Role of Nd:YAG Laser for Prevention of Neuroma Formation: An in-Vivo Study on Rabbit Facial Nerves

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ABSTRACT

Traumatic nerve transection is inherently associated with development of a neuroma at the end of the proximal stump. It can be very painful at exposed sites, which may arise spontaneously or after mechanical irritation, particularly after amputations. Clinically, neuroma and its recurrence might be a resisting problem against treatment. Frequently, various methods, including simple neuroma resection, have been proposed for the treatment, but none of these has provided satisfactory results. The present study was conducted to evaluate the use of Nd:YAG laser (1064nm) for nerve transection as regard the prevention of neuroma formation. Forty facial nerves from 20 Velander rabbits were divided into two equal groups. For group (A), 20 epsilateral facial nerves were subjected to Nd:YAG laser nerve-transection technique, while for group (B), the contralateral 20 facial nerves were subjected to scalpel nerve-transection technique. Eight weeks later, the transected nerves were re-explored and evaluated. The favorable results of Nd:YAG laser for nerve transection in the present study make Nd:YAG laser a safe and effective tool that could be applied for nerve transection in order to suppress neuroma formation with no harmful effects so far.

INTRODUCTION

Nerve injury is still representing a problem of considerable clinical importance. Traumatic nerve transection is inherently associated with development of a bulbous swelling or a neuroma at the end of the proximal stump [1-3]. Unlike classic neuroma, "neuroma in continuity" was found in association with an intact nerve trunk [4].

Although painful neuroma is reportedly uncommon (20-30%), it can be incapacitating. It can be very painful at exposed sites, which may arise spontaneously or after mechanical irritation, particularly after amputations [2,5]. Neuroma becomes painful when regenerating axons lose their proximal-to-distal orientation, after exiting from the proximal stump, and get lost in the scar tissue and grow in a tangle around the proximal stump. Histopathologically, neuroma is characterized by numerous minifascicles, growing in all directions, containing only a small group of collateral axonal sprouts [1].

Recently, patho-physiological and molecular biological basis of neuroma formation and its associated pain were suggested. Many investigators reported the essential contribution of Schwann cells hyperactivity in collaboration with regrowing axons during nerve regeneration [6]. Moreover, a higher glycosaminoglycans' content in the fibrous collagen matrix of neuroma (lodging myofibroblasts) was found [7]. These myofibroblasts are speculated to contribute to pain by contracting the collagen matrix around the sensitive non-myelinated axons.

Clinically, neuroma and nerve sheath neoplasms like acoustic neuroma (vestibular schwannoma) and schwannomas of the facial or peripheral nerves might be challenging problems against treatment. Frequently, simple neuroma resection, even after the introduction of gamma knife radiosurgery for the treatment of acoustic neuroma in order to minimize the associated facial paresis or paralysis and deafness, did not have a universal satisfactory outcome [8]. Redo surgery might offer a limited treatment option. Additional implantation of the proximal nerve stump in a nearby muscle after simple neuroma resection may be a good option for treatment of resistant neuromas [9].

Other methods, including heat in the form of electrocautery or laser, have been proposed to limit neuroma formation at the proximal stump of a transected nerve, but none of these has provided satisfactory results [10]. Over years, the use of Carbon Dioxide (CO_2) laser (wavelength, 10600nm) for prevention of neuroma formation has been attracting the interests but was found to give controversial results [11-14]. It produces a

limited amount of energy that is absorbed at the superficial layer of the nerve. This makes CO_2 laser, like bipolar electrocautery, is not suitable to induce homogeneous nerve coagulation [10]. Accordingly, other lasers with deeper energy penetration abilities may be proposed for prevention of neuroma formation. Theoretically, Neodymium: Yttrium Aluminum Garnet (Nd:YAG) laser (wavelength 1064nm) has more advantages. It is more tissue-selective and moreover, it can be delivered through an easier fiber-optic system.

As a consequence, the present study was conducted to evaluate Nd:YAG laser (1064nm) for nerve transection regarding the prevention of neuroma formation.

MATERIAL AND METHODS

Animal Model:

Twenty Velander rabbits (body weight, 3.0-3.5kg and age, 6-7 months) were anaesthetized by a single intramuscular injection of Ketamine hydrochloride (35mg/kg body weight) and Xylazine hydrochloride (5mg/kg body weight). Through bilateral transverse incisions in the cheeks below the eyes, facial nerves bilaterally (n=40) were exposed and dissected. The nerves were identified electro-physiologically by inserting two electrodes of the biotech data accusation device in the nerve substance (Fig. 1) to measure nerve action potential and excitation curves before and immediately after their transection (Fig. 2). The device parameters were kept constant at 500 pulses per second for a fixed time of 30 seconds. This device can detect nerve electric current of 90 millivolt. The nerves were then classified into two equal groups (A and B), 20 facial nerves for each.

Group (A): Nd:YAG Laser-Nerve Transection Technique:

Nd:YAG laser (1064nm, Premier Laser Systems, Inc., Model 815 XP), CW mode, at a power of 12 Watt, duration of few seconds, was used. Laser energy was delivered through a 600μm core diameter, bare quartz optical fiber, fitted with a SMA-905 connector.

20 epsilateral facial nerves were subjected to Nd:YAG laser nerve transection primarily by diffuse coagulation for a segment of about 7mm by defocused Nd:YAG laser beam, till whitening of the nerve segment was elicited. Subsequently, nerves were transected with additional focused laser beam just distal to the coagulated segment (Fig. 3).

Group (B): Scalpel-Nerve Transection Technique:

20 contralateral facial nerves were transected by sharp scalpel, and about 5mm portion of the distal ends were removed to prevent re-growth of axons into the distal stumps and to achieve the distance between the proximal and distal stumps the same as in group (A).

After wounds' closure, all rabbits were returned back to cages and were fed ad libitum with addition of a prophylactic antibiotic to their drinking water.

Evaluating Parameters:

I- *Gross Anatomy:*

Eight weeks later, the wounds were re-explored. The site of nerve transection including proximal and distal stumps in all nerves were inspected and evaluated as regard discolouration, bulbous swellings, and for the extensiveness of adhesions.

II- *Histopathological Evaluation:*

After gross evaluation, transected area including proximal and distal stumps were removed and marked as a proximal-to-distal orientation. From each group, randomly selected 10 specimens were examined by light microscopy while the other 10 specimens were examined by transmission electron microscopy (TEM).

A- *Light Microscopy:*

Specimens were trimmed and immediately fixed in 10% formaldehyde for 2 days, then washed by distilled water and left in 70% ethylalcohol overnight at room temperature. Dehydration of specimens was started by 96% ethylalcohol followed by complete dehydration by absolute ethylalcohol for an hour. Subsequently, specimens were immersed in 1% celloidin methyl benzoate overnight at room temperature then embedded in paraffin. From each paraffin block, 5 sections of 5μm thickness were obtained at the longitudinal plane and subjected to Haematoxylin and Eosin (Hx & E) stain and examined at a magnification of X 40.

B- *Transmission Electron Microscopy (TEM):*

Specimens were immediately fixed in 3% glutaraldehyde solution buffered with cacodylate, post-fixed with 1% osmium tetroxide, stained with uranyl acetate, dehydrated in acidified 2,2-dimethoxypropane, and embedded in epoxy resin. After hardening, cross-sections of 1.25μm thickness were cut with the use of an ultra microtome (Ultracut E, Reichert-Jung, Germany) and stained with toluidine blue and basic fuschine, and then examined by T.E.M. (Philips E.M. 420 Eindhoven, Netherlands) for up to X 7500 magnification.

RESULTS

I- *Gross Anatomy:*

Yellowish discolouration of proximal stumps was elicited among group (A) only. This was associated with small black particles of carbonization at the site of nerve transection.

Regarding neuroma formation, and its size, slight neuromas were found in 6 nerves (30%) among group (A), compared to moderate-to-severe neuromas at all nerves (100%) of group (B).

Regarding adhesions, slight adhesions were observed in 12 nerves (60%) among group (A), compared to moderate-to-severe adhesions at all nerves (100%) of group (B).

II- *Histopathological Evaluation:*

A- *Light Microscopy:*

Regarding group (A), Fig. (4A) shows extensive degenerative changes of axons and myelin sheaths along coagulated stumps as denoted by obvious excessive gappings. Axons showed reduced growth at the proximal stump, without true neuromas. No outgrowth of regenerating axons was found outside the coagulated proximal stumps.

On the other hand, regarding group (B), Fig. (4B) shows regenerating axons penetrating fibrous tissue and entering the intervening scar tissue and the surrounding adhesions. Minimal gappings of limited degeneration of axons and myelin sheaths could be also seen.

B- *Transmission Electron Microscopy (TEM):*

Regarding group (A), unmyelinated and partially myelinated nerve fibers showed a recognizable well-aligned appearance with intact perineurium and endoneurium. Thermally degenerated axons and myelin sheaths could be also seen as denoted by the partial and/or complete loss of the laminated outline collar pattern of the myelin sheaths, as well as, by the disappearance of neurofilaments, microtubules, and mitochondria from the axoplasms (Figs. 5,6). No Schwann cell hyperactivity could be elicited.

However, regarding group (B), numerous poorly-aligned partially myelinated axons were observed. Schwann cell hyperactivity was observed as denoted by the indentation of its nuclear membrane with condensation of heterochromatin at the inner surface of the nuclear membrane (Fig. 7). An extensive activity of the rough endoplasmic reticulum with excessive ribosomes could be also seen denoting a stimulated active nerve cell proliferation and neuroma formation (Fig. 8).

DISCUSSION

In clinical practice, neuroma formation might be a resisting problem against treatment. In such cases, re-operation might offer a limited treatment option [2,9]. Three conditions contribute to neuroma formation. These are, firstly; Schwann cells proliferation stimulating axonal growth starting at the proximal then at the distal stump [6,15,16]. Secondly; damaged perineurium, and thirdly; open endoneurial tubules that both facilitate axonal outgrowth [6,17,18]. All these three adverse events can be successfully avoided by use of Nd:YAG laser.

Many investigators reported the essential contribution of Schwann cells hyperactivity in collaboration with regrowing axons during nerve regeneration [6,15,16]. It was found that Schwann cell hyperactivity precedes axon regrowth. This is achieved along a laminin basement membrane to confer the directional specificity of the regenerating axons. Regarding this context, Nd:YAG laser could be of value. The observed prevention of Schwann cell's activity in association with degenerative changes of axons and myelin sheaths along the coagulated proximal stump among group (A) in the present study is most likely due to the homogeneous thermal effects of Nd:YAG laser. However, among group (B), Schwann cell's hyperactivity was observed as denoted by the indentation of its nuclear membrane with condensation of heterochromatin.

Although regenerating axons may force their way through dense fibrous tissue, a normal perineurium presents an impenetrable barrier against the passage of axons [6]. So that, procedures for neuroma prevention will only succeed if a normal perineurium is covering the nerve end before regenerating axons start to penetrate into the neighboring connective tissue, where their disorderly growth is responsible for neuroma formation. Regarding this issue, Nd:YAG laser, again, could be of value. It coagulates and seals the endoneurial tubules and allows perineurium to grow over the nerve stump before the axons reach the site of nerve transection as denoted by the intact endoneurial tubules and perineurium cover among group (A), in the present study, rather than among group (B).

The successfulness of Nd:YAG laser technique depends on the proposed theory that inhibited nerve cell growth is associated with an inhibition of the neuroma formation [6,17,18]. Accordingly, among group (B) rather than among group (A) in the present study, evidence of active protein synthesis

and mitosis continued to be observed as denoted by hyperactive rough endoplasmic reticulum and hyperactivity of Schwann cell nucleus.
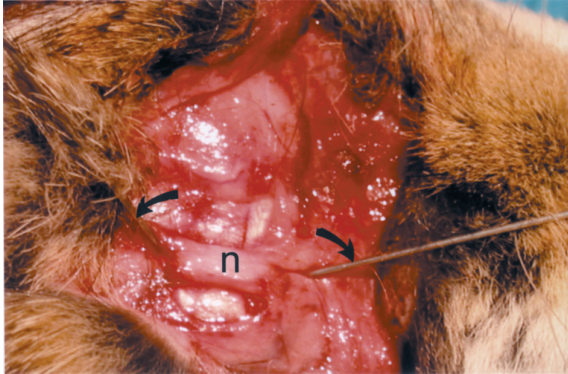

Fig. (1): Dissected and exposed facial nerve (n) with two electrodes (arrowhead) was inserted in its substance for electrophysiological identification.
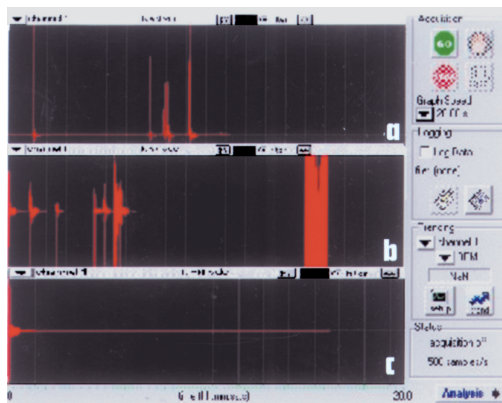

Fig. (2): Electrophysiological examination of the facial nerve. a- Normal action potential curve before nerve transection. b- Normal nerve excitation curve before nerve transection. c- Flat curve of lost nerve conductivity after nerve transection.
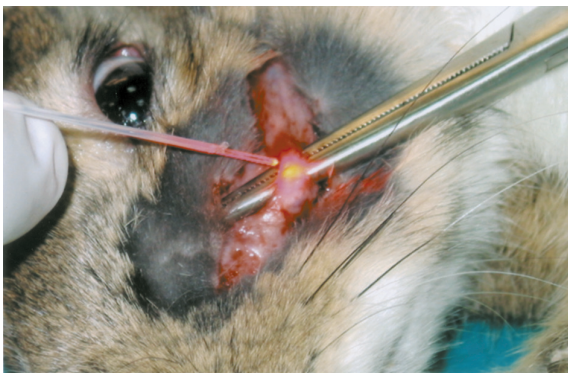
Fig. (3): Nd:YAG laser-nerve transection technique. Facial nerve was firstly coagulated for a segment of about ±7mm, followed by nerve transection just distal to the coagulated nerve segment.
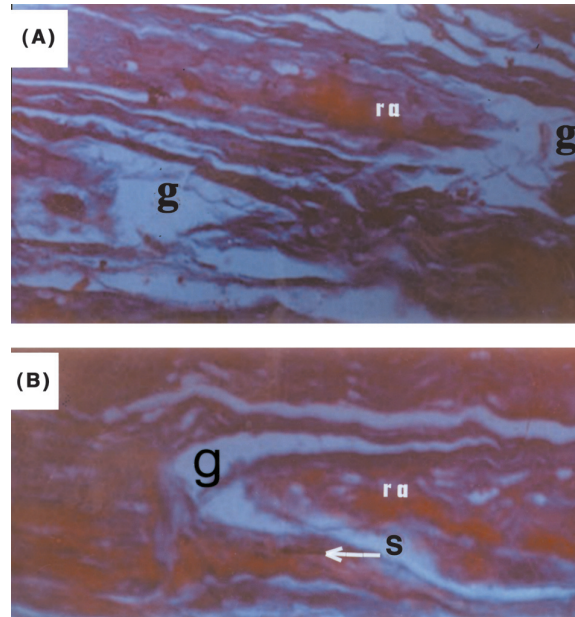
Fig. (4): Light microscopic examination using Hx & E stain. Fig. (4A,B) represents group (A), and group (B), respectively. Fig. (4A) shows an extensive degeneration of axons and myelin sheaths of the coagulated nerve as denoted by the presence of excessive gappings (g). No outgrowth of the regenerating axons (ra) outside the proximal stump. Fig. (4B) shows minimal gappings (g) of associated limited degeneration of the axons and myelin sheaths. Regenerating axons (ra) penetrating the scar tissue (s, arrowhead) could be elicited.
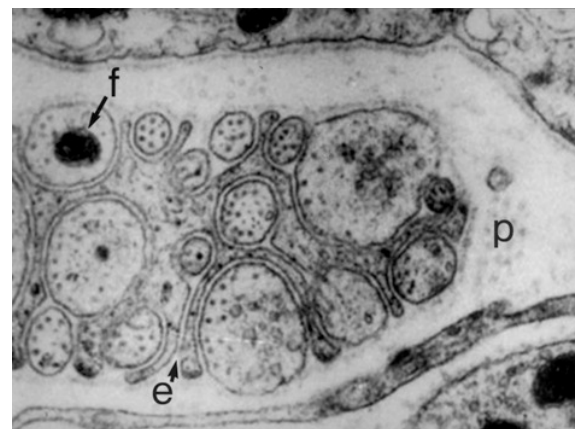

Fig. (5): TEM examination of group (A), of Nd:YAG laser-transected nerves. It shows a cross section of group of unmyelinated nerve fibers with intact perineurium (p) and endoneurium (e, arrowhead). The axoplasms show neurofilaments. A large central neurofilament (f, arrowhead) at the center of an axon could be also seen.
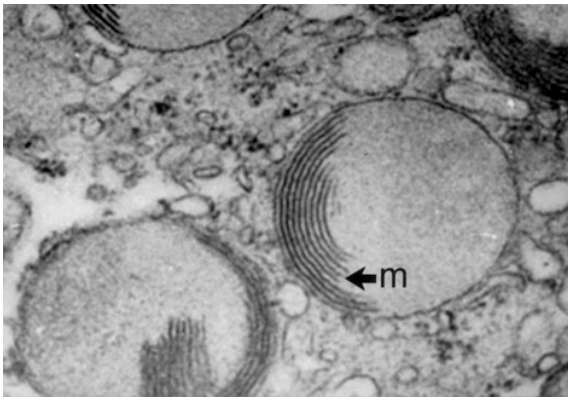
Fig. (6): TEM examination of group (A), of Nd:YAG laser-transected nerves. It shows partially myelinated axons as denoted by the presence of partially disentigrated laminated outline collar pattern of the myelin sheaths (m, arrowhead). The axoplasm is completely free from neurofilaments, microtubules, and mitochondria.
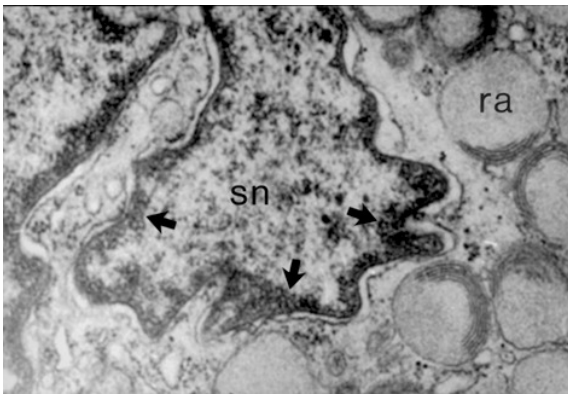

Fig. (7): TEM examination of group (B), of scalpel-transected nerves. It shows an indentation of the nuclear membrane of nucleus of Schwann cell (sn) (clasmatosis) with a condensation of heterochromatin (arrowhead) denoting the mitotic hyperactivity. Numerous poorly-aligned partially myelinated regenerating axons (ra) could also be seen.
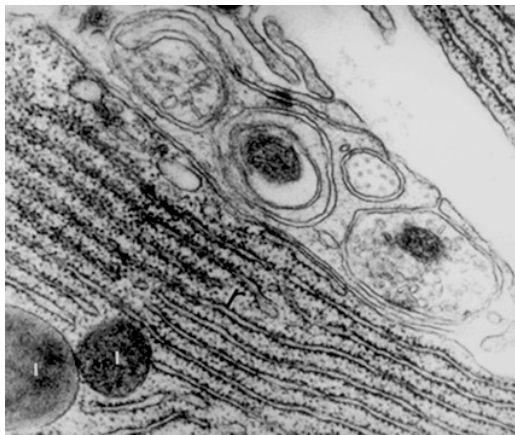

Fig. (8): TEM examination of group (B), of scalpel-transected nerves. It shows an extensive hyperactivity of the rough endoplasmic reticulum (r) of Schwann cell denoting a stimulated process of active nerve cell proliferation and neuroma formation. Lipofuscin inclusions (1) could be also seen as electron dense masses in Schwann cell's cytoplasm.

Collectively, according to the present findings, it is obvious that Nd:YAG laser could be an excellent tool for prevention of neuroma formation. Nd:YAG laser, through its deep optical penetration, allows coagulation of the entire nerve from one-sided irradiation. The selected laser power in the present study at 12 Watt for several seconds resulted into the desired diffuse homogeneous thermal coagulation. Powers below 10 Watt was found to produce unequal coagulation within the nerve, while powers higher than 14 Watt resulted into rapid vaporization and carbonization.

Conclusion:

Within the limitation of the present study, Nd:YAG laser was found to be a safe and effective tool that could be applied for nerve transection in order to suppress the formation of amputation neuroma with no harmful effects so far.

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