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Effects of Insulin-Like Growth Factor-1 and Platelet-Rich Plasma on Facial Nerve Injury in a Rat Model

Rat Modelinde İnsülin Benzeri Büyüme Faktörü-1 ve Plateletten Zengin Plazmanın Fasiyal Sinir Yaralanması Üzerine Etkileri

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ABSTRACT Objective: Insulin-like growth factor-1 (IGF-1) and platelet rich plasma (PRP) on nerve healing to evaluate electrophysiologically and histopathologically by experimental traumatic peripheral facial paralysis in rats. **Material and Methods:** Three groups of 7 rats in each were formed. Group 1: Control group. Group 2: Local IGF-1 applied. Group 3: Local PRP applied. After nerve identification, neuron excitability threshold (NET), latency and amplitude values of left facial nerve buccal branch (LFNBB) were recorded. The LFNBB of all rats in all groups was cut and sutured. PRF was applied to Group 2, IGF-1 and Group 3, respectively. Control measurements of LFNBB were performed at 12 weeks. Then traumatized part of LFNBB was removed and axonal degeneration, vascular congestion and macrovacuolization were evaluated histopathologically. **Results:** When all groups were compared, significant difference was observed in Group 3 (p1=0.002, p2<0.001, p3=0.017 respectively) in NET. Group 2 and 3 values were significantly different than control group (p1=0.011, p2=0.007) in latency values. There was no difference between Group 2 and 3. Group 2 and 3 values were significantly different in amplitude values compared to control group (p1<0.001, p2<0.001). While there was no difference between Group 2 and 3. In all histopathological examinations, a significant improvement was observed in Group 2 and 3 compared to control group, findings of histopathological improvement in the Group 3 was superior. **Conclusion:** The findings obtained from this study showed that local IGF-1 and PRP application had positive effects on nerve healing in traumatic facial palsy while PRP was more effective.

Keywords: Insulin-like growth factor-1;
platelet rich plasma;
traumatic facial nerve paralysis

ÖZET Amaç: Sıçanlarda deneysel travmatik periferik fasiyal paralizi ile sinir iyileşmesi üzerine insülin benzeri büyüme faktörü-1 [insulin-like growth factor-1 (IGF-1)] ve trombositten zengin plazma [platelet rich plasma (PRP)] elektrofizyolojik ve histopatolojik olarak değerlendirmektir. **Gereç ve Yöntemler:** Her biri 7 sıçandan oluşan 3 grup oluşturuldu. Grup 1: Kontrol grubu. Grup 2: Lokal IGF-1 uygulanan grup. Grup 3: Lokal PRP uygulanan grup. Sinir tanımlaması sonrası sol bukkal dalın nöron eksitabilite eşiği [Neuron Excitability Threshold (NET)], latans ve amplitüd değerleri kaydedildi. Her 3 gruptaki sıçanların hepsinin sol fasiyal sinirinin bukkal dalı kesilerek dikildi. Grup 2'ye IGF-1 ve Grup 3'e PRP uygulaması yapıldı. On ikinci haftada sol bukkal dalın kontrol ölçümleri yapılmıştır. Daha sonra, 12. haftada sol bukkal dalın travmatize olan kısmı çıkarıldı ve histopatolojik olarak aksonal dejenerasyon, vasküler konjesyon ve makrovakuolizasyon değerlendirildi. **Bulgular:** Her üç grup da kendi aralarında karşılaştırıldığında, Grup 3'te (sırasıyla p1=0,002, p2<0,001, p3=0,017) NET'te anlamlı farklılık gözlendi. On ikinci haftada kaydedilen latans değerleri, Grup 2 ve 3 değerleri kontrol grubundan anlamlı derecede farklıydı (p1=0,011, p2=0,007). Grup 2 ve 3 arasında fark yoktu. Grup 2 ve 3 değerleri kontrol grubuna göre amplitüd değerlerinde anlamlı farklılık gösterdi (p1<0,001, p2<0,001). Grup 2 ve 3 arasında fark bulunmazken, histopatolojik olarak değerlendirilen tüm incelemelerde, IGF 1 ve PRP ile tedavi edilen kontrol grubuna göre anlamlı iyileşme gözlendi, PRP ile tedavi edilen grupta histopatolojik iyileşme bulguları daha üstündü. **Sonuç:** Bu çalışmadan elde edilen bulgular, travmatik fasiyal felçte lokal IGF-1 ve PRP uygulamasının sinir iyileşmesi üzerinde olumlu etkilerinin olduğunu, PRP'nin ise daha etkili olduğunu göstermiştir.

Anahtar Kelimeler: İnsülin benzeri büyüme faktörü-1;
trombositten zengin plazma;
travmatik fasiyal sinir paralizisi

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The facial nerve (VII. CN) innervates the superficial facial muscles; surrounds the lips and eyes. It provides mimic movement and protects the lips as a sphincter when feeding. Compared to other cranial nerves, it is the most damaged cranial nerve due to the curvature in a very long and narrow bone canal.[1]

If the motor fibers of the VII. CN are damaged, facial paralysis occurs which affects all mimetic movements that are important for emotional and social life.[2,3]

The most common type of facial paralysis is idiopathic peripheral paralysis also known as Bell's paralysis, other types are traumatic, infectious, tumoral and congenital types.[2] Some types of traumatic paralysis occur after injuries, which are iatrogenic. The most valuable treatment procedure for this type of traumatic facial nerve injury and for many other traumatic incision injuries is to repair the nerve end to end by suturing. However, most of the time, this treatment is unable to provide satisfying physiological and functional recovery.[4] In addition to the surgical treatment, some other methods have also been tested such as the use of platelet rich plasma (PRP) and growth factors such as insulin growth factor (IGF) and human platelet-derived growth factor-BB (PDGF-BB).[5]

The effects of platelets on homeostasis have been known for a long time, the effects of growth factors and other substances on wound healing were discovered in the early 90s. After clinical studies of PRP in maxillofacial surgery performed by Whitman et al. in 1997, the number of studies investigating its effects on various tissues and nerve regeneration has increased and PRP has been used clinically.[6,7]

IGF-1 and IGF-2, also called somatomedins are growth factors that have anabolic effects. They have roles in tissue growth, development and regeneration. Besides their role in the development of central, peripheral and autonomic nervous systems, in nerve injury models IGF-1 has an important role in neuroregeneration in the peripheral nervous system. IGF-1 stimulates protein synthesis in neurons, glia, oligodendrocytes, and Schwann cells, and favors neuronal survival by inhibiting apoptosis.[8-11]

The technological improvement in neurosurgery and rehabilitation and the increasing use of microscopes has enabled us to better understand the mechanism of nerve healing. In our clinic, we have used hormones and certain medications locally and although these endeavors have been shown to be effective, we were not able to provide nerve healing like in the pre-injury period.

It is of upmost importance for ear nose throat specialists and other clinical branches that new treatment modalities are developed for the surgical treatment and post-treatment rehabilitation of facial nerve injury. In this context, the aim of this study was to compare the recovery efficacy of PRP and IGF-1 electrophysiologically and histopathologically on facial nerve injury.

MATERIAL AND METHODS

This study was conducted after receiving approval from the Health Sciences University of Türkiye, Ankara Training and Research Hospital, Experimental Animal Ethics Committee (date: February 15, 2016-0029, no: 391). We protected animal rights in our work in line with the principles of "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html). Our study was conducted in accordance with the principles of the Declaration of Helsinki. The study was performed on 21 adult female WistarAlbino rats weighing 250-300 grams from our hospital's experimental research and animal laboratory. The rats were divided into 3 groups with 7 animals in each group.

The buccal branch of the facial nerve was preferred because it is easily accessible, has homogeneous histological features and allows reproducible nerve conduction tests.[12,13] While the animals were under general anesthesia, the buccal branch of the facial nerve was stimulated with a nerve stimulator and neuron excitability thresholds (NETs) were established. To determine the excitability thresholds, electrical stimulation starting from 0.01 mA was given to the nerve and it was increased until contraction in the mimic muscles and waveform on the device monitor was observed. The value that created contraction in the mimic muscles was accepted as the excitability threshold. After making a full transection on the buccal branch of VII. CN in all rats, the nerve was sutured under a microscope.

Establishing the groups: The rats were then divided in 3 Groups. In Group 1, which was also the control group, we expected that rats would recover spontaneously after suturing and nothing else was given. The rats in Group 2 were given IGF-1 (IGF-1 LR3, Alley drug, Belgium). Saturated surgical spongeswere applied to the transected segment. The rats in Group 3 were given PRP. Follow-up measurements comprising of facial nerve excitability thresholds, latency and amplitude values were made at the 12th week. After the measurement, the traumatized part of the buccal branch was excised for histopathological examination.

Surgical Procedure: The same standard surgery procedure was performed for all rats by the same surgeon. 50 mg/kg of ketamine (Ketalar, Eczacıbaşı Pharmaceuticals, Türkiye) and 12.5 mg/kg xylazine (Rompun, Bayer Pharmaceuticals, Türkiye) were given intramuscularly to provide general anesthesia. Under sterile conditions, a horizontal incision of approximately 1.5 cm was made from the front of the outer ear and auricle to the first mustache line towards the mandible (Figure 1). Skin and subcutaneous tissues were dissected and the parotid gland and facial nerve truncus were made visible then the buccal branch of the facial nerve was exposed (Figure 2, Figure 3, Figure 4).

The first needle electrode was placed in the orbicularis oris muscle, the 2nd in the orbicularis ocular muscle, and the grounding electrode in the sternocleidomastoid muscle. A monopolar electrode was used for neural stimulations. Electrical stimulation was given from the proximal part of the part planned to be traumatized to the facial nerve starting from 0.01 mA with NerveIntegrity Monitor (NIM-Response 3.0 System, Medtronic Xomed, Jacksonville, Florida, USA). Right angle, constant current impulses were used with a 4Hz repetition rate and a duration of 100 µ.s. Intensities varied between 0 and 1 mA in 50 µ.A increments. Motor unit action potentials in the orbicularis oris and buccal muscles, were obtained. When determining the nerve excitability threshold

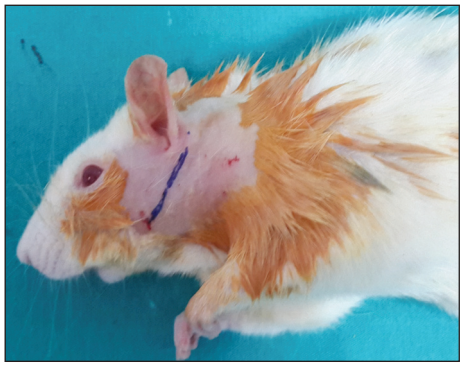

FIGURE 1: Approximately 1.5 cm horizontal incision site from the front of outer ear and auricle to the first mustache line towards the mandible.

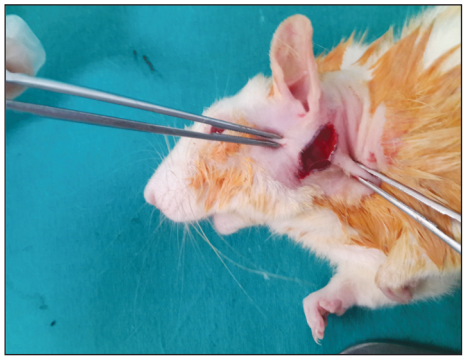

FIGURE 2: Skin incision and subcutaneous incision.

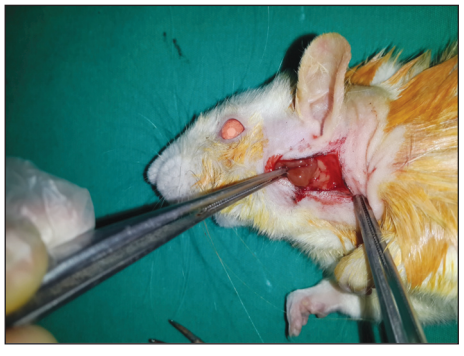

FIGURE 3: The parotid gland was made visible.

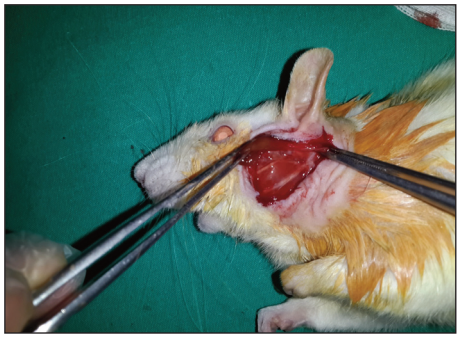

FIGURE 4: The branches of the facial nerve were exposed.

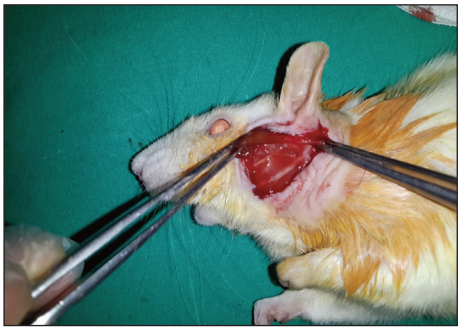

FIGURE 5: Cutting the buccal branch.

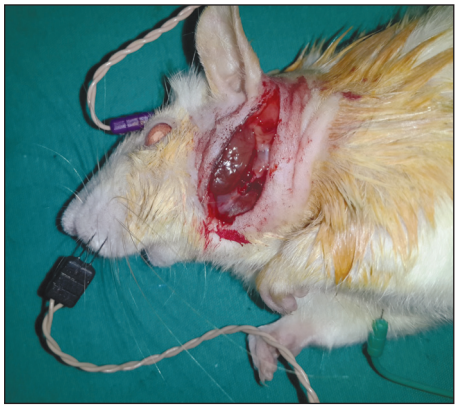

FIGURE 6: Suturing the buccal branch.

movement in the mimic muscles the formation of a wave pattern on the NIM-3 device monitor were taken into account. The buccal branch of the facial nerve of the subjects in all 3 groups was transected as a full layer with a scalpel (Figure 5). Next, it was sutured under a microscope (Takagi Operating Microscope, MFG.CO.LTD. Made in Japan) with a 8-0 monofilament suture (Figure 6). A single suture was administered to the transected buccal branch of all subjects. Then the incision site was sutured with 4/0 silk in all groups. At the 12th week, after the measurements were carried out, the traumatized part was removed by marking the distal part of the facial nerve and was fixated in a 10% formaldehyde solution.

PRP preparation: The blood of donor rats was drawn with an injector and poured into an anticoagulated glass tube (Vacutainer, No Additive, Becton Dickinson) after anesthesia was achieved. The tube was then centrifuged at 3000 rpm (Nf 200, Nuve industrial materials manufacturing, max speed 5,000 rpm, power 310 watts).[14] After centrifugation, the platelet-rich fibrin clot which formed on the top of the tube was gently removed.

IGF-1: As the half-life of IGF-1 is short (between 1-2 hours), in our study we preferred to use IGF-1LR3. IGF-1LR3, is a synthetic protein that maintains its pharmacological activity and has a half-life of 20-30 hours as well as being more potent comparedto IGF-1.[15]

Histopathological examination: All of the obtained facial nerve samples were put into process. After the samples were fixed in formaldehyde and processed in a closed system device (Leica ASP300, Leica Micro-systems GmbH, Wetzlar, Germany), 2 millimicron thick sections were taken from the paraffin blocks and prepared with microtome (Leica RM 2245, Leica Micro-systems GmbH, Wetzlar, Germany). Samples were stained with Hematoxylin eosin (HE) and May-Grunwald Giemsa (MGG) in the device Leica ST5020 (Leica Micro-systems GmbH, Wetzlar, Germany). The examinations were made with the Olympus BX51 (Olympus Optical, Tokyo, Japan) light microscope by the same pathologist who was blind to the groups. Axonal degeneration, vascular congestion, macrovacuolisation, axon diameter, and thickness of myelin were examined in all samples. Axonal degeneration, vascular congestion, and macrovacuolisation were graded as absent, mild, moderate and severe (Table 1). Axon diameter was graded as very narrow, narrow and normal; myelin thickness was graded as very thin, thin and normal.[16]

STATISTICAL ANALYSIS

Data were analyzed using the SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) program and results were considered statistically significant at $p<0.05$ level. Mean, standard deviation, median, minimum and maximum values were included indescriptive statistics. Normality of the data was tested. For inter-group comparisons Fisher Exact chi-square test, Kruskal Wallis test, and post-hoc tests were used.

RESULTS

Our study was carried out with a total of 21 experimental animals which were divided into 3 groups, 7 rats in each group. There were no systemic compli-

cations after surgery in the groups. The first measurement made after finding the buccal branch of the facial nerve under general anesthesia was the nerve excitability threshold which was initially found to be 0.04-0.08 mA (mean 0.05 mA) in all rats, and there was no statistical difference between the groups. The control measurements done at the 12th week showed the nerve excitability threshold to be 0.51±0.21 mA (0.3-0.9 mA) in Group 1, 0.23±0.08 mA (0.15-0, 40 mA) in Group 2 and 0.13±0.04 mA (0.08-0.20 mA) in Group 3. When the results were compared, a significant difference was found between Group 1 and Group 2, Group 1 and Group 3, and Group 3 and Group 2 in favor of Group 3 (p1=0.002, p2<0.001, p3=0.017) (Table 2).

In the first measurements made on the buccal branch of the facial nerve, there was no difference between the groups in terms of latency and amplitude values. The mean latency values recorded on the 12th week were found to be 6.85±2 ms in Group 1, 4.30±1.10 ms in Group 2 and 4.05±1.11 ms in Group 3. Based on the results, the measurements obtained from Group 2 and Group 3 were found to be significantly different from the control group (p1=0.011, p2=0.007). There was no significant difference between Group 2 and Group 3 (p=0.805). The mean amplitude values were also measured on the 12th week, and was found to be 113.28±12.27 mA in Group 1, 171.85±11.27 mA in Group 2 and 179.28±13.78 mA in Group 3. The results obtained for Group 2 and Group 3 were significantly different from the control group (p1<0.001, p2<0.001), and there was no significant difference between Groups 2 and 3 (p=0.383) (Table 2).

Severe macrovacuolization, axonal degeneration and vascular congestion were observed in Group 1. Axonal degeneration and macrovacuolization were observed less in Group 3 compared to Group 2 (Table 3, Table 4, Table 5). Less vascular congestion was observed in Group 2 compared to Group 3. Myelin thickness and axon diameter were found to be most preserved in Group 3 (Table 6, Table 7).

TABLE 1: Histopathological examination definitions.

	Absent	Mild	Moderate	Severe
Axonal degeneration	• No change observed	• Slight deterioration in nuclear polarization • Slight scatter in axon fibers	• Those between the characteristics defined in mild and severe	• Marked polarization disorder in the nucleus • Nuclear degeneration • Degeneration of axon fibers, prominent vacuole formation
Vascular congestion	• No change observed	• Presence of 1 or 2 capillaries containing 1 or 2 erythrocytes inside the nerve fiber	• Those between the characteristics defined in mild and severe	• More than two capillary vacuoles inside the nerve fiber whose lumen is filled with erythrocytes
Macrovacuolisation	• No change observed	• Presence of several vacuoles with a diameter equal to or slightly larger than the nucleus diameter	• Presence of 5 or 6 vacuoles with a larger diameter than the nucleus, observed individually	• Presence of large vacuoles forming groups

TABLE 2: Facial nerve excitability thresholds, latency and amplitude values in groups.

	Nerve excitability threshold (mA)		Latency (ms)		Amplitude (mA)	
	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
Group 1	0.05±0.01	0.51±0.21*	1.59±0.29	6.85±2*	240.71±34.41	113.28±12.27*
Group 2	0.05±0.01	0.23±0.08*	1.68±0.28	4.30±1.10*	224±21.54	171.85±11.27*
Group 3	0.05±0.01	0.13±0.04*	1.66±0.24	4.05±1.11*	224.28±28.63	179.28±13.78*

* p<0.001.

TABLE 3: Axonal degeneration crosstab.

			Axonal degeneration				
p=0.002			**Absent**	**Mild**	**Moderate**	**Severe**	**Total**
Group	1.00	Count % within group	0	0	3	4	7
		axonal degeneration	0.0%	0.0%	42.9%	57.1%	100.0%
			0.0%	0.0%	60.0%	100.0%	33.3%
	2.00	Count % within group	1	4	2	0	7
		axonal degeneration	14.3%	57.1%	28.6%	0.0%	100.0%
			25.0%	50.0%	40.0%	0.0%	33.3%
	3.00	Count % within group	3	4	0	0	7
		axonal degeneration	42.9%	57.1%	0.0%	0.0%	100.0%
			75.0%	50.0%	0.0%	0.0%	33.3%
Total		Count	4	8	5	4	21
		% within group	19.0%	38.1%	23.8%	19.0%	100.0%
		axonal degeneration	100.0%	100.0%	100.0%	100.0%	100.0

TABLE 4: Vascular congestion crosstab.

			Vascular congestion				
p=0.12			**Absent**	**Mild**	**Moderate**	**Severe**	**Total**
Group	1.00	Count	0	2	2	3	7
		% within group	0.0%	28.6%	28.6%	42.9%	100.0%
		Vascular congestion	0.0%	16.7%	100.0%	100.0%	33.3%
	2.00	Count	1	6	0	0	7
		% within group	14.3%	85.7%	0.0%	0.0%	100.0%
		Vascular congestion	25.0%	50.0%	0.0%	0.0%	33.3%
	3.00	Count	3	4	0	0	7
		% within group	42.9%	57.1%	0.0%	0.0%	100.0%
		Vascular congestion	75.0%	33.3%	0.0%	0.0%	33.3%
Total		Count	4	12	2	3	21
		% within group	19.0%	57.1%	9.5%	14.3%	100.0%
		Vascular congestion	100.0%	100.0%	100.0%	100.0%	100.0%

A significant improvement was observed in Groups 2 and 3 compared to the control group in the histopathologically evaluated axonal degeneration, vascular congestion, macrovacuolization, axon diameter and myelin thickness measurements in the experimental groups. In the comparison between Groups 2 and 3, the histopathological improvement in the group treated with PRP was superior to the group treated with IGF-1.

DISCUSSION

Facial paralysis, which occurs after facial nerve dysfunction causes several problems in the social life of the effected individual. By disrupting facial appearance, a person with facial deformity may suffer from decreased physical function and, importantly, experiences significant psychosocial distress.[2,17] Idiopathic peripheral facial paralysis has no cause. The most

TABLE 5: Axon diameter crosstab.

			Macrovacuolization				
p=0.014			**Absent**	**Mild**	**Moderate**	**Severe**	**Total**
Group	1.00	Count	0	1	2	4	7
		% within group	0.0%	14.3%	28.6%	57.1%	100.0%
		Macrovacuolization	0.0%	20.0%	40.0%	80.0%	33.3%
	2.00	Count	1	2	3	1	7
		% within group	14.3%	28.6%	42.9%	14.3%	100.0%
		Macrovacuolization	16.7%	40.0%	60.0%	20.0%	33.3%
	3.00	Count	5	2	0	0	7
		% within group	71.4%	28.6%	0.0%	0.0%	100.0%
		Macrovacuolization	83.3%	40.0%	0.0%	0.0%	33.3%
Total		Count	6	5	5	5	21
		% within group	28.6%	23.8%	23.8%	23.8%	100.0%
		Macrovacuolization	100.0%	100.0%	100.0%	100.0%	100.0%

TABLE 6: Axon diameter crosstab.

			Axon diameter			
p=0.002			**Very narrow**	**Narrow**	**Normal**	**Total**
Group	1.00	Count	2	4	1	7
		% within group	28.6%	57.1%	14.3%	100.0%
		% within axon diameter	100.0%	40.0%	11.1%	33.3%
	2.00	Count	0	5	2	7
		% within group	0.0%	71.4%	28.6%	100.0%
		% within axon diameter	0.0%	50.0%	22.2%	33.3%
	3.00	Count	0	1	6	7
		% within group	0.0%	14.3%	85.7%	100.0%
		% within axon diameter	0.0%	10.0%	66.7%	33.3%
Total		Count	2	10	9	21
		% within group	9.5%	47.6%	42.9%	100.0%
		% within axon diameter	100.0%	100.0%	100.0%	100.0%

TABLE 7: Myelin thickness crosstab.

			Myelin thickness			
p=0.010			**very Thin**	**Thin**	**Normal**	**Total**
Group	1.00	Count	5	1	1	7
		% within group	71.4%	14.3%	14.3%	100.0%
		% within myelin thickness	100.0%	11.1%	14.3%	33.3%
	2.00	Count	0	5	2	7
		% within group	0.0%	71.4%	28.6%	100.0%
		% within myelin thickness	0.0%	55.6%	28.6%	33.3%
	3,00	Count	0	3	4	7
		% within group	0.0%	42.9%	57.1%	100.0%
		% within myelin thickness	0.0%	33.3%	57.1%	33.3%
Total		Count	5	9	7	21
		% within group	23.8%	42.9%	33.3%	100.0%
		% within myelin thickness	100.0%	100.0%	100.0%	100.0%

known cause of traumatic facial nerve dysfunction is blunt trauma-induced temporal bone fractures or iatrogenic facial nerve injury.[18]

When the incision of the facial nerve occurs, the most effective treatment to regaining facial function is primary repair. Early recognition and early intervention play a key role in achieving postoperative facial function. The proximity of the injury to the cell body, type of neural damage, the time and type of repair, the patient's age, being under chemotherapy or radiotherapy, nourishment, and having underlying comorbidities also play key roles in nerve healing. The main prognostic factor is to provide a tension-free connection between the 2 ends.[19]

Even if the ideal conditions for primary nerve repairing occurs, full recovery is rarely seen. Loss of nerve cells due to trauma, final organ atrophy due to delayed innervation, or incorrect axon orientation during healing may cause failure in repair. Additionally, fibrosis and neuromas occurring at the injury site adversely affect nerve healing.[20]

Axon regeneration after nerve damage is a slow process. Nerve regeneration rate ranges from 0.6 mm/day to 4.2 mm/day, which is around 1 mm/day in humans. Increasing axonal branching rate is targeted in the regeneration of damaged nerve structures and recovery of nerve function in a short time.[16] To improve nerve regeneration and recovery rates, drugs, hormones and growth factors such as thyroid hormone, topical steroids, fibrin adhesives, human amniotic fluid, hyperbaric oxygen therapy, fluoxetine, gangliosides, vitamin D, vitamin E, androgens, riluzole, nimodipine, lithium, gingko biloba, memantine, erythropoietin, darbepoetin, tumuor necrosis factor alpha blocker,etanercept, nitric oxide and angiotensin-II have been used locally and systemically in clinical studies in addition to surgery. Some positive data have been obtained but the majority of these modalities have not been used in the clinic routinely.[16,21-24] Many studies continue in this field due to a better understanding of the molecular properties of nerve regeneration as well as technological advances. Similar studies in the literature inspired us to investigate the effects of PRP, which contains numerous growth factors and the exogenous administration of IGF-1 released by post-traumatic Schwan cells on nerve regeneration. Our contribution is not limited to the surgical treatment of facial nerve paralysis which is common in practice, but also to increase knowledge concerning the clinical benefits of PRP and IGF-1 to the peripheral nerve injury treatment process, which is vital for all clinical branches.

There are many studies in the literature investigating the effects of IGF-1 utilization on nerve regeneration. Thanos et al. showed that IGF-1 leads to increased axonal regeneration in the nerve graft and the average number of axons by 22%.[22] They observed that the corneal reflex improved and the opening between the eyelids decreased after local IGF-1 (50 µg/mL) application with cross facial nerve graft. In addition, light microscopy examination showed that IGF-1 increased axonal regeneration together with nerve graft, increased average nerve fiber count, length and thickness of myelin, and electron microscopic examination showed high density of microtubules that provide distribution within the axoplasm.[25] Similarly, Thanos et al. supported the improvement of facial paralysis with cross-facial nerve graft by demonstrating an increase in muscle reinnervation and functional improvement in general with the application of local IGF-1 treatment.[26] In the rat sciatic nerve transection model made by Mohammadi et al., it was found that intraperitoneal administration of IGF-1 (100 ng/kg/day) with allograft for a week provided more functional improvement compared to the group that only allograft was administered.[27] Tiangco et al. found a significant increase in musculocutaneous nerve axon count and myelin thickness/axon length ratio in experimental groups where local IGF-1 application was performed.[28] As stated above, besides the studies where IGF-1 was applied locally, studies investigating the effects of systemic administration on nerve regeneration were also conducted. Welch et al. demonstrated that systemic IGF-1 administration does not have an electrophysiological or histological effect on nerve repair in the short term after rat sciatic nerve transection repair.[29] In the study of Lutz et al., no significant difference was found between the experimental and control groups in the reinnervation of the flexor carpi ulnaris muscle after 14 days of postoperative application of rhIGF-1 (0.5 µg/kg)

following the transaction and repair of rats median nerve. In another study, IGF-1 was shown to be histochemically and electrophysiologically more effective when rhIGF-1 was administered via a mini pump following rat sciatic nerve damage.[30] Considering all the studies; the results obtained in our study are supported by the literature in that local IGF-1 applied to the incision line significantly increased the axon diameter and myelin thickness and decreased axonal degeneration. Our electrophysiological evaluation of the nerve excitability threshold was found to be low, the amplitude was found to be high and latency was found short. The values were statistically significant for the experimental groups revealing better nerve healing when compared to the control group.

After studies showed the positive effects of growth factors and other bioactive substances in platelets on wound healing, other studies have been conducted aiming to uncover their effects on nerve regeneration. In an experiment conducted by Cho et al., they administered PRP and mesenchymal stem cells after cut facial nerve injury and found this application enhances nerve regeneration.[31] Farrag et al. also found that in the rat model after facial nerve transection, application of PRP together with the facial nerve suturing provided a faster improvement in mustache movements, less latency prolongation and the highest increase in the number of axons in the histopathological examination was found in the PRP group.[32] When PRP was applied after end-to-end nerve repair, Sarıgüney et al. obtained better functional results. They showed an increase in myelin thickness and also an improvement in latency even though they were not able to show an increased fibrous proliferation in the epineuria histologically. They concluded that PRP application does not increase peripheral nerve regeneration however they concluded that it contributes to remyelination in the regenerating axons.[33] As a result of their studies, Giannessi and Küçük showed that PRP application caused an increase in motor function in the damaged nerve owing to an increase in the total number of axons both clinically, histologically and electrophysiologically.[34,35] Sabongi, Elgazzar and Ding also showed that PRP application after nerve damage can increase regenerated nerve fibers and have positive effects on functional recovery.[10,11] Studies have shown that PRP application after nerve injury increases the neurotrophic factors which contribute to axon regeneration, and functional and neurological recovery. It directly affects regeneration even in transected nerves and also allows the mesenchymal stem cells to be transformed into Schwan cells that release the factors that regulate axon regeneration and lastly it has been shown to regulate schwan cells activation and proliferation.

The findings of our study support all the studies mentioned above by demonstrating both electrophysiologically and histologically that PRP application after nerve repair increased axon diameter and myelin thickness, decreased axonal degeneration, and increased motor function.

Although many studies in the literature have examined the effects of IGF-1 and PRP on neuronal healing, only one study conducted by Emel et al. have compared the effects of these 2.[8] In that study, after crush injury of the sciatic nerve in rats, the effectiveness of PRP and IGF-1 was compared and nerve healing was evaluated with the sciatic functional index. They found that in the group treated with IGF-1, sensory functional recovery occurred faster than those treated with saline and PRP. They also found that wound healing was better in IGF-1 and PRP treated groups than in the control group.[8] The data from our study did not support these findings as we showed that the recovery of the group treated with PRP was better than both the IGF-1 and control group.

More recently, studies have been published investigating whether PRP increases IGF-1 expression and efficiency, rather than comparing IGF-1 and PRP. We think that the reason for this is that it is accepted that IGF 1 is a more effective growth and regeneration factor, and it is trying to find solutions that increase its effectiveness in the tissue. However, our study has revealed PRP to be a more effective healing factor than mentioned in theory. On the other hand, PRP can be used in tissue healing to increase the effectiveness of IGF-1.

CONCLUSION

The findings obtained from this study showed that local IGF-1 and PRP application had positive effects on nerve healing in traumatic facial palsy while PRP was more effective.

To strengthen and support the findings of our study, longer-term studies with a higher number of subjects examining the histopathologically and electrophysiologically changes that occurred as a result of IGF-1 and PRP administration to traumatic facial nerve injury are necessary.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Cemile Açıkgöz Yıldız;* ***Design:*** *Cemile Açıkgöz Yıldız;* ***Control/Supervision:*** *Necmi Arslan;* ***Data Collection and/or Processing:*** *Şule Demirci, Muzaffer Çaydere;* ***Analysis and/or Interpretation:*** *Cemile Açıkgöz Yıldız;* ***Literature Review:*** *Mehmet Şeneş, Cevdet Yılmaz;* ***Writing the Article:*** *Cemile Açıkgöz Yıldız;* ***Critical Review:*** *Necmi Arslan;* ***References and Fundings:*** *Cemile Açıkgöz Yıldız;* ***Materials:*** *Mehmet Şeneş, Cevdet Yılmaz, Cemile Açıkgöz Yıldız.*

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