Effects of Melatonin and Dexamethasone on Facial Nerve Neurorrhaphy

Deniz Tuna Edizer, Zehra Dönmez, Mehmet Gül, Özgür Yiğit, Birgül Yiğitcan, Turgut Adatepe, Nurten Uzun

Department of Otorhinolaryngology, Istanbul Training and Research Hospital, Istanbul, Turkey (DTE, ZD, ÖY)
Department of Histology and Embryology, İnönü University School of Medicine, Malatya, Turkey (MG, BY)
Department of Electrophysiology, Istanbul Training and Research Hospital, Istanbul, Turkey (TA)
Department of Neurology, Istanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, Istanbul, Turkey (NU)

OBJECTIVES: To investigate the effects of topical and systemic administrations of melatonin and dexamethasone on facial nerve regeneration.

MATERIALS and METHODS: In total, 50 male albino Wistar rats underwent facial nerve axotomy and neurorrhaphy. The animals were divided into 5 groups: control, topical melatonin, systemic melatonin, topical dexamethasone, and systemic dexamethasone. Nerve conduction studies were performed preoperatively and at 3, 6, 9, and 12 weeks after drug administrations. Amplitude and latency of the compound muscle action potentials were recorded. Coapted facial nerves were investigated under light and electron microscopy. Nerve diameter, axon diameter, and myelin thickness were recorded quantitatively.

RESULTS: Amplitudes decreased and latencies increased in both the melatonin and dexamethasone groups. At the final examination, the electrophysiological evidence of facial nerve degeneration was not significantly different between the groups. Histopathological examinations revealed the largest nerve diameter in the melatonin groups, followed by the dexamethasone and control groups (p<0.05). Axon diameter of the control group was smaller than those of the melatonin (topical and systemic) and topical dexamethasone groups (p<0.05). The melatonin groups had almost normal myelin ultrastructure.

CONCLUSION: Electrophysiological evaluation did not reveal any potential benefit of dexamethasone and melatonin in contrast to histopathological examination, which revealed beneficial effects of melatonin in particular. These agents may increase the regeneration of facial nerves, but electrophysiological evidence of regeneration may appear later.

KEYWORDS: Facial nerve, axotomy, neurorrhaphy, compound muscle action potential, regeneration

INTRODUCTION
Facial nerve (FN) injury is a relatively common clinical entity that results in both functional and cosmetic consequences [1, 2]. Its extracranial course and superficial location renders the FN susceptible to external damaging factors [3]. Transection of the FN should be repaired surgically by coaptation of the proximal and distal ends, an intervention known as neurorrhaphy [4]. To enhance the regenerating process, many experimental trials have been conducted studying the effects of a variety of agents [3-8].

Melatonin is involved in many physiological processes and has potent anti-inflammatory, antioxidant, and neuroprotective properties [4, 9-11]. Corticosteroids, commonly used in the treatment of nerve injuries, attenuate perineural inflammation and may prevent neuronal death and promote the recovery process [5, 10].

In the present study, we examined the effects of topical and systemic administrations of both corticosteroids and melatonin on nerve regeneration in an FN axotomy and neurorrhaphy model.
MATERIALS AND METHODS
Fifty male albino Wistar rats (200-250 g) were used in the experiments. The animals were exposed to a 12-h light/dark cycle and had free access to standard rodent diet and water. The study was approved by the Ethics Committee of Bezmialem University Animal Care and Use (2013/14) and conducted according to ethical standards.

The animals were anesthetized using Ketamine 30 mg/kg (Ketalar, Eczacibasi, Istanbul, Turkey) and Xylazine 6 mg/kg (Rompun, Bayer, Istanbul, Turkey), and all of the procedures except for intraperitoneal injections were performed under general anesthesia. After dissection of the surgical field, a 2-cm horizontal incision below the left auricle was made, and the FN trunk was identified and completely transected midway between the stylomastoid foramen and the point of bifurcation. Immediately thereafter, end-to-end anastomosis of the proximal and distal stumps was performed with 2 8-0 nylon epineural sutures in all animals. The right FN served as an innervated control. The surgical procedures were performed with an operating microscope. Before surgical intervention, all the animals received cefazolin sodium 40 mg/kg (Cefozin, Bilim, Istanbul, Turkey) intraperitoneally.

The rats were randomly divided into 5 groups, each containing 10 animals:

A. Topical administration of a saline-soaked gelfoam over the site of neurorrhaphy+intraperitoneal saline
B. Topical administration of a melatonin (20 mg/mL)-soaked gelfoam over the site of neurorrhaphy+intraperitoneal saline
C. Topical saline+intraperitoneal melatonin (20 mg/kg)
D. Topical administration of a dexamethasone (4 mg/mL)-soaked gelfoam over the site of neurorrhaphy+intraperitoneal saline
E. Topical saline+intraperitoneal dexamethasone (1 mg/kg)

Melatonin (Sigma Aldrich M5250, Milwaukee, WI, USA) solution was freshly prepared before injection by dissolving indoleamine in absolute ethanol and further dilution with normal saline; the final concentration of ethanol was 5%. Melatonin at a daily dose of 20 mg/kg body weight was administered intraperitoneally for 7 consecutive days. Intraperitoneal dexamethasone (Dekort 8 mg/2 mL amp; Deva Holding AS, Istanbul, Turkey) was administered at a dose of 1 mg/kg for 7 days. Topical drugs and saline were applied with a compressed gelfoam over the site of anastomosis.

Electrophysiological Evaluation
Nerve conduction study was performed with a Neuro-MEP 2-channel digital EMG device (Neurosoft, Russia) by one neurologist and evaluated by another in a blinded fashion. Superficial recording electrodes (Plaquette Adhesive Surface Electrodes, TE/K50431-002; Technomed, USA) were placed over the lip muscles, whereas the needle ground electrode (Subdermal Single Needle Electrodes, 13 mm in length-0.4 mm in diameter [27G], TE/S50716-002; Technomed) was inserted into the right posterior thigh region. The FN was stimulated by transcutaneous supramaximal stimulus intensity (10% above the level necessary to achieve maximal amplitude) distal to the site of anastomosis and neurorrhaphy. Latency (ms) and the peak amplitude (mV) of the compound muscle action potentials (CMAP) were recorded and compared with those of the healthy control site. Axonal degeneration was calculated using the following formula: 1-[mV (left)/mV (right)], and latency difference was calculated using the following formula: ms (left)-ms (right). Electrophysiological evaluation was performed before FN axotomy and 3, 6, 9, and 12 weeks after the end of drug administration.

Histopathological Examination
At the 12th week after drug administration, the skin incision was re-opened and the coapted segment was resected; the animals were sacrificed with sodium thiopental.

Light microscopy: FN samples were fixed in 10% formaldehyde solution for 48 h and then embedded in paraffin blocks for routine histologic processing. Of note, 6-μm-thick sections were prepared from the paraffin blocks, and these were stained with hematoxylin-eosin (H-E). The slides were examined under light microscopy (Nikon Optiphot-2), and images were captured and interpreted using a camera and a vision analysis system (NikonDS-FI2 camera and Nikon DS L3), respectively. The diameter of the FN was evaluated by the senior histologist who was blinded to the treatment groups.

Transmission electron microscopy (TEM): In this study, 2-mm-long FN tissue samples were taken for TEM (Leica EM AMW Automatic Microwave Tissue Processor; Leica Microsystems GmbH, Wetzlar, Germany) and fixed in 2.5% glutaraldehyde and 1% osmium tetroxide solution. The samples were embedded in araldite blocks by dehydration with acetone. Sections of 80-μm thickness were prepared with an ultramicrotome on a copper grid. Following contrasting with uranyl acetate and copper citrate, images were captured and examined (Carl-Zeiss, Oberkochen, Germany) by the senior pathologist who was blinded to the treatment groups. The axonal diameter and the thickness of the myelin sheath were measured quantitatively by examining a group of randomly selected 100 nerve fibrils.

Statistical Analysis
The Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. Mean, standard deviation, and median were used for descriptive statistics. Distribution of the variables was evaluated with Kolmogorov–Smirnov test. For the analysis of quantitative data, Kruskal–Wallis and Mann–Whitney U tests were chosen, and Wilcoxon test was used for continuous measurements. A p value <0.05 was considered statistically significant.

RESULTS
All the animals tolerated the surgical procedures and the electrophysiological evaluation well. There was no postoperative wound infection, and no animal was lost during the study period.

The mean amplitudes and latencies of each group are given in tables 1 and 2, respectively. Neither the mean amplitude nor the latency variables were significantly different in intergroup comparisons at the postoperative 3rd, 6th, 9th, and 12th weeks (p>0.05).

In all the groups, the mean amplitude of postoperative CMAPs measured at all time intervals decreased significantly compared with the preoperative period (p<0.05) (Table 1). The change in mean amplitude compared with the preoperative period was not significantly different.
between the 5 groups (p>0.05). FN degeneration was not significantly different between the groups at the 3rd, 6th, and 12th weeks (p>0.05), whereas at the 9th week, FN degeneration in the control group was significantly lower than that in the other groups (p<0.05) (Table 3).

In group A, the mean latencies of the postoperative electrophysiological evaluations at the 3rd, 6th, and 9th weeks were significantly longer than that of the preoperative period (p<0.05). However, the mean latency at the 12th week (1.1±0.2 ms) was not significantly different from the preoperative period (1.0±0.2 ms) (p=0.340). In all other groups, the mean latency values at all postoperative evaluations were noted to be longer than those of the preoperative evaluations (p<0.05). The change in mean latency compared with the preoperative period was not significantly different between the 5 groups (p>0.05). Latency differences between the surgery and nonsurgery sides of the groups were not significantly different (p>0.05) except for the findings of the systemic melatonin group at the 12th week, which was −0.2±0.1, indicating a shorter latency of the coapted FN in this group (Table 3).

### Histopathological Examination

The nerve diameter, axon diameter, and myelin thickness in each group are given in Table 4. The nerve diameter was significantly smaller in the control group (p<0.05), and animals in the melatonin groups (topical and systemic) had significantly larger nerve diameters than those in the dexamethasone groups (p<0.05). No significant difference was noted between topical and systemic adminis-
trations of melatonin as well as dexamethasone (p>0.05). The axon diameter was significantly smaller in the control group than in the melatonin (topical and systemic) and topical dexamethasone groups (p<0.05). No significant difference in axon diameter was detected between groups with topical and systemic administrations of both melatonin and dexamethasone (p>0.05); however, the axon diameter was significantly smaller in the systemic dexamethasone group than in the topical melatonin group (p<0.05). The control group had a significantly thinner myelin sheath than the other groups (p<0.05). The thickness of the myelin sheath was significantly less in the topical melatonin and topical dexamethasone groups than in the systemic melatonin group (p=0.033 and p=0.009, respectively).

Group A (Control, Figure 1): Prominent inflammatory cell infiltration and local hemorrhagic regions were noted in nerve sections under light microscopy. Axonal degeneration was widespread. Nuclei of the Schwann cells were heterochromatic and pyknotic with prominent cytoplasmic eosinophilia. Widespread degeneration and irregularity of the myelin sheath and prominent vacuolar formations in axoplasm sections were observed under TEM.

Group B (Topical melatonin, Figure 2): Minimal inflammatory cell infiltration was seen in nerve sections under light microscopy. Nuclei of the Schwann cells were moderately heterochromatic and pyknotic with prominent cytoplasmic eosinophilia. Widespread degeneration and irregularity of the myelin sheath and prominent vacuolar formations in axoplasm sections were observed under TEM.

Group C (Systemic melatonin, Figure 3): Nerve sections had generally normal histologic structure under light microscopy. However, sporadic axonal undulations were prominent. Nuclei of the Schwann cells were mostly normochromatic, but minimal heterochromatism was noted sporadically. Axons, neurofilaments, and myelin sheaths had normal ultrastructural architecture under TEM.

*Figure 1. a, b.* (a) Degeneration of the nerve tissue (asterisk) and pyknotic nuclei (arrow). Light microscopy, H–E, ×40. (b) Myelin degeneration (arrows) and myelin figures within the axoplasm (arrowhead). Transmission electron microscopy, ×10000. (H–E: Hematoxylin-eosin).
Group D (Topical dexamethasone, Figure 4): Nuclei of the endoneurial and perineural connective tissue cells were heterochromatic under light microscopy. Sporadic degeneration and distortion of the myelin sheaths were noted under TEM. Vacuoles comprising myelin figures were detected in axoplasms.

Group E (Systemic dexamethasone, Figure 5): Nuclei of the endoneurial and perineural connective tissue cells and Schwann cells were heterochromatic under light microscopy. Myelin sheaths had normal ultrastructural architecture under TEM. However, myelin figures, vacuoles, and sporadic electron-dense granules were detected in axoplasmic regions.

DISCUSSION
Traumatic FN injury may occur due to accidental trauma or in the surgical practice of otology and head and neck surgery, either as a complication or as a part of the procedure[2, 5, 12, 13]. If both the proximal and distal segments of the transected nerve are available, tension-free end-to-end anastomosis is considered as the gold standard for repair[14]. However, the results following end-to-end anastomosis are not satisfactory mainly due to poor axonal regeneration and synkinesis[1, 7].

Many papers have mentioned the potential action of melatonin against traumatic peripheral nerve injuries[11, 15-17]. Neuroprotective effects of melatonin, particularly at high doses, have been demonstrated in a sciatic nerve injury model[18]. Recently, an increase in the regeneration of the FN after neurorrhaphy was reported with systemic melatonin[4]. Turgut et al.[16, 19] also demonstrated that melatonin has a positive effect on the nerve regeneration process following sciatic nerve surgery. The authors stated that melatonin reduces neuroma formation[16, 19]. The beneficial effects of melatonin on nerve regeneration have been linked to the induction of Schwann cell proliferation, which provides the ideal environment for axonal regrowth[20]. Scar tissue formation between the transected segments can impede axonal growth, and the effects of melatonin in reducing collagen deposition may also contribute to its beneficial effects on recovery[17]. Kaptanoglu et al.[21] studied the effects of methylprednisolone and melatonin in an experimental spinal cord injury model and concluded that melatonin-treated animals show more obvious protection of neurons and subcellular organelles.

The effects of SCs for FN anastomosis are not well known[4]. Can the treatment principles of Bell’s palsy be applied to FN transection? This
question has not been examined in detail, and the number of articles is relatively low compared with that for Bell’s palsy [4, 5, 22-24]. Yanilmaz et al. [4] and Karlidag et al. [23] demonstrated that methylprednisolone does not provide any beneficial effect on FN regeneration following neurorrhaphy. Seth et al. [5] reported that the improvement of FN recovery might be possible with the use of high-dose systemic dexamethasone, if administered early following neurorrhaphy. Chen et al. [24] stated that high-dose methylprednisolone might enhance the survival time of motor neurons after FN transection. Yildirim et al. [22] concluded that SCs have no effect on the healing process in a nerve transection model. In contrast, in FN compression models, SCs have been reported to have the ability to decrease myelin degeneration, axonal denervation, and edema formation [22, 25]. Similar to nerve compression models, the effectiveness of SCs in the management of Bell’s palsy is well known [26, 27]. The main aim of SC administration is the reduction of edema formation [28]. Liberman et al. [28], in contrast, reported that corticosteroids might interfere with functional recovery following crush injury. They stressed that the immunosuppressive state achieved by SCs is responsible for the impaired regeneration process [28]. In our opinion, it is necessary to establish the dose of SCs to have a therapeutic effect following nerve injury.

In animal models of FN injury, the nerve is either crushed or transected (axotomy). Crush injury and transection can be considered as axonotmesis and neurotmesis, respectively [5, 29]. Because crush injuries can produce nonhomogeneous degrees of nerve damage among animals and show different healing mechanisms, we prefer FN transection that produces a more reproducible lesion [1, 5, 29].

It should be kept in mind that the rat FN has a higher regenerative capacity than that of humans [29]. The location and distance of peripheral nerve injury from the central nervous system has also been reported to affect the regeneration process. That is why the results of sciatic nerve injury models may not be applied to the FN [30].

In the present study, we investigated the effects of dexamethasone and melatonin on FN regeneration in an experimental model of axotomy and neurorrhaphy. Both systemic and topical administrations were evaluated. Regarding electrophysiological evaluation, latency and amplitude parameters between the groups did not differ significantly at any examination period. Moreover, a statistically significant decrease in amplitude and increase in latency compared with the preoperative period in all study groups at all examination periods may be interpreted.
as follows: neither melatonin nor dexamethasone had any beneficial effect on neural regeneration from the perspective of electrophysiological evaluation. At the 12th week, degeneration did not differ significantly between the groups; however, latency of the surgery side was less than that of the nonsurgery side in the systemic melatonin group. Although systemic melatonin seemed to have a promising effect, this finding was not in compliance with other electrophysiological parameters.

Histopathological results were somewhat different from those of electrophysiological evaluation. One of the most important findings was prominent inflammatory cell infiltration in the control group, which was minimal in the other groups. It was even absent in the systemic melatonin group. The findings of significantly smaller nerve and axon diameters and a thinner myelin sheath in the control group may point to the fact that both melatonin and dexamethasone had important effects in reducing nerve degeneration. Regarding the qualitative comparison of these 3 parameters between the melatonin and dexamethasone groups, the most notable difference was found in nerve diameter, which was significantly larger in the melatonin groups.

The findings of significantly larger axon diameter in the topical melatonin group than in the systemic dexamethasone group may be interpreted as follows: topical melatonin was more effective than systemic dexamethasone in terms of axonal regeneration.

Widespread myelin degeneration in the control group and normal myelin structure in the melatonin groups may reflect the protective effect of melatonin against myelin degeneration. Although the dexamethasone groups also had better myelin ultrastructure than the control group, these effects were not as prominent as in the melatonin groups. Thicker myelin sheath in the systemic melatonin group than in the topical melatonin and topical dexamethasone groups may be interpreted as follows: systemic melatonin had more important effects in preserving the thickness of myelin. We propose that systemic mediators may play a role in myelin regeneration because no statistically significant difference was detected between the systemic melatonin and systemic dexamethasone groups in terms of myelin thickness. However, myelin ultrastructure was also well preserved in the topical melatonin group, despite the presence of a significant difference between the topical and systemic melatonin groups in terms of myelin thickness. Although the dexamethasone groups had some advantages in terms of axon diameter (topical dexamethasone) and myelin thickness (systemic dexamethasone), these were not statistically significant compared with melatonin groups.

CONCLUSION

Electrophysiological evaluation did not reveal any potential benefit of dexamethasone and melatonin. In contrast, histopathological examination revealed beneficial effects of melatonin in particular. This can be interpreted as both corticosteroids and melatonin being able to increase FN regeneration following axotomy and end-to-end anastomosis, but electrophysiological evidence of regeneration may appear later.

Ethics Committee Approval: Ethics Committee Approval was received for this study from the Ethics Committee of Bezmialem University Animal Care and Use (2013/14).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.


Acknowledgements: The authors thank Pasa Basar for his tremendous effort in electrophysiological examinations.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES


