Comparison of Lyophylized Duramater and Autogenous Omental Wrappings of Grafting Sites in Experimentally Induced Facial Nerve Injury*

Part II Histologic and Histomorphometric Evaluation

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Abstract: The histologic and histomorphometric evaluation of wrapping the facial nerve by a lyophylized duramater (LD) or autogenous omental graft (AOG), on nerve regeneration, after experimentally induced facial paralysis, which was created by neurectomy and repaired by autogenous sural nerve grafts in dogs, was undertaken. The experimental design was reported. At the end of the observation period, specimens harvested from the main trunk of the facial nerve arising from the stylomastoid foramen, the repaired site and three branches of this nerve were examined histologically and histomorphometrically with respect to myelin area, axon number and nerve fiber diameter. Reduced fibrosis and improved nerve regeneration were found to be superior in the AOG wrapped group than the others. According to the histomorphometric evaluation of grafted site, the wrapped groups had better values than those of the control group. Wrapping was found to be useful for nerve regeneration in the grafting site and the omentum could be recommended for this purpose. Some reference values were obtained from histomorphometric evaluations that will be helpful for further studies, and traumatic injuries of facial nerves can be repaired by free cable grafts and regeneration seemed better when wrapped with AOG.

Key Words: Duramater, facial paralysis, graft, nerve regeneration, omentum

Introduction

Facial paralysis is well documented in dogs and cats, especially with regards its etiology and diagnosis. The most common cause of facial neuropathy was judged to be idiopathic, but traumatic facial nerve injury is not rare in small animal practice (1,2). If circumstances are suitable in humans, facial paralysis can be treated properly with direct repair or grafting (3-5). The regenerated nerve is observed by clinical, histomorphometric and electrophysiological evaluations. In the histomorphometric evaluations, myelin sheath thickness, nerve fiber diameter and axon counts are taken into account by many authors (6-8).

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The present paper describes histomorphometric aspects of the role of wrapping the grafted site by the LD and AOG with respect to nerve fiber diameter, myelin sheath thickness, and axon counts. Histologic examination of the repaired site was also evaluated. It was considered that the histologic and histomorphometric evaluations of the facial nerve of the adult dog would determine which wrapping material was suitable.

Materials and Methods

Case identification, experiment design, operative technique and observation period were reported previously (9).

The specimens were harvested from the grafted site, the three main branches (auriculopalpebral, dorsal buccal and ventral buccal branch) and the main trunk arising from stylomastoide foramen of the healthy side. All specimens were fixed separately in 2.5% gluteraldehyde solutions. The tissues were washed with cacodylate sodium in 0.1 molarity and they were fixed subsequently for 2 h in osmium tetroxide solution. The specimens were rewashed with cacodylate sodium (0.1 molarity) and then dehydrated in ethanol solutions (30, 50, 70, 90 and 100%). The specimens were kept in araldite (araldite CY 212 + araldite HY 964 + araldite DY 064 + Dibutyl phthalate) and propylene oxide mixture for 12 h. They were embedded in araldite. The prepared blocks were hardened at 40°C for 24 h and 60°C for 24 h consecutively. Sections of 1 µm thickness were cut using a 8800 LKB ultramicrotome.

Histomorphometric evaluation of the sections stained with Toluidine Blue was performed by a video camera connected to a light microscope and by a computer (Leica Q 500) having an Image Analyzing Program (KS400 Software). Myelin sheath thickness and nerve fiber diameter were calculated as the inner and outer border of myelin sheaths. They were randomly selected from 50 different areas (x100 magnification) and were drawn manually and calculated by a computer. Myelinated axon counts were estimated in at least 5 different areas of each section. Axons that appeared to be rectangular or square in shape were not estimated.

The outer diameter of a myelinated axon was taken into account to determine the nerve fiber diameter. The average value of each lesser and greater diameter was calculated by the computer to estimate nerve fiber diameter. Nerve materials, taken from grafted sites, were stained via the Klüver Barrera method (10).

The results were analyzed with the Chi-square test.

Results

Histomorphometric Analysis: The values of axon counts, nerve fiber diameter and myelin sheath thickness estimated from semi-thin sections of the main facial nerve trunk, the grafted site and the three branches are introduced in Figures 1-3.

Average axonal number was 5116.6, mean nerve fiber diameter was 18.19± 9.32 µm and myelin sheath thickness was 43.25 µm² in the main facial nerve trunk.

There were no significant differences between the groups on axon counts. Although significant difference was not present between the branches (P> 0.05), axon counts in the grafted sites were less than each of three branches (Figure 1). Nerve fiber diameters of the main trunk were greater than the grafted site and all three branches but the AOG group had a greater value than the others (P>0.05) (Figure 2).

A significant difference of myelin sheath thickness was not detected between the branches of each groups, but it was greater in the AOG group (P<0.05) (Figure 3). It was smaller in the grafting site than in the branches, but it was insignificant. Myelin sheath thickness was greater at the sections of the main trunk than in both the grafting site and the branches. In the LD group, myelin sheath thickness was smaller than the others (P<0.05).

The means of nerve fiber diameter and myelin sheath thickness were superior in the AOG, control and LD groups respectively, even though it was not significant (P>0.05).

Histologic Evaluations: Periepineural and interfascicular fibrosis with slight cellular reaction including macrophage and histiocytes were commonly seen in the two dogs of the control group. Vacuolar and

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\text{Total axon counts} = \frac{\text{Transected nerve area}}{\text{Evaluated area}} \times \text{Myelinated nerve numbers in evaluated area}
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granular structures were observed at the myelin layer in almost all nerve fibers and axonal necrosis (in one of these dogs). Free nerve fibers were seen extrafascicularly and were noted as irregular situations (Figure 4).

In the LD group, the repaired site was surrounded with slight fibrosis that contained multifocal inflammatory infiltration containing mostly lymphocytes and macrophages (Figure 5). Slight interfascicular
fibrosis, large numbers of free nerve fibers and neuroma formation were found in one dog of the LD group. Well myelinated axons and incomplete regeneration of the endoneurial tube in fascicular pattern were seen in the histologic section of the LD group (Figure 6). Foreign giant cell bodies and macrophages were detected around the suture material used for wrapping.

In the AOG group, there was fibrosis around the repaired and interfascicular region. However, both cellular reactions or fibrosis were lesser than in the other groups. Necrosis of some axons and Schwann cell proliferation were observed in one dog. A granular appearance was detected in the myelin layers of two dogs. Axons were well myelinated in different diameters but not in regular fascicular pattern in the histologic section of the AOG group (Figure 7).

Discussion

The nerve fiber diameter, myelin sheath thickness and axon counts, which are considered by many authors as a criterion in histomorphometric analysis (6,11,12) were used in the present study. To the authors’ knowledge, there have not been any histomorphometric studies about facial nerve grafting in dogs. The facial nerve fiber diameter of a healthy adult dog is reported to be 3.92 ±1.18 μm. In the same study, approximately 89% of the facial nerve fiber diameters were between 3 and 6 μm and ranged from 2 to 12 μm (10). In the present study, mean nerve fiber diameter was estimated to be 18.19 ±9.32 μm. In the study mentioned above (10), the nerve fibers of a circular shape and the diameters of lesser axis were taken into account, crenate fibers were not measured. In our study, nerve fibers every shape were
estimated and the average value of lesser and greater
diameter fibers were evaluated. The cause of the
disagreement with the other study (10) was a result of
different evaluation methods.

According to previous studies, axon counts are not
enough to evaluate peripheral nerve regeneration, despite
its extensive use for this purpose (4,11,12). Regenerated
axons can branch into as many as 20 distal fibers, and
axon counts in the distal segment may be 150% greater
than that in the proximal segment (11). In the present
study, axon counts were lower in distal parts of the
grafted site than the proximal in all of the groups as seen
in Figure 1. Not only were axon counts in the grafted site
lower than axons of total three branches, but also it was
less than the numbers of each one of the branches. It was
confirmed that, even though few axons pass through the
graft, they might be branched at the distal part of the
branches because of the signs of clinical improvement.
There were no significant differences between the groups
according to axon counts (P> 0.05). As the three main
branches of the healthy side, the results could not be
compared to the normal number of axons. At the same
time, there were no reference values about axon counts
for branches of canine facial nerves to the authors’
knowledge.

The relationship between nerve conduction velocity
and the diameter of myelinated nerves are known. The
fastest conduction is seen in the largest diameter nerve
fiber. Nerve fiber diameters are greater in the proximal
part of the grafted site than in both the grafted site (3)
and the distal part of it in the peripheral nerve of
extremity (11,12). Thanos and Terzis studied cross facial
nerve grafting on the basis of axon number, and nerve
fiber diameter and myelin sheath thickness (8). They
observed that the value detected in the proximal part
of the grafted site was the greatest, and the value in the
grafted site and distal part of the grafted site were
smaller. In a study on grafting facial nerve after
neurectomy, axonal diameter and myelin area in the
grafted site were reported to be smaller than the distal
part of the grafted site (13). The results of the study
showed that sections taken from the facial nerve trunk
had a greater diameter of nerve fiber than in the grafted
site or the branches. Mean fiber diameter in the AOG
group was greater than in the others.

Functionally, the myelin sheath serves as an insulator
controlling the leakage of current. Its area would have to
increase accordingly with nerve fiber diameter. Myelinization at the regeneration phase began when the
growing axon reached the distal tube and were increasing
from proximal to distal over time. This is a very
important criterion for the establishment of regeneration
(11,14). It is reported that regenerated and myelinated
nerve fibers have thinner sheaths than normal (6). Myelin
sheath thickness had a statistically smaller value in the
grafted site and branches of the nerves in the LD group
than in the others. It was observed that the appearance
of myelin sheath thickness was not normal in all three
groups. However, they appeared to be better in the AOG
group than the others. The histomorphometric value
of the three branches of the nerve fiber diameter, and
the myelin sheath thickness of the LD group was lower than
in the control group but it was thought that the results of
analysis was responsible for this. During estimation,
Schwann cell and vacuolar structure had to be taken into
account and affected the results. In histologic evaluation,
dispersion of the well myelinated axons was less in the
control group than in the wrapping groups. Extensive
degenerated axons and Schwann cell proliferation was
seen in the histologic sections of the control group.

Macrophage and fibroblast infiltration and adhesions
were observed upon histologic examination of the
repaired nerve. At neuroma formation, nerve fibers are
dispersed in fibrous tissue randomly (11). Adhesion of
repaired nerve to peripheral tissues, and fibrous tissue
that was localized at both the interfascicular area and
around the nerve in the histologic sections, was thought
to be the result of unwrapping the repaired site. The
results revealed the requirement of wrapping the grafted
site to prevent fibrous tissue invasion into the repaired
site. Well myelinated nerve fibers in an organized
fascicular pattern were superior in the AOG, LD and
control group concurrently. The superiority of the AOG
group was as a result of its structural property, which not
only protected the grafted site from peripheral invasion
of the fibrosis, but also could affect the nutritional
support of the graft.

Fibrous tissue formation was lower in the wrapped
groups than in the controls and interfascicular fibrosis
was limited at the LD wrapped area. It was evaluated
that suture material caused foreign giant cell body
formation and an inflammatory reaction which were
closered on the suture line. Lymphocyte invasion around
the case of the LD group was interpreted as a minimum
antigenic property and the xenograft of LD. Minimum antigenic property and the gradual development of fibrous tissue instead of LD, which acts as a natural tissue, are the advantages of the LD (13). Because of the union of LD with fibrous tissue, it could not be recognized in some sections. In the AOG group, the omentum was united with fibrous tissue, and fibrosis was lower than in other groups. It was believed that the omentum reduced fibrosis. Observed fat tissue residue at the operated site was thought to be the omental structure. Neurons exiting from the suture line might be the reason for excessive neuroma formation in the control group. Axons exiting from the suture line inclined towards the distal direction by the guide of wrapping material were considered to be the reason for little or no neuroma formation in the cases wrapped with AOG or LD. Natural autogene tissue and the easy to fold properties of omentum might be the reason for lower neuroma formation than in the other groups. Excessive interfascicular fibrosis in the control group might be the result of an invasion of fibrosis that came from around the repaired site.

In conclusion, injury of facial nerves can be repaired by sural nerve graft, but wrapping the grafted site should be considered with a suitable material. The results of the previous and present study indicate that AOG could be preferred for this purpose. There was no significant difference in three parameters between the branches of facial nerve. It was observed that almost equal regeneration was present in all the three branches.

References