TOPICAL WRAPPING OF OMENTAL TRANSPOSITION AROUND TRANSECTED SCIATIC NERVE PREVENTS EPINEURAL SCAR FORMATION IN RABBITS

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Abstract

The role of topical wrapped of omental pedicle in preventing postoperative perineural fibrosis was examined by gross anatomical dissection and histological analysis in rabbits. The aim of study was to evaluate the role of omental pedicle in preventing postoperative perineural fibrosis of injured sciatic nerve in rabbits. The left sciatic nerve was exposed and transected into 12 adult male New Zealand white rabbits. The animals were divided into two groups six animals each, which named epineural nerve sutures group (ENS) as a positive control that the transected sciatic nerves was coaptation using epineural nerve sutures, whereas the omental pedicle wrapped around coaptated nerve in the omental pedicle transposition group (OMPT). The right sciatic nerves (non-operated) were collected from three animals of each group as a negative control group. Sixteen weeks post operation (PO), the operation site was evaluated by blinded surgical dissection. The results showed the there was graded adhesions of perineural into surrounded tissues using a numerical grading scheme. Morphometric analysis of distal segments of OMPT was statistically significant at P<0.05 difference compared with ENS group. Ultrastructural study showed OMPT group less collagen fibers proliferation than ENS group. It was concluded that OMPT is effective in decreasing epineural scar formation.

Keywords: Omental pedicle, Perineural fibrosis, Peripheral nerve, Rabbit

1. Introduction

The omentum has been used in different areas of surgery for more than 30 years. Omental transposition has been demonstrated to be beneficial in the surgical treatment of neurological injuries, as a graft material in treating chronic leg ulcers, necrotizing fasciitis and soft tissue defects, and also used to wrap bowel anastomosis site [1].

The omentum has been extensively used in neurosurgery since the beginning of the 1970s. In 1974, [2] described an experimental model on the transplant of the greater omentum into the brain of animals. Revascularization of a transected peripheral nerve begins from vessels of the nerve stumps and from surrounding tissue [3]. Goldsmith’s group was demonstrated that omental transposition promotes healing and even regeneration of neurons across a transected spinal cord in experiments in cats [4,5]. Perineural scarring is one of the factors affecting the clinical results in peripheral nerve surgery. This phenomenon causes tethering of peripheral nerves, which restricts nerve mobility during limb movement [6]. Tethering of nerve, which if severe and prolonged, it can gives rise to ischemia and further nerve injury [6,7]. Reduction of perineural scar formation not only might improve the outcome after peripheral nerve surgery but also would not facilitate for secondary operation and thereby decrease the risk of complications. Therefore, the aim of study was to evaluate the role of omental pedicle in preventing postoperative perineural fibrosis of injured sciatic nerve in rabbits.

2. 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), which named epineural nerve sutures group (ENS) as a positive control that the transected sciatic nerves was coaptation using epineural nerve sutures, whereas the omental pedicle wrapped around coaptated nerve in the omental pedicle transposition group (OMPT). The right sciatic nerves (non-operated) were
collected from three animals of each group as a negative control group. All animals were acclimatized for 3 weeks in individual cages and they also fed with commercial rabbit pellets and given water *ad libitum*. Completed 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).

**Epineural Nerve Sutures Group (ENS)**

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 lata 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 coaptation with the aid of a magnifying glass (X3), both ends of the nerve were immediately sutured after transection. An end-to-end anastomosis sutured pattern using 8-0 nylon with simple interrupted suture, was placed in the epineurium and perineurium (Fig. 1)

**Omental Pedicle Transposition Group (OMPT)**

A similar procedure was performed as in Group ENS, but the sciatic nerve was wrapped in omental pedicle transposition after an end-to-end coaptation. 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 stitches ((Fig. 2). 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 morphometric analysis. A 1cm in length was collected from the distal segments of the operated 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. Right sciatic nerve of non-operated right hind limb was collected from three animals of each group. Six nerve samples were obtained and trimmed corresponding to the transected sites of the left sciatic nerves which constituted the negative control.

Figure (1): Photograph showing the coaptated proximal segment (thin arrow) and distal segment (thick arrow) of sciatic nerve in rabbit.

Figure (2): Photograph showing the coaptated sciatic nerve of proximal segment (thin arrow) and distal segment (thick arrow) wrapped with omentum pedicle (asterisk) in a rabbit.
Postoperative observations

Clinical Signs Evaluation (Motor Functions Evaluation)

All animals were examined daily from the first day to the end of the study. The onset and ability of walking were recorded for all animals. The gait was graded as normal, crouched, or crawl on heel. Knuckling was graded based on normal, mild, moderate to severe. Muscle contraction force was graded based on weak, moderate, and strong. Muscle mass was graded as normal, mild, moderate, and severe muscular atrophies.

Sensory Functions Evaluation

Nerve sensory functions were recorded weekly starting from the end of the 3rd week to the end of the study. Sensory functions and clinical signs scoring were evaluated using the grading criteria of toe spreading reflex, lateral aspect leg sensation, toe pinch and toe prick as a present (+) or absent (–). Foot withdrawal and vocalization tests were evaluated by lateral aspect leg sensation, toe pinch and toe prick, which were recorded as positive response, indicative of recovery.

Gross Anatomical Evaluations

The neurolysis sites of animals in the OMPT group and the ENS group were evaluated by blinded surgical dissection under general anesthesia 4 weeks after surgery. Perineural adhesions during anatomical dissection were evaluated according to the numerical grading scheme described by [4] (Table 1).

Histological Study

The entire sciatic nerve and surrounding tissues were removed en bloc and immersed in 10% neutral buffered formalin overnight. Tissues were embedded in paraffin and cross-sectioned at 5 μm. Sections were stained with Masson trichrome stain and the connective tissue was evaluated using. The thicknesses of scar and nerve tissue were measured under light microscopy (Olympus BX50, Tokyo,
Japan) using an ocular micrometer (Olympus) and the scar tissue formation index was obtained by dividing the value of the thickness of the scar tissue by the value of the thickness of the nerve tissue.

Table (1): Numerical grading scheme for gross evaluation described by (4)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and muscle fascia</td>
<td>1</td>
<td>Skin or muscle fascia entirely closed</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Skin or muscle fascia partially open</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Skin or muscle fascia completely open</td>
</tr>
<tr>
<td>Nerve adherence and nerve separability</td>
<td>1</td>
<td>No dissection or mild blunt dissection</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Some vigorous blunt dissection</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Sharp dissection required</td>
</tr>
</tbody>
</table>

Morphometric and Ultrastructural Study

The specimens for semithin section were fixed with 4% glutaraldehyde overnight at 4°C. The specimens were dehydrated, infiltrated with resin and polymerized. Following polymerization, the samples were semi thin (1 μm) and ultrathin sectioned using an ultra microtome (Leica).

Statistical Analysis

All data were expressed as mean and standard error (M±SD). Statistical comparison between all groups was performed using Statistical Package for the Social Sciences (SPSS) 16.0 software (non-parametric tests), Kruskal Wallis and Mann-Witney tests for clinical observation and for morphometric analysis, independent-sample T test for gross and histological evaluation were used. P value ≤ 0.05 was considered significant.

3. Results

Sciatic Nerve Functions Evaluation

Motor Observations

The onset and ability to walk of the OMPT group showed significantly earlier improvement (p≤0.05) compared to the ENS group. The type of gait (crouching and crawl) and knuckling disappeared in all animals of OMPT with significant difference (p≤0.05) compared to the ENS. The muscle contraction
force became stronger in the OMPT with significant difference ($p \leq 0.05$) compared to the ENS. When the animals of OMPT were used, the operated limb and the muscle mass recovered to mild with significant difference ($p \leq 0.05$) compared to the ENS group on day 112 PO.

Sensory Clinical Observations

Sensory clinical signs including toe spread, lateral leg sensation; toe pinch and toe prick in the OMPT animals showed that sensory reflexes were regained with significant difference ($p \leq 0.05$) compared to ENS on day 112 PO.

Anatomical Evaluation

At the 16 weeks, the original incision sites were carefully reopened for staging and evaluation. There was no infection or inflammatory reaction. The entire OMPT group showed significantly less perineural adhesions than controls did (Fig. 3 a, b). According to the Petersen numerical grading scheme, the skin, muscle, and deep fascia closure were almost completed ($p>0.05$). In OMPT-treated animals, nerve adherence and nerve separability were significantly less than in the control group ($p<0.05$). The results of gross evaluation of both groups are summarized in Table 2.

Figure (3): Photographs demonstrating the macroscopic appearance of sciatic nerves at 16 weeks PO. A. Nerve of ENS group surrounded by scarring and tethered to the surrounding tissue (thin arrow). In addition, the fused peroneal and tibial components could not be separated with blunt dissection (thick arrow). B. Nerves of OMPT group were easily dissected from the surrounding tissue (arrow), and the tibial and peroneal components were easily separated with blunt dissection.
Table (2): Results of the perineural adhesion scores

<table>
<thead>
<tr>
<th>Animal No</th>
<th>Skin closure</th>
<th>Muscle fascia</th>
<th>Nerve tissue</th>
<th>Separability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OMPT (treated)</td>
<td>ENS (Control)</td>
<td>OMPT (treated)</td>
<td>ENS (Control)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

M±SE 1.00±0.00 a 1.00±0.21 a 1.16±0.16 a 1.50±0.22 a 1.00±0.00 a 2.50±0.22 b 1.00±0.00 a 2.50±0.22 b

^a,b^ Means ±SE (n=6) with different superscript within same row are significantly different at (p < 0.05) compared to control.

Histopathological Evaluation

Epineural of the nerve in animals of ENS group consistently demonstrated a thick band of dense epineural connective tissue surrounding the sciatic nerve in site of anastomosis (Fig. 4a). In contrast, nerves treated with OMPT were surrounded by thin, bands of connective tissue (Fig. 4b). Quantification of the dense connective tissue surrounding the nerves revealed a statistically significant (p<0.05) reduction around nerves treated with OMPT group compared with ENS group (Table 3).

Figure (4): Photomicrographs of cross-sections of nerves and surrounding tissue. Sections were stained with Masson trichrome stain for collagen. A. Photomicrograph of tissue from ENS group demonstrated a dense band of darkly-stained connective tissue (arrows) surrounding the sciatic nerve and encasing the nerve bundles (×40). B. Photomicrograph of tissue from a rabbit in the OMPT group showed the nerves demonstrate a significantly less epineurial connective tissue composed of thin layers (arrows) (×40).
Morphometric analysis

Histomorphometric analysis of the distal segment of the ENS group showed a decreased in the number of myelinated nerve fiber, fiber diameter, myelin sheath thickness, axon diameter, and g ratio were (8072, 4.63 µm, 1.60 µm, 3.03 µm, 0.65 respectively, compared to the negative control (right limb). The distal segment of the OMPT group showed a decrease in the number of myelinated nerve fiber, fiber diameter, myelin sheath thickness, axon diameter and g ratio were (10923, 6.64 µm, 2.36 µm, 4.28 µm, 0.64 respectively, compared to the negative control (Table 4)

Statistical analysis of the distal segment showed that the number of myelin nerve fibers was not significantly different (p> 0.05) in the OMPT group compared to the negative control group. Fiber diameter, axon diameter and myelin sheath thickness of the ENS, and OMPT groups were significant (p< 0.05) reduce compared to that of negative control group (Table 4).

Table (3): Results of the quantitative histological analysis of thickness perineural scar tissue

<table>
<thead>
<tr>
<th>Animal No</th>
<th>OMPT</th>
<th>ENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>0.246</td>
</tr>
<tr>
<td>2</td>
<td>0.115</td>
<td>0.170</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>0.173</td>
</tr>
<tr>
<td>4</td>
<td>0.153</td>
<td>0.235</td>
</tr>
<tr>
<td>5</td>
<td>0.163</td>
<td>0.230</td>
</tr>
<tr>
<td>6</td>
<td>0.166</td>
<td>0.285</td>
</tr>
<tr>
<td>M±SE</td>
<td>0.132±0.012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.234±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means thickness of scar (n=6) with different superscript within same row are significantly different at (p < 0.05) compared to animal control.

Ultrastructural Study

Ultra structural changes at distal segment of the ENS group appeared low and thin myelinated between majority of unmyelinated nerve fibers and thick bundle of collagen fibers surrounding of nerve fibers(Fig.5 A). Ultrastrutural of the distal segment of sciatic nerve in OMPT group showed good
myelination of regenerative nerve fibers, activated Schwann cells through increased numbers of mitochondria, well refind of endoneurium and less of collagen fibers (Fig. 5 B).

Table (4): Statistical analysis and mean values of the number of fibers, fiber diameter, myelin thickness, axon diameter and g ratio of the negative control and treated groups on day 112 PO

<table>
<thead>
<tr>
<th>Time</th>
<th>Negative Control</th>
<th>ENS Group</th>
<th>OMPT Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fibers</td>
<td>13292±1091&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8072±52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10923±570&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diameter of the fibers</td>
<td>10.352±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.63±1.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.64±1.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thickness of the sheath</td>
<td>3.472±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.36±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diameter of the axons</td>
<td>6.86±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.03±1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.28±1.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>g ratio</td>
<td>0.66±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means ±SE (n=6) with different superscript within same row are significantly different at (p < 0.05) compared to negative control (right hind limb).

Figure 5. Electromicrograph of distal segment of sciatic nerve on day 112 PO (A). ENS group demonstrates the thin myelinated nerve fibers (arrow), thick collagen fibers at endoneurium (arrow head). UA&LCX 12500. B. OMPT group showed good myelination of regenerated nerve fibers, activated Schwann cells through increased numbers of mitochondria (arrows) and prominent basement membrane (arrow heads). UA&LCX 7500.
4. Discussion

Results of this study showed that the onset of movement of limb and animals walking early developed in the OMPT compared to the ENS. The omentum used in this study could secrete analgesic substances such as opioids, neurotransmitters including gamma aminobutyric acid (GABA), norepinephrine and other monoamines in their roles in the modulation of pain. Anger et al. [8] reported the role of analgesic substance in the mechanism of modulation of pain. However, the knuckling disappeared in all animals in the OMPT and this might be due to the role of omentum effeciveness functional recovery on the transected sciatic nerve on day 112 PO, which enhanced early innervation of the extensor and flexor muscle that controls normal locomotion of the limb. Harman-Boehm et al. [9] reported that the omentum is rich in macrophages and mononuclear cells and that the macrophage is essential for successful nerve regeneration by release growth factors and cytokines that stimulate Schwann cell to proliferation.

The degree of sensory reflexes included spreading of toe which increased from day to day, gradually regaining function involving the second, third and fourth toes, which is in agreement with the report by [10] who described the gradual return of the peroneal nerve function. This index might be applicable to the monitoring of recovery in an animal to express differences in the final degree of recovery. Therefore, in a study of the recovery of the sciatic nerve in rabbits, the reliability and desensitivity of the toe-spreading reflex is evaluated as a sign of functional recovery alongside concurrent muscle weights. Improvement of motor and sensory functions could indicate that regenerating nerve fibers grow out through the site of coaptation due to low intraneural fibrosis. The absorption action of the omentum decreases the level of fibrinogen and fibrin production derived from fibrinogen, which leads to decreased fibrosis (scar) at the coaptation site of the transected sciatic nerve. Decreased fibrosis will facilitate the extension and direction of axons to align and orientate correctly. This result is similar to
the findings of [11] who showed that omental pedicle transposition had more newly developed nerve fibers and less scar tissue.

The histological sections findings of the coapated sciatic nerve in the OMPT showed considerable improvement and acceleration of transected sciatic nerve compared to the ENS. Meanwhile, the ENS histology of transection of injured sciatic nerve showed that the nerve fibers challenged both regeneration and axon path finding to the target organ because of the disruption of the endoneurial sheath with loss of axon alignment. This increased the proportion of extra-fascicular regenerating axons, and more fiber misdirection to the target organs. The continuous degeneration and deposition of collagen induced a retardation of the myelination process. This result is consistent with [12] which reported that the extraneural fibrosis and wound-bed adhesions may tether the suture site and adjacent nerve bed. The coaptated sciatic nerve in the OMPT showed more progress of normal parallel orientation of the nerve fibers, few collagen fibers, increased vasculature and increased number of Schwann cells. The increase of Schwann cells resulted from the high concentration of stem cells in the omental adipose tissue. It is reported that adipose tissue contains a large number of stromal stem cells [13], which are directly released from this tissue to the transected sciatic nerve. Effectively, increased angiogenesis, vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF) and proximity to associated nerve tissue combined to stimulate differentiation of stem cells into Schwann cells.

Total number of myelinated nerve fibers in the proximal and distal stumps of the nerve fibers of ENS group studied showed a significant decrease (p≤0.05) in the number of fibers across day 112 PO in all groups. Assessment of the healing process of the distal segment is very vital in the interpretation of the outcome of treatments given. This is because the distal segment is more severely affected by the degenerative (Wallerian) effects of incision as well as inflammatory and collagenation response to such nerve injuries [14]. The proximal stumps generally have higher nerve fiber number values above those
of the distal. Such difference results from the anatomical positioning of the stumps in direct relation to the neuron, which aids in the axoplasm transport for the nourishment of the neuron by serving as a source of neurotrophic factors [15]. Nerve fiber diameter is an indicator of the level of maturation of a nerve fiber [16].

This mechanism was observed in this study where there was a general decrease across groups and times below the normal recorded values for the negative control in both the proximal and distal stumps. The improved healing observed in the OMPT is the result of the ability of the omental pedicle to secrete neurotrophic and neurotropic factors as well as clear the injury site of scar tissues and debris (rich phagocytic activity).

Ultrastructural studies showed OMPT group had many activated Schwann cells, remyelination, good basal laminae and less deposition of collagen fibers. Basement membrane of the Schwann cells in the OMPT group remained intact in the injured region due to the matrix metalloproteinase gelatinase B (MMP-9) and the tissue inhibitor of MMPs (TIMP)-1 were induced in coaptation site and distal segments of sciatic nerve after transection. The TIMP-1 may protect basement membrane from uncontrolled degradation after injury [17]. When omental pedicle was wrapped around coaptation site of sciatic nerve the TNT-α and TGF- β1 produced by omentum [18] may participate in the regulation of TIMP-1 levels during nerve repair. The BM of the Schwann cells is used as a guide for the growth cone at the tip of each axon sprouts from proximal segment which contain multiple filopodia that adhere to the basal membrane. The growth cone was directing in the BM by both contact and chemotactic guidance into appropriate fascicle leading to their original targets organ [19].

ENS group showed only thin myelination of regenerated nerve fibers, numerous unmyelinated fibers and neuroaxon with few mitochondria. Internal endoneurial fibrosis at the suture site might inevitably divert extraneural or intraneural regrowing axons and scar formation which compromises the microvascular bed of a nerve potentially provoking secondary axonal degeneration [20,21]. The result
was in agreement with other previous studies which showed the fibrous ingrowths at the repair site due to use of epineural nerve sutures [22, 23].

5. Conclusion

In OMPT group, the surgical and motor and sensory clinical signs were more improved compared with ENS. The anatomical evaluation showed less perineural adhesions with surrounded tissue. The histopathological examination showed minimal scar tissue formation and good myelination in OMPT group. The morphometeric analysis showed increase in the number of myelinated nerve fibers, fiber diameter, axon diameter and myelin sheath thickness compared with ENS group, ultrastructural study of the OMPT group showed less collagen fibers at endoneurium and thick myelination of regenerated nerve fibers. The result shows that OMPT group was better in peripheral nerve regeneration and functional recovery due to decreased of scar tissue formation at the site of coaptation.

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References


