (2191 ± 82 myelinated axons, mean±SEM) compared to the group with empty tube implants (1597 ± 80). This enhanced regeneration was not observed in the group implanted with tubes containing high-viscosity HA (1643 ± 69). The stimulatory effect of exogenous HA on nerve regeneration could be due to its activity on non-neural cell proliferation, migration and differentiation which would lead to faster ingrowth of regenerating axons.

Key words: Chamber model, extracellular matrix, hyaluronic acid, nerve regeneration, tubulization repair

INTRODUCTION

In current standard clinical practice for the repair of human peripheral nerve injuries, direct anastomosis of the proximal and distal nerve stumps is preferred [16]. However, there are many clinical situations in which direct realignment of the nerve fascicles is impossible, such as when a segment of nerve has been damaged or removed and the proximal and distal nerve stumps are separated by a distance that must be bridged. Such bridging is usually achieved using a nerve autograft. Unfortunately, the results of such nerve grafting are not entirely satisfactory [1], and there is a need for alternative nerve repair strategies. Bridging a nerve gap with a tubular prosthesis (entubulation repair) offers a promising alternative approach for nerve repair.

Various studies using the tubulization technique for peripheral nerve repair have shown that the repair process can be significantly improved by manipulating the microenvironment of the regenerating nerve fibers within a prosthetic tube at the time of implantation [6,7,12]. Thus, tubulization appears to be a useful model for assessing the possible effects of exogenously applied substances on nerve repair since it allows the quantification of several parameters.

Specific modifications of the early microenvironment within tubular prostheses at the time of implantation improve nerve regeneration. Among the substances tested so far are fibrin adhesives [22], collagen type I gel [10], laminin gel [11] and hyaluronic acid preparations [14,19]. Highly purified, medical-grade hyaluronic acid is currently used to reduce the incidence of postoperative adhesions, and as a viscoelastic agent in intraocular surgery, a synovial replacement device, and in various cosmetic applications [15]. In this work, we examined the effect on peripheral nerve regeneration of two commercial preparations of HA which differed in their viscoelastic properties.

This paper is dedicated to the memory of our colleague Prof. Gregorio Santiago Montes.

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MATERIAL AND METHODS

Twelve male C57BL/6J mice, approximately 3 months old at the time of operation, were used in this study. All operative procedures were done under Avertin anethesia (0.5 g of tribromoethanol dissolved with 0.25 g of 2-methyl-2-butanol in 19.5 ml of distilled water; 0.02 ml/g body wt., i.p.). After surgery, the mice were housed in a temperature- and humidity-controlled room with a 12 h light/dark cycle, and were given food and water *ad libitum*.

The left sciatic nerve was exposed and transected at mid-thigh level, and the proximal and distal nerve stumps were sutured into a polyethylene tube (PT) 6 mm long (inner diameter 0.75 mm), to give a final nerve gap length of 4 mm. The mice were divided into three groups of 4 animals each. One group received implantation of a PT alone, another group was implanted with tubes filled with a low-viscosity extract of hyaluronic acid (HA) (Polireumin®, MW = 450 - 1000 kDa), and the third group was implanted with tubes filled with a high-viscosity extract of HA (Synvisc®, MW = 7000 kDa).

After a post-operative interval of 4 weeks, the mice were anesthetized and perfused transcardially with 150 ml of 1% paraformaldehyde plus 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The PT with the enclosed regenerated nerve cables were then dissected out, immersed in the same fixative overnight, postfixed in 2% osmium tetroxide, and processed for Epon embedding. One-micrometer-thick transverse sections were cut from the middle portion of the regenerated nerve cables and stained with alkaline toluidine blue. The total number of myelinated axons in these sections was determined with a computer-controlled system (Biographics Inc., Winton-Salem, NC, USA).

RESULTS

The cross-sectional appearance of the regenerated nerve cables obtained in the experimental groups is illustrated in Fig. 1. The tissue cables were well vascularized and many myelinated axons were present. Figure 2 shows the number of myelinated axons in

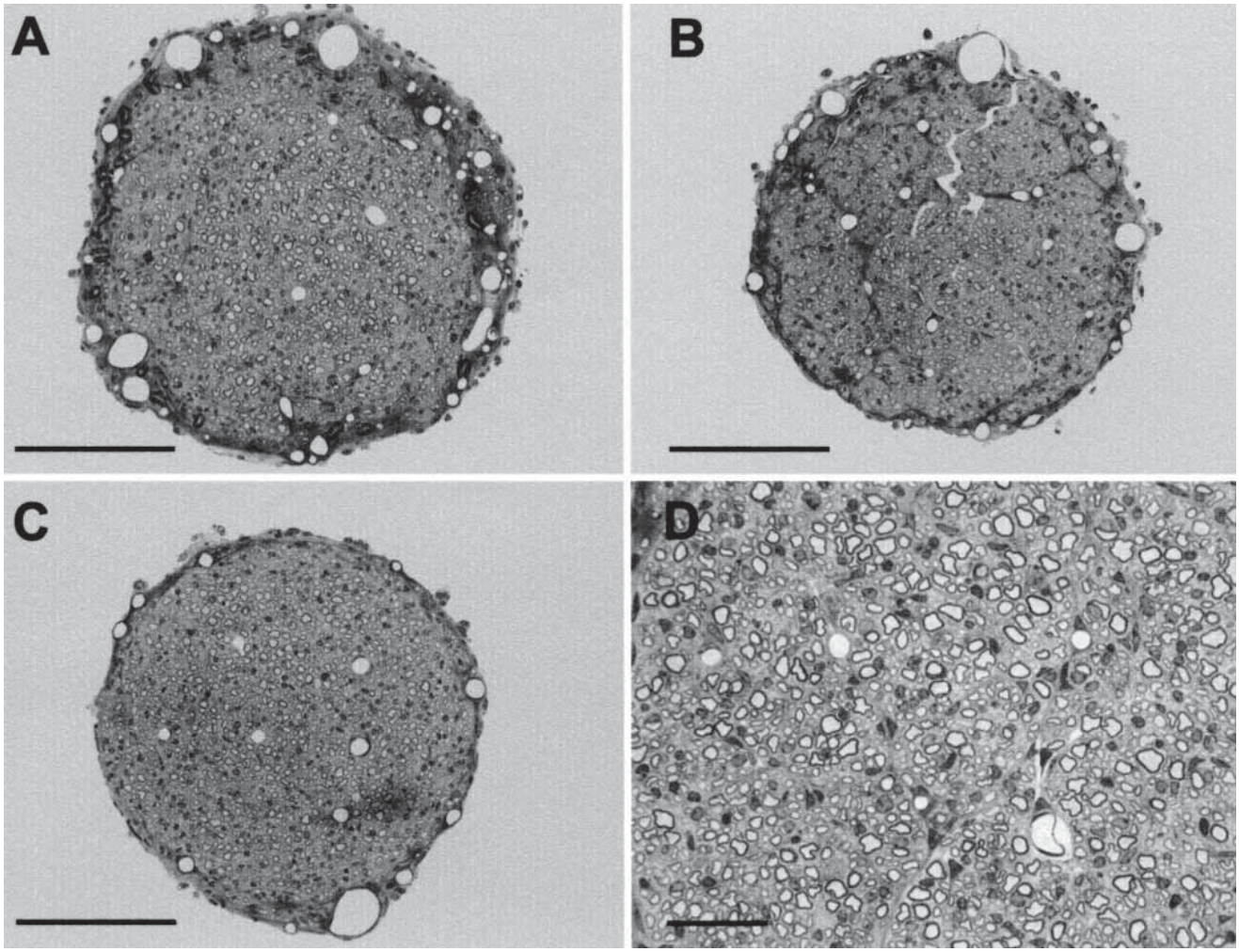

Figure 1. Light micrographs of cross-sections at the midpoint of regenerated sciatic nerve cables 4 weeks after polyethylene tube implantation. Numerous myelinated axons and blood vessels are seen in the nerve tissue. **A**, Low-viscosity HA (Polireumin®); **B**, High-viscosity HA (Synvisc®); **C**, Empty tube; **D**, higher magnification of myelinated nerve fibers from A. Bars: A, B, C = 100 μm; D = 30 μm.

the experimental groups. The animals in the low-viscosity HA group had significantly more myelinated axons (2191 ± 82, mean ± SEM) compared to the high-viscosity HA (1643 ± 69) and the empty PT (1597 ± 80) groups ($P<0.05$, ANOVA followed by Neuman-Keuls test). There was no significant difference between the latter two groups.

DISCUSSION

Tubulization is a process in which two stumps of a sectioned nerve are introduced into a tube to allow regrowth of the injured nerve axons toward the distal end. This technique of nerve repair is a useful model for investigating the cellular and molecular events during regeneration in the peripheral nervous system [3], especially since it provides quantifiable parameters that allow the evaluation of exogenous agents which may influence peripheral nerve regeneration [2].

It is unclear whether the stimulatory effect of low-viscosity HA on nerve regeneration observed here resulted from a direct action on the regrowing axons or an indirect effect on the non-neuronal components of the regenerating nerves. The high-viscosity HA preparation appeared to retard the ingrowth of axons and non-neuronal cells, probably by acting as a mechanical barrier to their migration.

Williams *et al.* [23] described the initial ingrowth of tissue elements in an empty silicone tube bridging a transected sciatic nerve. These authors observed that the lumen of the tube was initially filled with extracellular matrix containing many fibrin strands oriented along the chamber's longitudinal axis. This matrix served as the substrate for the migration of Schwann cells and fibroblats which preceded the appearance of any observable axons. The early insertion of a preformed HA matrix into the tube lumen could provide these cells with a suitable substrate for ingrowth.

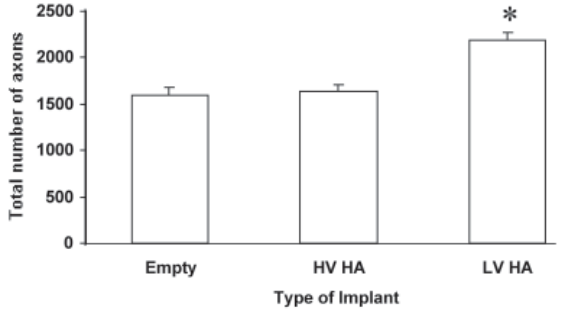

Figure 2. Total number of myelinated axons 4 weeks after nerve repair. The columns represent the mean ± SEM of 4 mice. LV HA, low-viscosity HA (Polireumin®); HV HA, high-viscosity HA (Synvisc®); Empty, empty polyethylene tube. *$P<0.05$ compared to the other groups (ANOVA followed by the Neuman-Keuls test).

HA, a normal component of intact and regenerating peripheral nerves [9,17], is a fibroblast-derived glycosaminoglycan which is believed to play an important role in wound healing. The early presence of an HA-rich matrix favors the infiltration of migratory cells into injured tissue [13]. HA is involved in the detachment process of the cell cycle that allows cell migration [18], but also inhibits cell differentiation, thus creating an environment that promotes cell proliferation [5]. The degradation products of HA modulate the inflammatory response and stimulate angiogenesis [4,20,21]. Additionally, the prolonged presence of HA in fetal wounds may provide the mesenchymal signal for healing by regeneration rather than by scarring and fibrosis [8].

Our results may have therapeutic implications in that local administration of HA could modulate the healing and enhance the regeneration of severed peripheral nerves. The choice of an appropriate HA preparation should take into consideration its viscoelastic properties since the local application of high-viscosity HA solution appears to diminish nerve regeneration.

ACKNOWLEDGMENTS

The authors thank Jennifer A.B. Ribeiro for her excellent technical assistance.

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Received: February 28, 2003
Accepted: April 3, 2003