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Effects of Omental Pedicle Transposition on Regeneration of Neurotmesis Sciatic Nerve in Rabbit

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ABSTRACT

The present study was aimed at providing an understanding of the role of omental pedicle transposition in peripheral nerve regeneration by utilizing an established rabbit sciatic nerve regeneration model. Twelve adult New Zealand White rabbits (2-2.3kg) were divided into two groups (n=6) and acclimatized for 3 weeks. Complete blood examination, liver and kidney function tests were carried out during this period. In Group A, an end-to-end of sciatic nerve segment anastomosis was done, while that of Group B, the nerve anastomosis wrapped with omental pedicle was performed. The nerve specimens were collected from both groups for histopathological and ultrastructural evaluation after 16 weeks post surgery. Results showed that omental pedicle transpositioned (Group B) had more newly developed nerve fibres and less scar tissue. Ultrastructural examinations showed neuronal sprouting, whereas directions of regenerative nerve fibres were intraneural, but in the end-to-end anastomosis of group B showed that some of nerve fibres had extraneural.

Keywords: Histopathology, nerve regeneration, omental pedicle transposition, ultrastructure

INTRODUCTION

Traumatic peripheral nerve injuries are common in companion animals due to trauma, iatrogenic lesions, and surgical misadventure (Risio, 2005). There are many current conventional techniques of nerve repair such as epineural suturing, perineural suturing, perineurol nerve grafting, and free vascularized nerve grafting (Alluin *et al.*, 2008), often with disappointing results (Saunderland, 1991). Misdirection of regeneration axons is also a factor which may explain poor functional recovery (Brushart, 1991). The omentum has been used in neurosurgery since the early seventies. Successful results have been obtained with the transplant of the omentum to the brain or spinal cord in both animals and humans (Cucca *et al.*, 1980; de la Torre and Mussivand, 1993; Pappas *et al.,* 1996).

The omentum has also been used in several situations of ischemia of the extremities, such as Buerger's disease and peripheral vasculopathies, due to presence of lipid fractions in the omentum promoting vascular perfusion and angiogenesis (Goldsmith *et al.,* 1984; Goldsmith *et al.,* 1986). In addition, it has been demonstrated that omental transposition promotes healing, regeneration, and neurons across a transected spinal cord in the experimental studies in cats and human (de la Torre and Goldsmith, Goldsmith and de la Torre, 1992; Goldsmith *et al.,* 2000). The need for a good method of repairing transected nerve was the basis of

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the present study. Thus, the objective of this study was to evaluate morphological changes in omental pedicle transposition technique in regenerating sciatic nerve.

MATERIALS AND METHODS

Laboratory Animals and Surgical Protocol

Twelve male adult New Zealand White rabbits (2-2.3 kg) were divided into two groups (n=6) and they were acclimatized for 3 weeks in individual cages. They were also fed with commercial rabbit pellets and given water *ad libitum*. Complete blood examination, liver and kidney function tests were performed during the period of acclimatization. Meanwhile, broad spectrum antibiotics (Pencillin Streptomycin) and antihelmintic (Ivermectin) were administered prior to the start of the experiment. The experimental procedures were performed as approved by the Faculty's animal care and use committee (08 R13/Dec08-Nov-09). Induction was done by an intramuscular injection of ketamine (Bioketan, Vetquinol), xylazine (Ilium Xylazil. 100) and acepromazine (Calmivet, Vetoquinol) and maintained by on halothane (Isoflurane).

Group A

A 6-8 cm in length caudo-lateral skin incision was made parallel to and 2 cm caudal to the left femur bone. The fascia latae was incised and the biceps femoral muscle was separated from the semitendinosus by blunt dissection. The left sciatic nerve was separated from its surrounding tissue using a pair of ophthalmic scissors and a jeweller's forceps. The nerve was transected using a surgical blade #15.

Nerve Anastomosis

With the aid of a magnifying glass (X3), both ends of the nerve were immediately sutured after transection. An end-to-end anastomosis pattern, using 8-0 nylon suture with simple interrupted suture, was placed in the epineurium and perineurium.

Group B

A similar procedure was performed as in Group A, but the sciatic nerve was wrapped in omental pedicle transposition after an end-to-end anastomosis. The omentum was detached from the transverse colon, and the omental pedicle transposition was done through the abdominal wall muscle by blunt dissection using a pair of artery forceps. The extended omental pedicle was held and pulled through the tunnel between the semi-membranous and adductor muscles. The omental pedicle was then wrapped around the anastomosed sciatic nerve, and fixed to the muscles using two sutures. The two skin incisions were closed in a routine manner with 3-0 Vicryl using the sub-cuticular pattern.

All the animals in both groups were euthanized at 16 weeks post operation by intracardiac injection of pentobarbitone (Dolethal). The anastomosed left sciatic nerve was exposed, examined grossly, and then harvested for histopathological and ultrastructural studies. Three specimens of 1cm in length were collected from the proximal, middle (anastomosis site), and distal segments of the co-opted sciatic nerve. Each specimen was divided into two parts, and these were then fixed and processed in the routine manner for histopathology and electron microscopy, respectively.

RESULTS

Histology

Group A

The longitudinal sections of the proximal nerve stumps demonstrated normal arrangement of the nerve fibres with high concentration of Schwann cells. Occasionally vacuolated, degenerated nerve fibres, and scanty of fibrous tissue were seen perineurally (*Fig. 1a*). The middle portion of the sutured line segment revealed a normal parallel arrangement of the newly regenerated nerve fibres with several vacuolations and degenerated fibrous tissues in the perineural region close to the sutured site (*Fig. 1b*). The distal nerve stump demonstrated parallel distribution of the new nerve fibres. There were few Schwann cells with several vacuolations, degenerated nerve fibres, and slight fibrosis perineurally (*Fig. 1c*).

Group B

The longitudinal sections of the proximal nerve stumps showed an increase in the number of Schwann cells, and the nerve fibres resembled the normal parallel pattern arrangement of the nerve fibre with normal stain affinity (*Fig. 2a*). The mid portion section revealed an increased number of nerve fibres and Schwann cells, and a slight peripheral fibrosis. (*Fig. 2b*). The section of distal nerve stumps demonstrated occasional vacuolation and fibres degeneration with moderate increased in the number of nerve fibres and Schwann cells (*Fig. 2c*). The cross-section of distal nerve stumps demonstrated a high vascularization close to the omental pedicle (*Fig. 2d*).

Ultrastructure

Degenerated nerve fibres with extra-neural and atrophied band of Bungner bands were observed in the distal segment of nerve in Group A (*Fig. 3a*). Meanwhile in Group B, the Schwann tube structures or basement membrane re-formation and re-distribution (*Fig. 3b*), active Schwann cells and thick myelinated nerve fibres (*Fig. 3c*) were seen.

DISCUSSION

This study demonstrated that the omental pedicle promoted and improved the rate of functional recovery within a short period of time (16 weeks), as compared to the end-to-end anastomosis. In addition, it was also observed that the omental pedicle enhanced the onset and acceleration of nerve fibres regeneration. The histological examinations showed a plenty of nerve fibres regeneration in the omental pedicle group. Misdirected axonal growth at the repair site might have occurred, leading to poor functional outcome post-operation; this might not necessarily imply total recovery of the nerve function (Sobeski *et al*., 2001; Meck *et al*., 1999). The histological findings, such as nerve fibre density, myelin sheath thickness, number of Schwann cells, and supportive tissues

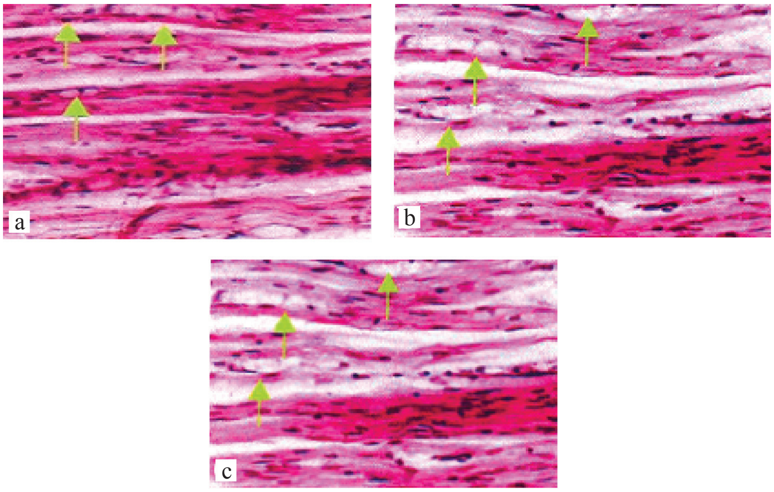

Fig. 1: Photomicrograph of the sciatic nerve in Group A (H&E, X100). (a) Note the proximal part with degenerative nerve fibres (arrows) and poor fibrous tissue perineurally; (b) The middle part contained several degenerated nerve fibres (arrows) and increased number of Schwann cells; (c) The distal part with several degenerated nerve fibres (arrows) and focal concentration of Schwann cells

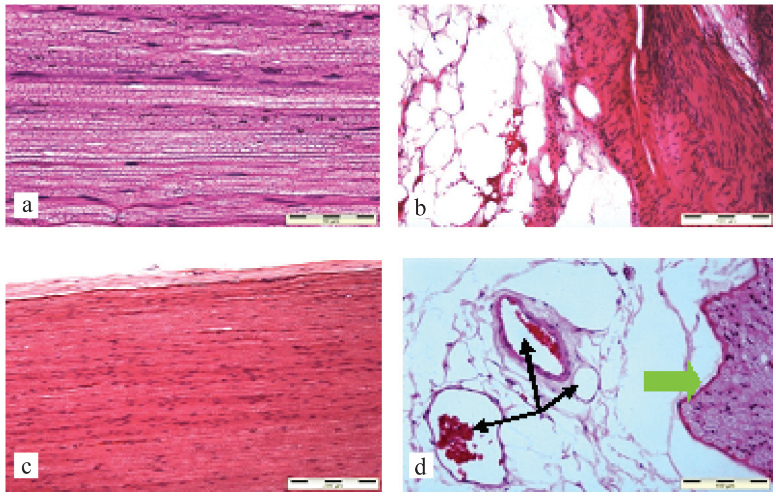

Fig. 2: Photomicrograph of the sciatic nerve in Group B (H&E). (a) No significant pathological changes were seen except for the slight increase in the number of Schwann cells; (b) The middle part showing good axonal alignment, no intra-neural scarring and an increase in the number of nerve fibre and Schwann cells. (c) The distal part has an increase number of Schwann cells. The sciatic nerve showed vascularisation (arrows) of omentum around the anastomosed site (thick arrow) H&E

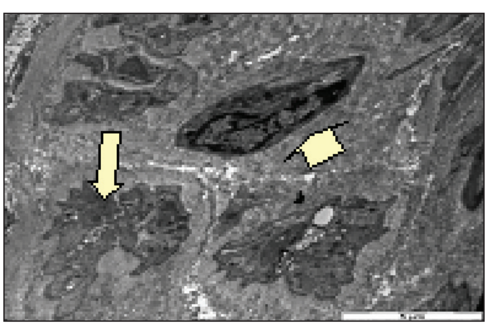

Fig. 3a: Electrograph of ultrastructural of rabbits' sciatic nerve in Group A showing atrophied band of Bungner in distal segment (arrows) and degenerative nerve fibre (arrow heads), Uranyl acetate and lead stain

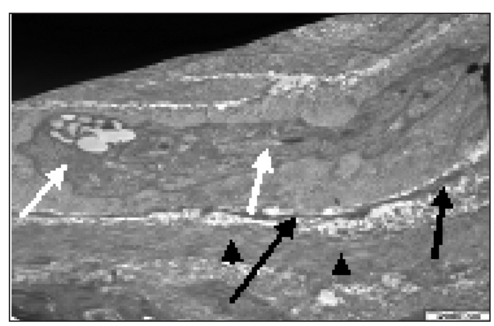

Fig. 3b: Electromicrograph of sciatic nerve treated with omental transposition showing Schwann cells in endoneural tube (white arrows), basement membrane (black arrows) and collagen fibres (arrow heads), Uranyl acetate and lead stain

like blood vessel and fibrous sheath, showed that they played different roles in promoting nerve regeneration. One possible explanation for the improvement in the function of regenerative nerve fibres is that it easily grows throughout the omentum due to lack of intra-neuronal fibrosis (scar formation) and the ability of the omentum to stimulate vascularization (Chamorro *et al*., 1993).

Moreover, omental pedicle also has several advantages for the treatment of transected peripheral nerves as an autogenous transposition,

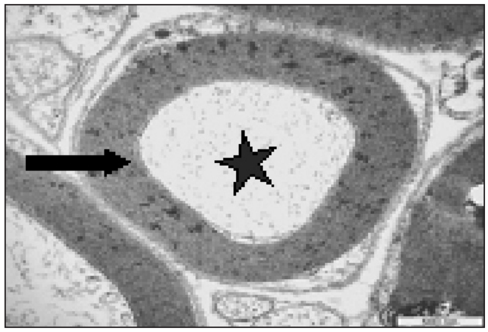

Fig.3c: Photograph of ultrastructural of rabbit sciatic nerve treated with omental transposition showing a good myelin axon (asterisk) with Schwann cells, Axon surrounded by myelin sheath (arrows), Uranyl acetate and lead stain

and does not cause any intra-abdominal defect. It can be extended through minor laparoscopic intervention without complication (Domene *et al*., 1998; Kamei *et al*., 1998) and no injury occurs at the donor site, compared to nerve transplantation.

Ultrastructural examinations showed that the group of omental pedicle demonstrated developed Schwann tube or basement membrane which is very important as a guide for axonal outgrowth due to the presence of extra-cellular matrices such as a collagen, fibronectin, lamanine, and integrine, in addition to active Schwann cells and thick myelinated nerve fibres (Dahluin, 2008). Another study reported that the mechanism of omental pedicle might promote healing when applied to the injured site, because of the rich blood vessel density, blood content, growth and angiogenetic factors, vascular endothelial growth factor (VEGF), chemotactic factors (stromal-cell-derived factor SDF-1α), progenitor cells (WT-1, CXCR4) to clear tissue debris and clotted blood, assuring a fresh blood supply, and thus preventing tissue death by ischemia. Moreover, the re-vascularized injured tissue is thus supplied with a potent mixture of growth factors and progenitor cells which further helps in the recruitment of progenitor cells from the bone marrow and local tissue to accelerate tissue repair (Liberg *et al*., 2007).

In conclusion, omental pedicle promotes healing when it is applied to injured sites because of the rich blood density, blood content, as well as growth and angiogenic factors. The ultrastructural examinations demonstrated developed Schwann tube and thick myelinated nerve fibres which are very important as guides for axonal outgrowth.

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