EXPERIMENTAL STUDY

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Effects of platelet-rich plasma on nerve regeneration in a rat model

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Objective: The aim of this study was to determine whether platelet-rich plasma has a regenerative effect on a sciatic nerve injury model in rats.

Methods: A sciatic nerve cut model was created in 24 nerves of 12 rats. All nerves were repaired with epineural sutures by the same surgeon. Rats were randomly divided into two groups; platelet-rich plasma was applied to the injury site in the platelet-rich plasma group and saline only to the same area in the control group. Motor and electromyographic assessments were performed at the end of 12th postoperative week and all rats were euthanized for histological specimens.

Results: Motor recovery was significantly better in the platelet-rich plasma group than the control group. The differences in electromyographic and histomorphometric findings between the groups were significant ($p<0.05$).

Conclusion: Our experimental study demonstrated positive effects of platelet-rich plasma on nerve regeneration.

Key words: Growth factor; nerve regeneration; peripheral nerve injury; platelet-rich plasma; sciatic nerve.

The primary objective of surgical treatment in peripheral nerve injury cases is to restore the structural integrity of the injured neurons. Notwithstanding the developments in understanding the mechanisms of nerve regeneration and surgical techniques and materials, full functional recovery has not been possible in serious injuries of large nerve trunks.[1]

With regard to the numerous factors related to neurons, the processes of nerve regeneration and target organ reinnervation are very complicated.[1] The majority of previous studies focused upon mechanical factors such as surgical techniques and suture materials. However, the impact of chemical and biological agents on nerve regeneration continues to be an object of interest.[1-6]

Blood platelets contain many different growth factors (GFs) that are released when activated, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1),

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fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF).[1,7-10] As a result, platelet-rich plasma (PRP) prepared by centrifugation of the patient's own blood contains various GFs which have an effect on tendon, bone, ligament and muscle tissues.[1,7,9,11] All of these GFs initiate angiogenesis and contribute to tissue healing by increasing the chemotactic and mitotic properties of undifferentiated cells. Recent research on PRP's contribution to nerve healing have brought to successful conclusions.[1,4,8,10]

The aim of this study was to show that PRP can enhance nerve regeneration when locally applied in primary repair of the peripheral nerve transection in rats. Possible effects were evaluated using mechanical, electrophysiological and histological methods.

Materials and methods

This study was inspected and approved by the local ethical committee of animal experiments. Twelve Wistar albino rats weighing between 200 and 240 g were included in the research. Rats were randomly divided into two equal groups; the autologous PRP and control groups (6 rats, 12 sciatic nerves each).

One ml of blood drawn from the tail vein was put in a citrated tube. After 7 minutes of centrifuge at 700 RCF, the resulting plasma at the top was equally applied to both repaired nerves of the same rat.

Surgery was performed to create the sciatic nerve cut model. A ketamine–xylazine mixture (75 mg/kg ketamine + 5 g/kg xylazine) was given intraperitoneally for general anesthesia. After the rats were fixed to the operation table in the prone position, the surgical areas were cleaned. Both sciatic nerves were exposed from 1 cm distal to the sciatic notch to 1 cm distal of the trifurcation of the nerve in the posterior of the knee joint. The soft tissues were completely removed by carefully dissecting the nerve segment of 3 to 3.5 cm proximal to branching. The sciatic nerve was transversely cut with micro scissors 1.5 cm proximal of the trifurcation. Nerves were repaired by the same surgeon (LK) with epineural sutures (10-0 Ethilon®; Ethicon Inc., Somerville, NJ, USA).

In the PRP group, the prepared PRP was injected to an absorbable gelatin sponge (0.5x0.5x0.1 cm, Spongostan®; Ethicon Inc., Somerville, NJ, USA) and put on the repair area. In the control group, saline-injected absorbable gelatin sponge (Spongostan®) was applied to the repair line. The wound was closed with absorbable suture material (3-0 Vicryl®; Ethicon Inc., Somerville, NJ, USA). Rats were put in their cages and allowed to perform normal activities.

At the end of the 12th week, all the sciatic nerves were taken for histological evaluation, after motor and electrophysiological evaluations. Following the receipt of nerve samples, the rats were sacrificed.

Fig. 1. **(a)** Inclined plate; side view. **(b)** Side view during motor evaluation. **(c)** Inclined plate; rear view. **(d)** Rear view during motor evaluation. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 1. Results of electrodiagnostic evaluation, motor function, histology and microscopy.

	PRP group	Control group	p*
	Mean±SD	Mean±SD	
Climbing angle (degree)	63.6±0.89	38.33±2.58	0.02
CMAP amplitude of the gastrocnemius muscle (mV)	14.01±5.02	5.78±2.39	0.01
CMAP amplitude of the interdigital muscle (mV)	0.85±0.54	0.24±0.42	0.01
Number of axons	1,969.50±93.02	879±34.81	0.001

*Mann-Whitney U-test.
CMAP: Compound muscle action potential; PRP: Platelet-rich plasma; SD: Standard deviation.

The inclined plate, defined by Rivlin and Tator, was used to evaluate the motor power of the rats on the 12th postoperative week.[12] The highest slope angle the rats could stand still on the plate was noted (Fig. 1).

All electrodiagnostic evaluations were performed by the same evaluator (FA) using the same electromyography device (Nicolet Viking IIE EMG/EEG Electromyography System; Nicolet Biomedical Inc., Memphis, TN, USA) immediately following the motor evaluation while the rats were under general anesthesia (75 mg/kg ketamine + 5 g/kg xylazine). Filter setting was 5 Hz to 10 kHz, stimulation time was 0.1 ms and stimulation frequency was 1 Hz. Subcutaneous platinum needle electrodes (Grass; Astro-Med, Inc., West Warwick, RI, USA) were used for stimulation and recording. The sciatic nerve was stimulated supramaximally at the sciatic notch point. Compound muscle action potentials (CMAPs) were recorded from the gastrocnemius and the 2nd and 3rd interdigital muscles.

The distal of the damaged sciatic nerve was fixed at 3.6% glutaraldehyde in 0.1 mol/L Sorensen's phosphate buffer (pH=7.3). Post-fixation was made in the same buffer solution with 1% osmium tetroxide. Following the dehydration with graded ethanol solution, the epoxy was embedded in resin (Fluka; Sigma-Aldrich Corp., St. Louis, MO, USA). Specimens were sectioned into 0.5-mm-thick serial sections with ultra-microtome (20 sections from each specimen). The sections were stained with thionine and samples were photographed under a light microscope (Olympus BX51; Olympus Corp., Tokyo, Japan). The number of axons was measured by examining 6 randomly selected fields (1 center, 5 periphery) with the help of a digital counter with x10 magnification.

SPSS for Windows v.13.0 (SPSS Inc., Chicago, IL, USA) software was used for statistical analysis. Results were recorded in the form of mean±SD. Mann-Whitney U-test was used in comparison of the groups. Any probability coefficient with a p value less than 0.05 were considered statistically significant.

Results

Angle of climb was 63.6±0.89 degrees in the PRP group and 38.33±2.58 in the control group. The difference between the two groups was statistically significant ($p<0.05$) (Table 1).

In the PRP group, CMAP amplitudes of the gastrocnemius and interdigital muscles were 14.01±5.02 mV and 0.85±0.54 mV, respectively. In the control group, CMAP amplitudes of the gastrocnemius and interdigital muscles were 5.78±2.39 mV and 0.24±0.42 mV, respectively (Fig. 2). The differences between the two groups were statistically significant ($p<0.05$) (Table 1).

The average number of total axons in the control

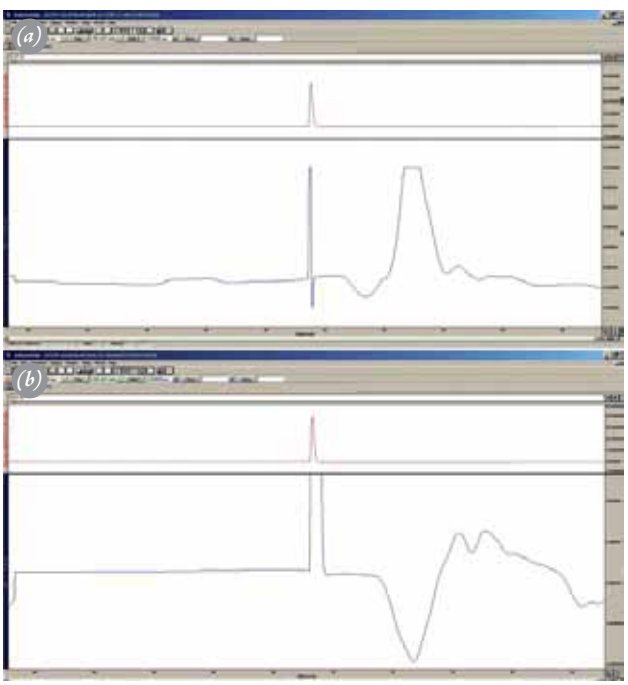

Fig. 2. EMG recording samples obtained from the gastrocnemius muscle. **(a)** PRP group. **(b)** Control group. It can be observed that CMAP amplitude is higher in the PRP group than the control group. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

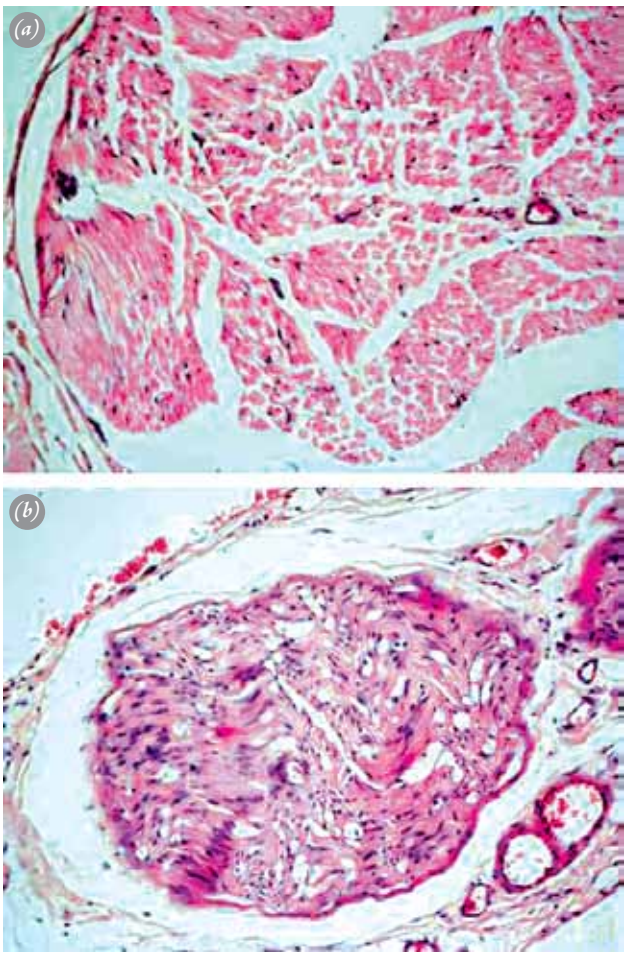

Fig. 3. **(a)** Histological section from the PRP group (Thionine, x200). **(b)** Histological section from the control group (Thionine, x200). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

group was 879.3±34.81 (x100 magnification) and 1,969.50± 93.02 in the PRP group (Fig. 3). The difference between the two groups was statistically significant ($p<0.05$) (Table 1).

Discussion

Following peripheral nerve damage, demyelination and axonal degeneration occur in the distal segment.[13] Despite the regenerative response of the nerve and current developments in peripheral nerve surgery, functional healing after nerve repair remains insufficient.[14] Irreversible damages can occur in the target organ as a result of the delay of nerve healing process, particularly in cases in which the nerve damage occurs in the proximal part of the extremity. In addition to surgical and mechanical factors, biological and chemical factors affecting the healing process should also be studied and may pave the way for positive results in nerve repair.

In a normal integrated peripheral nerve, trophic factors which affect nerve tissue are generated in the target organ and transported in a retrograde fashion along the cell body. Schwann cells (SCs) are not only the actors of Wallerian degeneration that occurs following the nerve damage but are also responsible for the production of trophic factors that regulate regeneration. The GF generated by SCs, such as nerve growth factor, brain-derived neurotropic factor, ciliary neurotropic factor and glial cell line-derived neurotropic factor, are involved in the modulation of recovery.[1] Neurotrophins diffusely distribute around the damaged axons after releasing from the SCs. Regenerating axons tend to extend towards the distal segment with a high concentration of neurotrophin.

Alpha granules of platelets contain growth factors with mitogenic and chemotactic characteristics, such as PDGF, TGF-β, IGF-1, FGF and VEGF.[8,9] Although they are not classical neutrophic factors, the effect of these GFs on nerve regeneration has been extensively studied.

IGF-1 receptors exist in axons of peripheral nerve cells, nerve endings, SCs and cell bodies of the motor neurons. Acting as a neurotropic factor, IGF-1 initiates the formation of bud growth, supports the forward extension of the nerve and inhibit apoptosis in motor, sensory and sympathetic neurons.[15,16]

FGF is a factor with numerous bioactivities. It stimulates the proliferation and differentiation of mesoderm cells. Additionally, it contributes to embryonic development, tissue repair, wound healing, neuroprotection and nerve regeneration.[17,18] In peripheral nerve injuries, the number of FGF receptors and affinities increase in the area of injury.[11]

TGF-β2 and TGF-β3, two of the three forms of TGF-β, play an important role in SC proliferation and differentiation. In addition, they are necessary for many neurotropic factors to be effective.[19]

VEGF, an angiogenic factor, stimulates cell proliferation and mediates the antiapoptotic effect through a variety of receptors. These receptors exist in neural tissues, especially in SCs and in axonal buds, where damaged axons begin to sprout.[20]

In connection with these factors, the effect of PRP on peripheral nerve regeneration has been studied in recent years. Farrag et al. concluded that the application of PRP produces better results than the application of fibrin glue or no biological agent following surgical nerve repair in a facial nerve transection model on rats.[3]

Ding et al.[21] applied PRP on the field of injury af-

ter creating crush injury on the cavernous nerve in their study of 24 rats divided into 3 groups. The authors concluded that the functional healing and histological parameters in the PRP group were significantly better than the others. A similar study in which a crush injury was created on the sciatic nerve also showed the positive effects of PRP.[4]

Sariguney et al. concluded that PRP is effective when applied following the ideal surgical nerve repair in a nerve transection model and was ineffective in cases of insufficient surgical repair.[22]

In addition to the aforementioned positive effects of GF, recent studies have reported unsatisfactory results. Welch et al. reported no significant effect of GFs on a transection and direct repair model.[23] Piskin et al. concluded that GFs did not improve nerve regeneration after microsurgical reconstruction of a gap with collagen tubes.[24]

The results of our study showed that a single dose of PRP applied on the repair site increases recovery of motor function in clinical, histomorphometrical and electromyographical terms by increasing the number of total axons. The low number of subjects might be considered the weakness of the study. However, given that this was an animal model, working with a greater number of animals than is statistically necessary would be violation of animal rights and considered unethical. In addition, the application of the nerve injuries to both legs of the subjects instead of leaving the second leg as a control group can be considered another weakness of our study. Nonetheless, we believe that both legs should be in the same group in order to obtain reliable results on the inclined plate during the motor evaluation.

In conclusion, PRP appears to have a positive effect on nerve regeneration. However, further studies supporting PRP's effect on nerve regeneration and suggesting standard treatment models are necessary before clinical application is adopted.

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Conflicts of Interest: No conflicts declared.

References

1. Yu W, Wang J, Yin J. Platelet-rich plasma: a promising product for treatment of peripheral nerve regeneration after nerve injury. Int J Neurosci 2011;121:176-80.
2. Guntinas-Lichius O, Wewetzer K, Tomov TL, Azzolin N, Kazemi S, Streppel M, et al. Transplantation of olfactory mucosa minimizes axonal branching and promotes the recovery of vibrissae motor performance after facial nerve repair in rats. J Neurosci 2002;22:7121-31.
3. Farrag TY, Lehar M, Verhaegen P, Carson KA, Byrne PJ. Effect of platelet rich plasma and fibrin sealant on facial nerve regeneration in a rat model. Laryngoscope 2007;117:157-65.
4. Emel E, Ergün SS, Kotan D, Gürsoy EB, Parman Y, Zengin A, et al. Effects of insulin-like growth factor-I and platelet-rich plasma on sciatic nerve crush injury in a rat model. J Neurosurg 2011;114:522-8.
5. Lundborg G. A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg Am 2000;25:391-414.
6. Huang W, Begum R, Barber T, Ibba V, Tee NC, Hussain M, et al. Regenerative potential of silk conduits in repair of peripheral nerve injury in adult rats. Biomaterials 2012;33:59-71.
7. Cervelli V, Lucarini L, Spallone D, Brinci L, de Angelis B. Use of platelet rich plasma and hyaluronic acid on exposed tendons of the foot and ankle. J Wound Care 2010;19:188-90.
8. Cho HH, Jang S, Lee SC, Jeong HS, Park JS, Han JY, et al. Effect of neural-induced mesenchymal stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model. Laryngoscope 2010;120:907-13.
9. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plast Reconstr Surg 2004;114:1502-8.
10. Gordon T, Sulaiman O, Boyd JG. Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst 2003;8:236-50.
11. Grothe C, Nikkhah G. The role of basic fibroblast growth factor in peripheral nerve regeneration. Anat Embryol (Berl) 2001;204:171-7.
12. Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg 1977;47:577-81.
13. Federici T, Liu JK, Teng Q, Yang J, Boulis NM. A means for targeting therapeutics to peripheral nervous system neurons with axonal damage. Neurosurgery 2007;60:911-8.
14. Fenrich K, Gordon T. Canadian Association of Neuroscience review: axonal regeneration in the peripheral and central nervous systems--current issues and advances. Can J Neurol Sci 2004;31:142-56.
15. Apel PJ, Ma J, Callahan M, Northam CN, Alton TB, Sonntag WE, et al. Effect of locally delivered IGF-1 on nerve regeneration during aging: an experimental study in rats. Muscle Nerve 2010;41:335-41.
16. Koriyama Y, Homma K, Sugitani K, Higuchi Y, Matsukawa T, Murayama D, et al. Upregulation of IGF-I in the

goldfish retinal ganglion cells during the early stage of optic nerve regeneration. Neurochem Int 2007;50:749-56.
17. Ohta M, Suzuki Y, Chou H, Ishikawa N, Suzuki S, Tanihara M, et al. Novel heparin/alginate gel combined with basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve. J Biomed Mater Res A 2004;71:661-8.
18. Yao CC, Yao P, Wu H, Zha ZG. Absorbable collagen sponge combined with recombinant human basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve. J Mater Sci Mater Med 2007;18:1969-72.
19. Haas SL, Fitzner B, Jaster R, Wiercinska E, Gaitantzi H, Jesnowski R, et al. Transforming growth factor-beta induces nerve growth factor expression in pancreatic stellate cells by activation of the ALK-5 pathway. Growth Factors 2009;27:289-99.
20. Rosenstein JM, Krum JM. New roles for VEGF in nervous tissue-beyond blood vessels. Exp Neurol 2004;187:246-53.
21. Ding XG, Li SW, Zheng XM, Hu LQ, Hu WL, Luo Y. The effect of platelet-rich plasma on cavernous nerve regeneration in a rat model. Asian J Androl 2009;11:215-21.
22. Sariguney Y, Yavuzer R, Elmas C, Yenicesu I, Bolay H, Atabay K. Effect of platelet-rich plasma on peripheral nerve regeneration. J Reconstr Microsurg 2008;24:159-67.
23. Welch JA, Kraus KH, Wells MR, Blunt DG, Weremowitz J. Effect of combined administration of insulin-like growth factor and platelet-derived growth factor on the regeneration of transected and anastomosed sciatic nerve in rats. Am J Vet Res 1997;58:1033-7.
24. Piskin A, Kaplan S, Aktaş A, Ayyildiz M, Raimondo S, Aliç T, et al. Platelet gel does not improve peripheral nerve regeneration: an electrophysiological, stereological, and electron microscopic study. Microsurgery 2009;29:144-53.