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Original Article

The Effectiveness of Ebselen in Facial Nerve Crush Injury: An Experimental Study

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BACKGROUND: Ebselen is a neuroprotective drug that protects cellular components from oxidative damage by modulating enzymatic cofactors, metalloproteins, gene expression, antioxidant-anti-inflammatory effects, and immunological systems. Our goal was to compare the efficacy of Ebselen and methylprednisolone on rat facial nerve crush injury.

METHODS: Thirty healthy male Wistar rats (mean weight of 245 g) were used in this study. The rats were randomly divided into four groups: Group 1 (ebselen group), Group 2 (methylprednisolone group), Group 3 (control group), and Group 4 (sham group (the right side of the control group)). Except for the sham group, all groups had their left facial nerve crushed. Three weeks after surgery, prospective functional, histologic, and electrophysiologic recovery was reported.

RESULTS: The ebselen group and methylprednisolone group had similar and more significant recovery at Nerve Excitability Thresholds (NET) at the end of three weeks. These groups also showed similar features in terms of histopathological parameters such as axonal degeneration, vascular congestion, axon diameter, and myelin thickness. Except for the macrovacuolization parameter, both showed statistically better results than the control group. Although there was an earlier improvement in the whiskers and blink tests in the ebselen group compared to the methylprednisolone and control groups, complete recovery was observed in all groups on the 21st day.

CONCLUSION: Ebselen was found to be similarly effective to methylprednisolone in nerve regeneration in a rat model of experimental facial nerve crush. Considering that methylprednisolone has serious systemic side effects, ebselen may be a good alternative.

KEYWORDS: Facial nerve, crush injury, neuroprotective agents, methylprednisolone, ebselen, experimental rat model

INTRODUCTION

Traumatic facial nerve damage occurs as a consequence of head or maxillofacial trauma as a result of surgery or most accidents and has been the topic of several studies due to the functional and psychological difficulties it causes. Among these cases, there is a wide range of patients with mild post-traumatic paresis on one end and total facial paresis on the other end due to a complete nerve incision. Especially in experimental studies, it is attempted to be treated with many agents and surgical techniques. However, because of the diversity of injuries, experimental models of facial nerve injury are also diverse, and there is no standardized method for these injury models.[1] The most commonly used method is crush injury, which has been applied using various techniques and durations.[2,3]

Ebselen, 2-phenyl-1,2-benzisoselenazol-3[2H]-one; PZ-51; DR-3305, is a lipid-soluble seleno-organic compound that catalyzes the reduction of reactive oxygen species (ROS), interacts with peroxynitrite (ONOO3), inhibits lipoxygenase, NO synthase, NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase, protein kinase C, and H-/K-ATPase, mimicking glutathione

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peroxidase.[4,5] With these properties, through the control of enzymatic cofactors, metalloproteins, gene expression, antioxidant-ant i-inflammatory effects, and immunological processes, the agent ebselen can prevent cellular components from oxidative damage.[6] Although several studies have investigated the effects of ebselen in various diseases, including brain injury, cardiovascular disease, and hearing loss,[7-16] its neuroprotective impact on facial nerve injuries has not been previously examined. Additionally, since ebselen does not release selenium, its toxicity is low.[17] In this study, we evaluated the effect of ebselen on facial nerve regeneration and chose it over methylprednisolone due to its lower systemic side effects.

Facial nerve injuries affect millions of people globally, leading to the development of various treatments, including medications, electrical stimulations, and surgical procedures.[18,19] While surgical procedures are frequently performed for nerve transection injuries,[20,21] electrical stimulation can be challenging, prompting research into medical treatments for facial nerve crush injuries. Steroids are the only agents proven to be effective in humans and have been extensively studied in this context.[22-26] However, the systemic side effects of steroids and their less obvious utility in treating facial nerve crush injuries compared to Bell palsy have led to the exploration of alternative agents. Many studies have tested various agents for efficacy in facial nerve injuries,[3,27-37] with some comparing these agents to steroids[3,27,29,31,37] and others not.[28,30] Despite the systemic side effects, steroids should be included in comparative studies because of their known effectiveness in humans. In addition, the agent under investigation should aim to reduce or eliminate the damage caused by trauma, ischemia, oxidative stress, and inflammation, and ideally, it should be at least as effective as, if not superior to, steroids. Furthermore, the selected agent's effectiveness must be thoroughly evaluated through functional, electrophysiological, and histopathological assessments, which are limited in the literature.[3,27] Based on these considerations, we chose to compare ebselen, known for its antioxidant, anti-inflammatory, and neuroprotective properties, with methylprednisolone in a comprehensive study. In this study, we aimed to evaluate the potential neuroprotective effect of ebselen on functional, electrophysiological, and histological recovery in an experimental facial nerve crush injury model by comparing it with methylprednisolone.

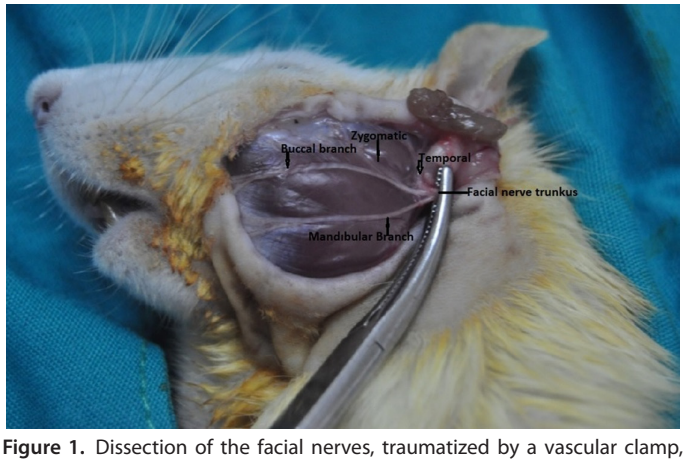

Figure 1. Dissection of the facial nerves, traumatized by a vascular clamp, was performed from the stylomastoid foramen main trunk to the zygomatic, buccal, and marginal branches.

METHODS

The experiment follows the rules outlined in the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, updated 1996). The Institutional Ethics Committee on Animal Experiments (#56.2020) authorized this study, which was carried out at the Marmara University School of Medicine, Experimental Animal Laboratory, İstanbul, Türkiye.

Subjects

Thirty male Wistar albino rats (mean weight of 245 g, 230-280 g) with an average age of 13 weeks old were used in this study. Throughout the period of the study, the rats were placed in a semi-acclimatized room with a 12-hour light/dark cycle, humidity-controlled (40–60% relative humidity), temperature-controlled (21–22°C), and ventilation-controlled environments. One rat was kept in each cage, and cages were cleaned daily. Tap water and standard rat pellets were given, and rats were allowed free access to them (ad libitum). All rats were handled for more than two weeks. They were then randomly separated into four groups of ten rats each: Group 1 (ebselen group), Group 2 (methylprednisolone group), Group 3 (control group), and Group 4 (sham group (the right side of the control group)).

Surgical procedure and Anesthesia

All 30 rats underwent the same standard surgical procedures. 100 mg/kg Ketamine hydrochloride (Ketalar® 500 mg/10 ml, Pfizer Medicine Türkiye) and 10 mg/kg Xylazine HCl (Rompun® 2%, 25 mg/ml, Bayer Medicine Türkiye) were used in conjunction to provide anesthesia throughout the surgical procedure, electrophysiological measurements, and euthanization.

Before anesthesia and surgery, bilateral corneal reflexes and blink reflexes (vibrissae orientation and movements) of the experimental animals in all groups were checked and observed normally.

Following anesthetic administration, the areas corresponding to the trace of the left facial nerve were shaved and painted with povidone-iodine (Poviiodeks® 1000cc Kim-Pa Medicine Türkiye). A 2 cm long horizontal skin incision was made from the front of the external auditory meatus to the first whisker's level at the corner of the mouth. The facial nerve trunk was revealed after passing the epidermis and subcutaneous tissues (Figure 1). Before the crush injury, electrophysiological assessments were performed to measure facial nerve stimulation thresholds in milliamperes (mA) using a neural integrity monitor (Medtronic NIM-Response 3.0 System) (NIM-2; Medtronic Xomed, Jacksonville, Fla, USA) (the evaluation method is detailed in the electrophysiological assessment section). The rats' facial nerve trunks were then traumatized for 60 seconds by a vascular clamp in all groups except the sham group (Figure 1). For the sham group, only incisions and dissections were made on the right side of the rats in the control group; facial crush injury was not performed. After the crush injury, electrophysiological assessments were performed in all groups again to verify complete paralysis in the injury groups and to verify no paralysis in the sham group. The incision site was sutured appropriately with a subcutaneous 4-0 vicryl (Ethicon, Germany) suture to prevent the rats from scratching the incision area.

Groups and Treatments

To avoid the consumption of research subjects, the injury group was assigned to the left side of the rats, while the sham group was assigned

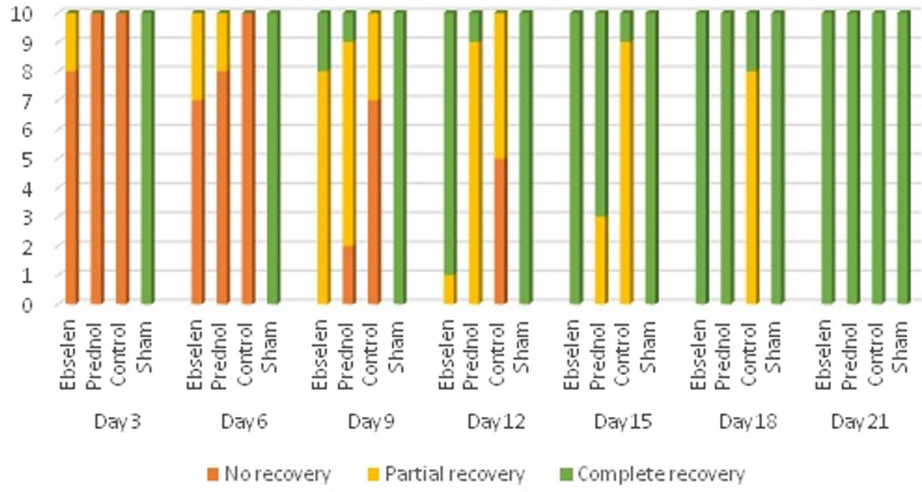

Figure 2. Distributions of blink test recovery status according to the groups in 21 days.

to the right side. After getting injured, **Group 1** (*Ebselen*) rats were given Ebselen (10 mg/kg/day, Sigma, St. Louis, Mo, USA) orally for 10 days. A prior study was used to determine the Ebselen dosage.[38]

Group 2 (*Methylprednisolone*) rats were injured and administered 1 mg/kg/day methylprednisolone intraperitoneally for 10 days.

Group 3 (*Control*) rats were injured and administered isotonic NaCl solution via the oral route for 10 days.

Group 4 (*Sham*) rats were not injured; only incision and dissection were made on the right side of the rats in the control group and then closed.

20 mg/kg cefazolin sodium (Cefozin, Bilim Medicine ,Türkiye) was administered i.p. before and on the postoperative first day.

Evaluation of Nerve Regeneration

At the end of 3 weeks, after evaluating the eye blink reflexes and vibrissae orientation and movements of all rats, the right and left facial nerves were exposed again under anesthesia, and nerve stimulation thresholds were measured.

Then, for histopathological evaluation, the facial nerve was cut proximal to the crush injury, and the main trunk of the facial nerve was excised together with the buccal muscle, including the buccal and mandibular branches. After the procedure, the experiment was concluded by the euthanasia of the animals.

Functional Recovery Assessment

Bilateral eye-blink reflexes and vibrissae orientation and movements of rats were checked before crush injury. Daily comparisons of the uninjured right side's intact function to the left side's recovering facial nerve function were made. On a three-point scale, the blink reflex's recovery was rated: 1 for no recovery, 2 for minimal recovery, and 3 for full recovery.

Electrophysiological Assessment

The same approach of nerve integrity monitoring was used to assess the stimulation thresholds of the facial nerve. Nerve Integrity Monitor (Medtronic NIM-Response 3.0 System) electrophysiological monitoring was carried out. The orbicularis oris muscle received one of the intramuscular needle electrodes for the nerve integrity monitor, while the sternocleidomastoid muscle received the grounding electrode. For each evaluation of nerve conduction, a bipolar needle electrode was implanted in the region of the vibrissal muscle, and

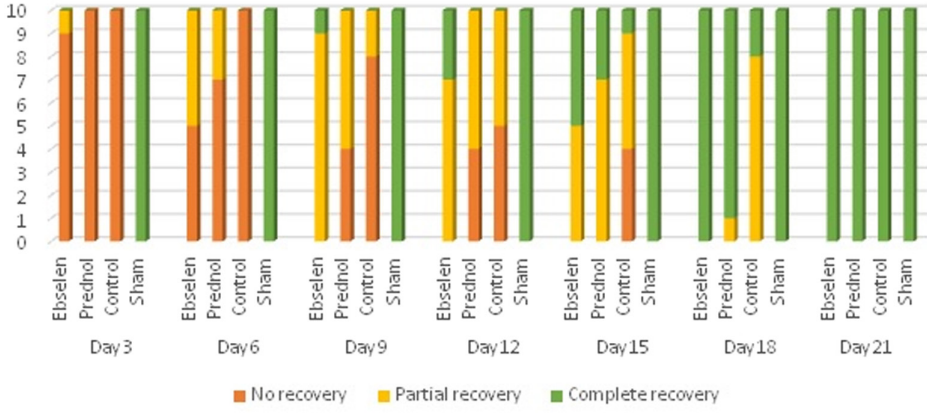

Figure 3. Distributions of whisking test recovery status according to the groups in 21 days.

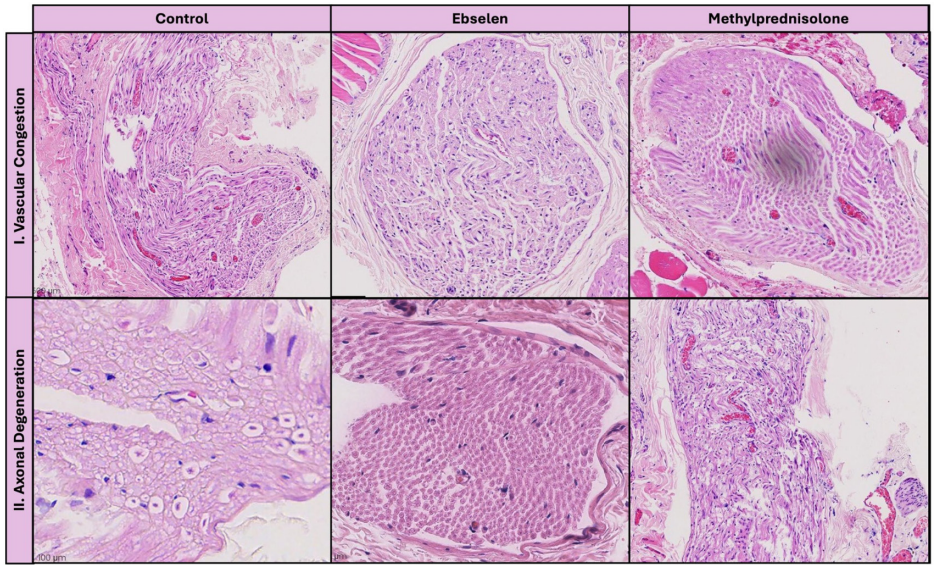

Figure 4. Histopathological images of injury groups with hematoxylin and eosin staining. I. Vascular congestion in control, ebselen, and methylprednisolone groups. II. Axonal degeneration in control, ebselen, and methylprednisolone groups.

a grounding electrode was placed in the back. A monopolar stimulating electrode was inserted at the buccal branch's proximal end. Excitability thresholds are the levels of stimulation that can produce a compound muscle action potential (CMAP) that can be detected. The nerve stimulation thresholds of every animal in each group were calculated using nerve integrity monitoring, starting at 0.01 mA. The stimulation strength was gradually raised until the device's screen displayed a waveform and the face muscles began to contract. After surgery, the incision site was sutured in all groups.

Histopathological Evaluation

After electrophysiologic testing, a minimum 1 cm-long nerve segment with two branches (the buccal and marginal mandibular) as well as the buccal muscle was extracted for histopathologic evaluation. The specimen was embedded in blocks of paraffin and fixed in formalin for 24 hours to produce 4 µm sections. Giemsa and hematoxylin-eosin were used to stain each sample. Then, using light microscopy (Axio Vert, Zeiss, Germany), an expert pathologist evaluated the degree of vascular congestion, macrovacuolization, axon diameter, axonal degeneration, and thickness of the myelin sheath. The degree of axonal degeneration, macrovacuolization, and vascular congestion was classified as none, mild, moderate, or severe. The axon diameter was classified as very small, small, or normal. The classification of the myelin sheath's thickness was very thin, thin, or normal. Then the results in all groups were compared statistically.

Statistical Analysis

All the statistical calculations were completed using IBM SPSS Statistics 25, Statistical Package for the Social Sciences, on a computer. The Shapiro-Wilk test was used to assess the parameters' suitability for normal distribution when analyzing the research data. The Mann-Whitney U test was used to determine which group was responsible for the difference, and the Kruskal-Wallis test was used to compare quantitative data across groups that did not have a normal distribution. The Wilcoxon signed-rank test was used to compare non-normally distributed parameters within groups. Because several statistical tests were used, the Bonferroni correction procedure was used to reduce the probability of a type I error. The level of statistical significance was set at $P < .05$.

RESULTS

Functional Recovery; Eye Blink Reflex

In the evaluation of the eye blink reflex, all groups showed similar characteristics on the 21st day when compared to each other ($P = 1$). On the days before the 21st day, at least one group showed different characteristics from the others. Although faster recovery was observed with ebselen, there was no significant difference between the groups on the 3rd and 6th days ($P > .05$) (Figure 2) (Table 1). In summary, full recovery was achieved on the 21st day in all groups. The ebselen and methylprednisolone groups were similar to each other, except on the 12th day, and showed better eye blink reflex recovery than the control group (Figure 2).

Functional Recovery; Vibrissae Movement

In the evaluation of the whisking function, all groups showed similar characteristics on the 21st day when compared to each other ($P = 1$). Although faster recovery was observed with ebselen, there was no statistical difference on the 3rd, 6th, and 9th days when compared with the methylprednisolone group ($P > .05$), (Figure 3) (Table 2). In summary, full recovery was achieved on the 21st day in all groups. The ebselen and methylprednisolone groups were similar to each other, except on the 12th day, and showed better vibrissae movement recovery than the control group (Figure 3).

Electrophysiological Recovery

All electrophysiological threshold values for each group were presented in Table 3. When these values were compared for all groups, the difference between the sham group and the three injury groups was significant in terms of both before-trauma and after-trauma, and after-treatment comparisons (Table 4). The lowest median values were seen in the ebselen and methylprednisolone groups, and there was no significant difference between these two groups when comparing the after-treatment median value of NET findings with the

Table 1. Comparison of Blink Test Recovery Status According to the Groups

	Ebselen	Prednol	Control	Sham	*P*1*	*P*2*	*P*3**
Day 3					**<.001**	.126	.481
No recovery	8	10	10	0			
Partial recovery	2	0	0	0			
Complete recovery	0	0	0	10			
Median	1 (1-2)	1 (1)	1 (1)	3 (3)			
Day 6					**<.001**	.197	.739
No recovery	7	8	10	0			
Partial recovery	3	2	0	0			
Complete recovery	0	0	0	10			
Median	1 (1-2)	1 (1-2)	1 (1)	3 (3)			
Day 9					**<.001**	**.003**	.353
No recovery	0	2	7	0			
Partial recovery	8	7	3	0			
Complete recovery	2	1	0	10			
Median	2 (2-3)	2 (1-3)	1 (1-2)	3 (3)			
Day 12					**<.001**	**<.001**	**.002**
No recovery	0	0	5	0			
Partial recovery	1	9	5	0			
Complete recovery	9	1	0	10			
Median	3 (2-3)	2 (2-3)	1.5 (1-2)	3 (3)			
Day 15					**<.001**	**<.001**	.28
No recovery	0	0	0	0			
Partial recovery	0	3	9	0			
Complete recovery	10	7	1	10			
Median	3 (3)	3 (2-3)	2 (2-3)	3 (3)			
Day 18					**<.001**	**<.001**	1.00
No recovery	0	0	0	0			
Partial recovery	0	0	8	0			
Complete recovery	10	10	2	10			
Median	3 (3)	3 (3)	2 (2-3)	3 (3)			
Day 21					1.00	1.00	1.00
No recovery	0	0	0	0			
Partial recovery	0	0	0	0			
Complete recovery	10	10	10	10			
Median	3 (3)	3 (3)	3 (3)	3 (3)			

*Kruskal-Wallis test, **Mann-Whitney U test.
*P*1: Comparison of ebselen, prednol, control, and sham groups.
*P*2: Comparison of ebselen, prednol, and control groups.
*P*3: Comparison of ebselen and prednol groups.

control group ($P = .42$) (Table 4). We expect the difference between before and after trauma values to be statistically significant, as well as the sham group values not to be significant anyway. However, the electrophysiological threshold values could not reach the before-trauma values in all injury groups even if treatment was given.

While the ebselen and methylprednisolone groups had lower values compared to the control group, they had higher values compared to the sham group. There was a statistically significant difference in all comparisons of after-treatment values except for the ebselen and methylprednisolone comparison (Table 4).

Histopathological Recovery

Median score values and comparison of macrovacuolization, vascular congestion, axon diameter, axonal degeneration, and thickness of the myelin sheath were presented in Table 5. There was a statistically significant difference in all parameters between the injury groups (group 1, 2, 3) and the sham group ($P < .05$) (Table 6) (Figure 4).

Table 2. Comparison of Whisking Test Recovery Status According to the Groups

	Ebselen	Prednol	Control	Sham	*P*1*	*P*2*	*P*3**
Day 3					**<.001**	.368	.739
No recovery	9	10	10	0			
Partial recovery	1	0	0	0			
Complete recovery	0	0	0	10			
Median (min-max)	1(1-2)	1 (1)	1 (1)	3 (3)			
Day 6					**<.001**	.044	.481
No recovery	5	7	10	0			
Partial recovery	5	3	0	0			
Complete recovery	0	0	0	10			
Median (min-max)	1,5 (1-2)	1 (1-2)	1 (1)	3 (3)			
Day 9					**<.001**	**.001**	.089
No recovery	0	4	8	0			
Partial recovery	9	6	2	0			
Complete recovery	1	0	0	10			
Median (min-max)	2 (2-3)	2 (1-2)	1 (1-2)	3 (3)			
Day 12					**<.001**	**.007**	.029
No recovery	0	4	5	0			
Partial recovery	7	6	5	0			
Complete recovery	3	0	0	10			
Median (min-max)	2 (2-3)	2 (1-2)	1.5 (1-2)	3 (3)			
Day 15					**<.001**	.021	.481
No recovery	0	0	4	0			
Partial recovery	5	7	5	0			
Complete recovery	5	3	1	10			
Median (min-max)	2.,5 (2-3)	2 (2-3)	2 (1-3)	3 (3)			
Day 18					**<.001**	**<.001**	.739
No recovery	0	0	0	0			
Partial recovery	0	1	8	0			
Complete recovery	10	9	2	10			
Median (min-max)	3 (3)	3 (2-3)	2 (2-3)	3 (3)			
Day 21					1.00	1.00	1.00
No recovery	0	0	0	0			
Partial recovery	0	0	0	0			
Complete recovery	10	10	10	10			
Median (min-max)	3 (3)	3 (3)	3 (3)	3 (3)			

*Kruskal-Wallis test, **Mann-Whitney U test.
*P*1: Comparison of ebselen, prednol, control, and sham groups.
*P*2: Comparison of ebselen, prednol, and control groups.
*P*3: Comparison of ebselen and prednol groups.

Ebselen and methylprednisolone groups showed similar features in terms of histopathological parameters ($P < .05$) (Table 5) while both showed statistically above-average results when compared to the control group ($P < .05$) (Table 6) except for the macrovacuolization parameter ($P = .062$) ($P = 1$) (Table 6).

DISCUSSION

In this study, our findings suggested that ebselen can accelerate functional, electrophysiological, and histopathological recovery following facial nerve crush injury, showing comparable or even superior results to methylprednisolone in certain instances.

Functional recovery in facial nerve crush injuries is typically reported to occur between days 9 and 21 in the literature.[27,30,39] In our study, the assessment of the eye blink reflex revealed that all treatment groups achieved full recovery by the 21st day post-injury. Notably, both the ebselen and methylprednisolone groups exhibited faster recovery compared to the control group, particularly evident from

Table 3. Evaluation of Electrophysiological Threshold Values According to the Groups Before-Trauma, After-Trauma, and After-Treatment

		Before Trauma (BT)	After Trauma (AT)	After Treatment (ATre)	Difference (BT-ATre)	*P**	Difference (BT-AT)	*P**	Difference (AT - ATre)	*P**
Ebselen	Mean	0.028	0.245	0.094	0.066	**.004**	0.217	**.005**	0.151	**.005**
	Standard dev.	0.013	0.06	0.021	0.02		0.047		0.039	
	Median	0.03	0.225	0.09	0.06		0.195		0.135	
	Min-Max	0.01-0.05	0.2-0.35	0.07-0.14						
Prednol	Mean	0.028	0.215	0.087	0.059	**.005**	0.187	**.005**	0.128	**.005**
	Standard dev.	0.016	0.091	0.022	0.02		0.075		0.069	
	Median	0.025	0.175	0.085	0.06		0.15		0.09	
	Min-Max	0.01-0.05	0.12-0.35	0.06-0.11						
Control	Mean	0.03	0.234	0.127	0.097	**.005**	0.204	**.005**	0.107	**.005**
	Standard dev.	0.015	0.076	0.032	0.02		0.061		0.044	
	Median	0.03	0.2	0.125	0.095		0.17		0.075	
	Min-Max	0.01-0.05	0,14-0,35	0,09-0,2						
Sham	Mean	0.026	-	0.029	0.003	.879				
	Standard dev.	0.014	-	0.015	0.01					
	Median	0.025	-	0.025	0					
	Min-Max	0.01-0.05	-	0.01-0.05						
	p**	.942	.47	.001						

*Wilcoxon signed rank test, **Kruskal-Wallis.

the 12th day onward. Although the differences between ebselen and methylprednisolone were not statistically significant on the 3rd and 6th days, the outcomes on other days suggest that ebselen may facilitate a more rapid functional recovery in the early phases post-injury. These findings are consistent with previous studies indicating that antioxidants and anti-inflammatory agents can expedite neural functional recovery following injury.[28,31,39] The early improvement in eye blink reflex underscores ebselen's potential in enhancing quality of life by reducing the duration of functional impairment.

The restoration of vibrissae movement is particularly important as it reflects the functional integrity of motor neurons and muscle coordination. Similar to the eye blink reflex outcomes, vibrissae movement assessments showed complete recovery in all groups by day 21. Ebselen and methylprednisolone treatments resulted in better recovery trajectories compared to the control group, with significant improvements observed from the 12th day onward. The early-stage recovery, although not statistically significant between ebselen and methylprednisolone, again favored ebselen, suggesting its efficacy in promoting neuromotor function restoration.

Table 4. Post Hoc Comparison of Electrophysiological Threshold Values According to the Groups.

	BT (*P**)	AT (*P**)	ATre (*P**)
G1-G2	.969	.246	.421
G1-G3	.758	.549	.011
G1-G4	.728		**.001**
G2-G3	.758	.466	**.004**
G2-G4	.786		**.001**
G3-G4	.579		**.001**

AT, after-trauma; ATre, after-treatment; BT, before-trauma; G1, ebselen group; G2, prednol group; G3, control group; G4, *Mann-Whitney U test.

Functional recovery was also evaluated objectively by electrophysiological evaluation. Electrophysiological evaluations using a Nerve Integrity Monitor showed similar threshold values between the ebselen and methylprednisolone groups, both of which exhibited positive effects on facial nerve regeneration, indicating the potential efficacy of ebselen comparable to methylprednisolone. The control group had the lowest values. Finally, our functional evaluations have shown that ebselen is at least as effective as methylprednisolone in facial nerve regeneration.

Histopathological analysis revealed significant differences between the injury groups and the sham group across all evaluated parameters, confirming the impact of the crush injury. Both ebselen and methylprednisolone treatments resulted in substantial improvements in vascular congestion, axon diameter, axonal degeneration, and myelin sheath thickness compared to the control group. However, no significant difference was observed in macrovacuolization between the treatment groups and the control group. This suggested that ebselen and methylprednisolone improved other parameters thanks to their anti-edema, antioxidant, and anti-inflammatory effects, whereas other mechanisms were not sufficient for addressing macrovacuolization. Another thought is that it may be related to the dose and/or the method of administration of ebselen. Further studies are needed for this. However, all these results showed that ebselen and methylprednisolone were positively effective in nerve regeneration, and ebselen was at least as effective as methylprednisolone.

Table 5. Evaluation of Histopathologic Parameters According to the Groups

	Ebselen	Prednol	Control	Sham	*P*1*	*P*2*
Axonal Degeneration					**<.001**	**.001**
None	4	2	0	0		
Mild	5	4	1	0		
Moderate	1	4	2	0		
Severe	0	0	6	0		
Median (min-max)	1 (0-2)	1 (0-2)	3 (1-3)	0 (0)		
Vascular Congestion					**<.001**	**.001**
None	4	1	0	0		
Mild	6	7	2	0		
Moderate	0	2	4	0		
Severe	0	0	4	0		
Median (min-max)	1 (0-1)	1 (0-2)	2 (1-3)	0 (0)		
Macrovacuolization					**<.001**	.089
None	3	0	0	0		
Mild	5	5	5	0		
Moderate	2	5	5	0		
Severe	0	0	0	0		
Median (min-max)	1 (0-2)	1.5 (1-2)	1.5 (1-2)	0 (0)		
Axonal Diameter					**p<0,001**	**p=0,001**
Normal	4	3	0	0		
Small	6	6	2	0		
Very small	0	1	8	0		
Median (min-max)	1 (0-1)	1 (0-2)	2 (1-2)	0 (0)		
Myelin Sheath Thickness					**<.001**	**.001**
Normal	4	4	0	0		
Thin	5	4	1	0		
Very thin	1	2	9	0		
Median (min-max)	1 (0-2)	1 (0-2)	2 (1-2)	0 (0)		

*Kruskal-Wallis test,
*P*1: Comparison of ebselen, prednol, control, and sham groups.
p2: Comparison of ebselen, prednol, and control groups.

However, the study has its limitations. First, the absence of electron microscopy in the histological assessments restricts our ability to analyze ultrastructural changes in nerve regeneration, which could provide deeper insights into the cellular-level effects of the treatments. Additionally, while both Ebselen and methylprednisolone were tested individually, the combination of the two treatments was not explored. Given the shared anti-inflammatory and neuroprotective properties of both agents, future studies should investigate whether combining these treatments could result in synergistic effects, offering even greater therapeutic benefit.

Table 6. Post Hoc Comparison of Histopathologic Parameters According to the Groups.

	Axonal Degeneration (*P**)	Vascular Congestion (*P**)	Macrovacuolization (*P**)	Axonal Diameter (*P**)	Myelin Sheath Thickness (*P**)
G1-G2	.144	.06	.062	.483	.805
G1-G3	**.001**	**.001**	.062	**.001**	**.001**
G1-G4	**.005**	**.004**	**.002**	**.004**	**.005**
G2-G3	**.003**	**.005**	1.000	**.002**	**.002**
G2-G4	**.001**	**.001**	**.001**	**.002**	**.005**
G3-G4	**.001**	**.001**	**.001**	**.001**	**.001**

G1, Ebselen group; G2, Prednol group; G3, control group; G4, Sham group.
*Mann-Whitney U test.

Another limitation to consider is the inherent regenerative capability of the animal model used. Rats are known to have a higher capacity for nerve regeneration compared to humans, which may limit the direct translatability of these findings to clinical practice. However, the use of Ebselen, a compound already in clinical use for other conditions, offers a significant advantage. Unlike many agents tested in experimental settings that cannot be applied to human patients, Ebselen's proven safety in humans suggests that the transition from experimental models to clinical trials could be smoother. Future research should focus on evaluating its effectiveness in human facial nerve injury models, with larger sample sizes, longer follow-up periods, and more advanced imaging and evaluation techniques.

CONCLUSION

In conclusion, our study demonstrates that Ebselen is at least as effective as methylprednisolone in promoting functional, electrophysiological, and histopathological recovery after facial nerve crush injury. Given its lower toxicity and promising results, Ebselen may be a valuable alternative to steroids in the treatment of facial nerve injuries. Further studies with morphological and molecular analysis are needed to fully explore its potential in clinical settings, including dosage optimization and the exploration of combined treatment strategies.

Ethics Committee Approval: The experiment adheres to the guidelines of the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). This study was conducted at the Marmara University School of Medicine, Experimental Animal Laboratory, İstanbul, Türkiye, and was approved by the Institutional Ethics Committee on Animal Experiments (#56.2020).

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