

Original Article

Electrophysiological and Functional Evaluation of Peroneal Nerve Regeneration in Rabbit Following Topical Hyaluronic Acid or Tacrolimus Application After Nerve Repair

Agon Y Mekaj, Arsim A Morina, Suzana Manxhuka-Kerliu¹, Burim Neziri², Shkelzen B Duci³, Vera Kukaj⁴, Iliriana Miftari⁵

From the Clinic of Neurosurgery, Faculty of Medicine, University of Prishtina, ¹Institute of Pathology, Faculty of Medicine, University of Prishtina, ²Institute of Pathophysiology, Faculty of Medicine, University of Prishtina, ³Clinic of Plastic and Reconstructive Surgery, Faculty of Medicine, University of Prishtina, ⁴Clinic of Neurology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo, ⁵Department of Landscape Ecology and Resources Management, Justus Liebig University Giessen, Giessen, Germany

ABSTRACT

Aims and Objectives: To investigate and compare the effects of hyaluronic acid (HA), tacrolimus (FK-506) and saline on peripheral nerve regeneration *in vivo* after topical application at the site of nerve repair.

Materials and Methods: In the present study, 48 adult male European rabbits (*Oryctolagus cuniculus*), ranging in weight from 2.5 to 3 kg, were randomly assigned to three experimental groups: Group I (saline), Group II (HA) and Group III (FK-506). After transection and immediate repair of the right sciatic nerve of each rabbit, the nerve repair sites were wrapped with an absorbable gelatin sponge soaked that contained saline, HA and FK-506 in Groups I, II and III, respectively. The left hind leg was used as a control. To evaluate the effects of HA and FK-506 on nerve regeneration, electrophysiological measurements were acquired at 6 and 12 weeks after nerve repair and toe-spreading index (TSI) experiments were conducted at 4, 8 and 12 weeks after nerve repair.

Results: Motor nerve conduction velocity (MNCV) was improved in Groups II and III compared to Group I, but no differences between Groups II and III were observed. After 12 weeks, however, the MNCV in Groups I, II and III was 40.04%, 51.16% and 50.42%, respectively, of that in the control group (100%). In addition, at 12 weeks, Grade 4 TSI scores were observed in Groups II and III.

Conclusion: Electrophysiological analyses and functional evaluations based on the TSI indicate that HA and FK-506 exert similar, positive effects on nerve regeneration that are superior to those observed in response to saline treatment.

KEY WORDS: Hyaluronic acid, nerve injury, peroneal nerve regeneration, rabbits, tacrolimus

INTRODUCTION

Nerves are complex organs composed of motor and sensory neurons and are present in nearly all parts of the human body.^[1] Peripheral nerve trauma is a relatively common health problem (affecting approximately 3% of all trauma patients) and can result in significant disability and permanent functional deficits depending on the severity of injury.^[2,3] Peripheral nerves are fragile and can be easily damaged by stretching, compression, crushing, or transection.^[4] The recovery of nerve function after damage depends on many factors, including the type, site and extent of nerve injury, the age of the patient, the timing of repair after nerve injury and the surgical technique used.^[5-7] One of the main factors that prevents the regeneration of injured nerves and full recovery of function is scar formation.^[8-10] To prevent scar formation following the repair of injured peripheral nerves, pharmacological agents such as hyaluronic acid (HA) and tacrolimus (FK-506) are topically applied to sites of nerve repair. These pharmacological agents prevent scar formation by suppressing fibroblast proliferation and

promoting lymphocyte migration, granulocyte phagocytosis and macrophage motility.^[11-14]

Peripheral nerve regeneration after nerve repair can be evaluated by electrophysiological, morphological and functional methods. Electrophysiological tests provide quantitative measurements in normal and pathological states^[15] and can be invasive (insertion of needles into muscles) or non-invasive.^[16] Nerve regeneration can also be evaluated by the toe-spreading reflex, an excellent non-invasive examination method.^[17,18]

MATERIALS AND METHODS

Forty-eight adult male 8–10-month-old European rabbits (*Oryctolagus cuniculus*), ranging in weight from 2.5

Address for correspondence:

Dr. Agon Y Mekaj, E-mail: agon.mekaj@uni-pr.edu

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Mekaj AY, Morina AA, Manxhuka-Kerliu S, Neziri B, Duci SB, Kukaj V, et al. Electrophysiological and functional evaluation of peroneal nerve regeneration in rabbit following topical hyaluronic acid or tacrolimus application after nerve repair. Niger Postgrad Med J 2015;22:179-84.

Access this article online

Quick Response Code:



Website: www.npmj.org

DOI: 10.4103/1117-1936.170738

to 3 kg (mean weight of 2.8 kg), were used in the present study. Before experimentation, the rabbits were placed in separate cages and raised under conventional laboratory conditions. All procedures were performed at the Experimental Animals Breeding and Research Center. Animal care was conducted following approval by the Animal Experimental Ethics Committee of Medical Faculty, University of Prishtina, Prishtina, Kosovo (No. 1551).

Equal numbers of animals were randomly assigned to three experimental groups (16 animals in each group).

Group I – After sciatic nerve transection and repair, the site of nerve repair was wrapped with an absorbable gelatin sponge soaked (AGSS) with 0.5 ml of saline.

Group II – After sciatic nerve transection and repair, the site of nerve repair was wrapped with AGSS with 0.5 ml of HA (Orthovisc 16 mg/2 ml).

Group III – After sciatic nerve transection and repair, the site of nerve repair was wrapped with AGSS with 15 µl of FK-506 (Prograf, Astellas Pharma). From the original ampule of 5 mg/ml, two dilutions were prepared to obtain a final concentration of 10 ng/ml.^[13] The left hind leg, which was not subject to surgery, was used as a control.

SURGICAL PROCEDURE

All rabbits (48 total) were anaesthetized by intravenous injection of 30 g/L sodium pentobarbital (1 mg/kg) into the ear vein. The rabbits also received 20 mg/kg of cephazolin intravenously before surgery to prevent postoperative infection. All procedures were performed aseptically. After anaesthesia had been induced, the animals were placed in the lateral decubitus position. A longitudinal incision was made in the lower of 2/3 of the thigh. First, the right sciatic nerve was exposed using a splitting procedure to separate the semitendinosus and biceps muscles.^[19] The sciatic nerve was transected transversely approximately 2 cm proximal to the bifurcation point using a surgical blade (No. 15). After the nerve had been transected, it was immediately repaired through end-to-end neurorrhaphy with four equidistant epineural 10-0 nylon sutures (Ethicon, Inc., Somerville, NJ, USA), which were placed 1 mm from the transected site. After neurorrhaphy, the site of nerve repair was wrapped with AGSS (Spongostan®; Ethicon, Inc., Somerville, NJ, USA) with 0.5 ml of saline (Group I), HA (Group II) or FK-506 (Group III). The wounds were then closed in layers, and the rabbits were allowed to move around freely to recover from anaesthesia. All surgical procedures were performed by the same surgeon using microsurgical instruments under a loupe with a magnification of ×3.5.^[11]

ELECTROPHYSIOLOGICAL MEASUREMENTS

Electrophysiological measurements were acquired at 6 and 12 weeks after the nerve repair procedure on maximally extended hind limbs. During the electrophysiological recording, the room temperature was maintained at 24°C. Electrophysiological measurements were obtained using an electromyograph (EMG) machine.

The peroneal nerve was stimulated with a surface stimulator at the fibular head (distal to the site of nerve repair) and at the

sciatic notch (proximal to the site of nerve repair). Hair was removed from the rabbit's leg (using clippers and depilatory cream), and the recording electrodes, which were self-adhesive with gelled surfaces, were attached over the tibialis anterior muscle in a belly tendon.^[20,21] For electrical stimulation, a square pulse with a frequency of 1 Hz and a duration of 0.2 ms was used. The intensity was gradually increased to a supramaximal value to evoke the maximal compound muscle action potential (CMAP). Because the results varied, the average value obtained from five electrical stimulations was used.^[22] Three parameters of the CMAP were analysed for each rabbit: Motor nerve conduction velocity (MNCV), signal amplitude and CMAP latency. The CMAP is an EMG measure that indicates the extent of neural regeneration and represents the summation of the action potentials of all of the excited muscle fibres that respond to the nerve stimulation. The MNCV was calculated for the nerve segment between two stimulation sites by dividing the difference between the latencies by the distance between the stimulation points.^[17] The CMAP latencies were measured from the onset of the initial negative deflection, whereas the CMAP amplitudes were measured from the onset of the initial negative deflection to the negative peak.^[21]

TOE-SPREADING REFLEX

The toe-spreading reflex is a simple, repeatable, reliable, *in vivo* and non-invasive method for assessing the recovery of peroneal nerve function after injury.^[18,23] The motor function of the right hind paw and peroneal nerve regeneration, as expressed by the peroneal function index (PFI), were evaluated at 4, 8 and 12 weeks after injury using the toe-spreading index (TSI) described by Gutmann.^[23] The TSI test was performed by holding rabbits in the air by the skin on their backs. The rabbits then attempted to move forward toward the operating table but were prevented from touching the table with their feet because of their suspended position. Rabbits in which reinnervation had occurred spread their second, third and fourth toes in a reflexive manner. The motion of the toes was documented by photography and classified into four grades as described by Gutmann^[23] [Table 1].

STATISTICAL ANALYSIS

Descriptive statistics (mean and standard deviation [SD]) were used to analyse the quantitative data. Differences between the control, HA and FK-506 groups were assessed at weeks 6 and 12, and significant differences were determined using two sample *t*-test when comparing different independent samples and a paired *t*-test when comparing the same groups at different time points (weeks 6 and 12). *P* < 0.05 was considered statistically significant. The data were analyzed using SPSS Statistics 21.00 software (IBM; Chicago, IL, USA).

Table 1: PFI-the TSI for behavioral analysis

Grade	Clinical symptoms
Degree I	Just visible spreading of the 4 th toe alone (also the 2 nd and 3 rd)
Degree II	Slight spreading of all three toes
Degree III	Spreading of all three toes less forceful than normal
Degree IV	Full spreading of all three toes equal to normal

PFI: Peroneal function index, TSI: Toe-spreading index

RESULTS

In this study, electrophysiological peroneal nerve evaluations were performed in 48 rabbits between 6 and 12 weeks after transection and immediate repair of the right sciatic nerve. The non-transected left hind leg was used as the control group. Functional evaluation of the PFI was performed at 4, 8 and 12 weeks after injury using the TSI. The postoperative condition of the rabbits was good, and the rabbits did not exhibit infection or other complications.

ELECTROPHYSIOLOGICAL MEASUREMENTS

The electrophysiological recordings included measurements of MNCV (m/s) as well as the amplitude (mV) and latency of the MNCV (m/s).

For experimental Group I (saline), Group II (HA) and Group III (FK-506), the mean value and the SD of MNCV after 6 weeks of nerve repair was 16.25 ± 1.14 m/s, 19.82 ± 1.62 m/s and 20.1 ± 1.49 m/s, respectively. The mean value of the MNCV at week 12 for the three groups increased considerably to an average of 20.50 ± 1.79 m/s, 26.19 ± 1.73 m/s and 25.81 ± 1.63 m/s, respectively. The mean value of the MNCV in the control group, which was recorded only at week 6 was 51.19 ± 2.32 m/s. Differences in the means between two independent samples (the control group compared with Groups I, II or III) and paired samples (comparing measurements obtained at week 6 and week 12 in Groups I, II and III) were assessed using the *t* distribution. Highly significant differences were observed among the means for the three groups; each of the *P* values for the three paired *t*-tests was 0.000.

The average differences in the mean value of the MNCV between the control and treated groups at weeks 6 and 12 are presented in Figure 1. However, no significant differences were observed between Groups II and III at weeks 6 or 12; the *P* values for these tests were 0.614 and 0.339, respectively. The greatest differences were observed between weeks 6 and 12 for Groups I, II and III, with *P* = 0.000.

COMPOUND MUSCLE ACTION POTENTIAL AMPLITUDE

The mean CMAP amplitude 6 weeks after nerve repair was 3.4 ± 0.44 mV, 6.5 ± 0.66 mV and 6.56 ± 0.65 mV in Groups I, II and III, respectively. The mean amplitude for

Group I after week 12 doubled to 6.97 ± 0.7 mV, whereas for Groups II and III, the amplitudes were nearly 50% higher compared to the average values at week 6 with values of 9.65 ± 1.7 mV and 9.73 ± 1.23 mV, respectively. The mean amplitude of the control group at week 6 was 18.24 ± 2.81 mV, which was significantly higher and was also characterized by higher within-subject variability differences [Figure 2]. The mean amplitudes for Groups I, II and III were 5.36-, 2.8- and 2.78-fold lower, respectively, than the mean amplitude for the control group. The difference between the mean value of the control group and each of the treated groups (I, II and III) at week 12 was notably smaller with decreases of 2.61-, 1.89- and 1.87-fold, respectively. These differences were not significant because the *P* values of the independent two-sample *t*-tests between the control group (at weeks 6 and 12) and Groups II and III at weeks 6 and 12 were 0.967 and 0.842, respectively. However, highly significant differences were observed between the paired samples at weeks 6 and 12 for Groups I, II and III, with *P* = 0.000.

MOTOR NERVE CONDUCTION VELOCITY LATENCY

In Groups I, II and III, the average MNCV latency 6 weeks after nerve repair was 3.2 ± 0.18 m/s, 2.59 ± 0.25 m/s and 2.53 ± 0.045 m/s, respectively. These values slightly decreased at week 12 compared to week 6, to 2.52 ± 0.08 m/s, 1.93 ± 0.024 m/s and 1.96 ± 0.07 m/s, respectively. Compared to the three experimental groups (I, II and III), the mean latency in the control group was lower with a value of 1.51 ± 0.035 m/s [Figure 3]. The difference between the mean values of the control group and experimental Group I at week 6 was significant with *P* = 0.000. Highly significant differences were also observed for all independent two-sample *t*-tests for Groups I, II and III, with *P* = 0.000. Similar results were obtained for paired *t*-tests for Group I at weeks 6 and 12, with *P* = 0.000. No significant differences were observed between Groups II and III when they were compared as independent samples at weeks 6 and 12 (*P* = 0.345 and *P* = 0.146, respectively).

The electrophysiological results are presented in Table 2 and include the mean MNCV and CMAP amplitudes. The mean values of these same parameters are also presented as a percentage of the mean values of the control group; for each parameter, the value of the control group was designated as

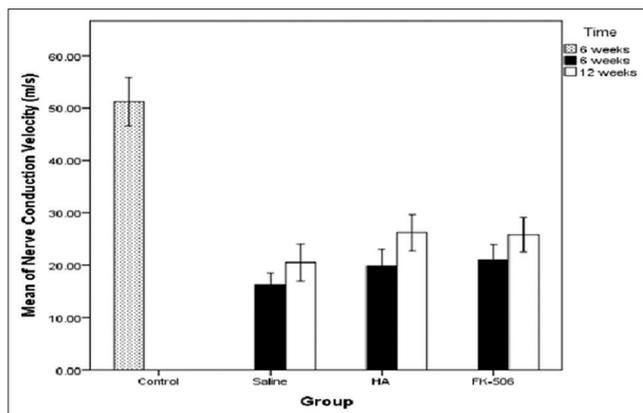


Figure 1: Group data showing the mean nerve conduction velocity (m/s) at weeks 6 and 12

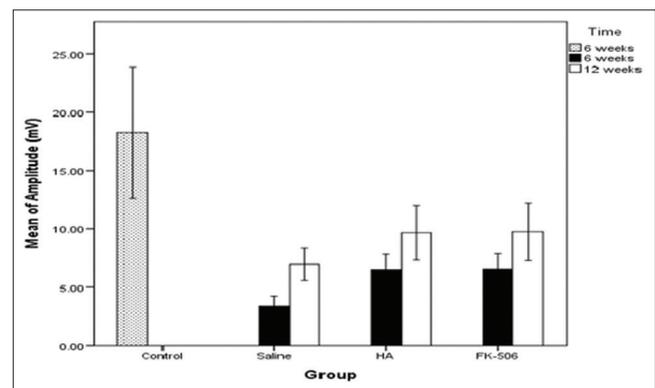


Figure 2: Group data showing the mean amplitude (mV) at weeks 6 and 12

100%. The average MNCV for the control group after 6 weeks was 51.19 m/s (100%), whereas for Groups I, II and III, the average values were 16.25 m/s (31.74%), 19.82 m/s (38.7%) and 20.1 m/s (39.3%), respectively. The average MNCV values after 12 weeks were 20.50 m/s (40.04%), 26.19 m/s (51.16%) and 25.81 m/s (50.42%) for Groups I, II and III, respectively. These results reveal that the improvement in MNCV in Groups II and III was greater than that in Group I after 6 and 12 weeks. Similarly, the nerve regeneration observed in Groups II and III was superior to that observed in Group I. At 12 weeks, amplitude increases of 52.90%, 53.34% and 38.21% relative to the control group value (set to 100%) was found in Groups II, III and I, respectively [Table 2]. Significant differences in the mean values of all parameters were identified by comparing the treatment groups with the control group and by comparing different time points within the same treatment group. Highly significant differences ($P = 0.000$) were observed in all cases, with the exception of the comparisons between Groups II and III ($P > 0.05$), as noted above.

FUNCTIONAL EVALUATION

Assessments of right hind paw motor function and peroneal nerve regeneration were performed based on the PFI [Table 1] at 4, 8 and 12 weeks after nerve repair using the TSI. After

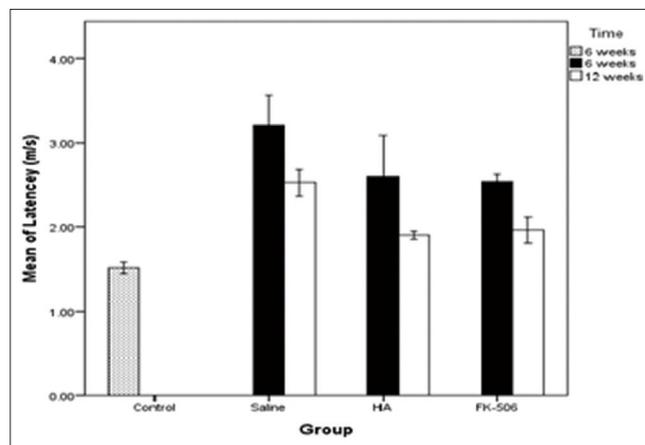


Figure 3: Group data showing the mean latency (m/s) at weeks 6 and 12

4 weeks, the ratio of Grade 1 cases to Grade 2 cases was 14:2, 13:3 and 13:3 for Groups I, II and III, respectively. At week 8, no Grade 1 cases were observed; however, the TSI scores gradually increased in all groups so that the ratio of Grade 2 to Grade 3 cases was 11:5, 9:7 and 10:6 for Groups I, II and III, respectively. Finally, after 12 weeks, equal numbers of Grades 3 and 4 cases (eight each) were observed in Group I, whereas the ratio of Grade 3 to Grade 4 cases was 3:13 and 4:12 for Groups II and III, respectively [Table 3].

DISCUSSION

Peripheral nerve trauma is a relatively common health problem and is often complicated by scar tissue formation around microsurgical repair sites. Numerous studies in animal models have sought to improve operative techniques for nerve repair to prevent scar formation and enhance nerve regeneration through the use of different pharmacological agents.^[9-13,24] HA and FK-506 prevent perineural scar formation and peripheral nerve adhesion when they are topically applied at the site of nerve repair.^[11-14] These effects of HA and FK-506 on scar formation may occur through the suppression of fibroblast proliferation and the promotion of lymphocyte migration, granulocyte phagocytosis and macrophage motility.^[11-15,25] Konofaos and Terzis demonstrated that FK-506 exhibits neurotrophic effects in experimental models; these effects are mediated by neurite elongation and the enhancement of nerve regeneration both *in vitro* and *in vivo*.^[26] However in most animal studies, pharmacological agents are applied locally following various types of surgeries. We previously summarized several experimental studies that demonstrated the ability of these agents to prevent failed back surgery syndrome.^[27] In addition to other methods (such as macroscopic, histomorphometric and immunohistochemical methods), post-operative success and the rate of nerve regeneration in the experimental animals were evaluated using electrophysiological measurements and by assessing functional recovery.^[11,18,21,22,25,28]

In the present study, we used an electrophysiological approach to evaluate the function of the peroneal nerve after topical application of the pharmacological agents HA and FK-506. This is the first study to indirectly evaluate (through

Table 2: Average MNCV and MNCV amplitude in the experimental groups and their percentages of the value of the control group

Data	Control group	Saline group		HA group		FK-506 group	
	6 weeks	6 weeks	12 weeks	6 weeks	12 weeks	6 weeks	12 weeks
MNCV (m/s) (%)	51.19 (100)	16.25 (31.74)	20.50 (40.04)	19.82 (38.7)	26.19 (51.16)	20.1 (39.3)	25.81 (50.42)
Amplitude (mV) (%)	18.24 (100)	3.4 (18.64)	6.97 (38.21)	6.5 (35.63)	9.65 (52.90)	6.56 (35.96)	9.73 (53.34)

MNCV: Motor nerve conduction velocity, HA: Hyaluronic acid, FK-506: Tacrolimus

Table 3: The time course of motor function recovery assessed by TSI

Grade	Saline group			HA group			FK-506 group		
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks
1	14	0	0	13	0	0	13	0	0
2	2	11	0	3	9	0	3	10	0
3	0	5	8	0	7	3	0	6	4
4	0	0	8	0	0	13	0	0	12

HA: Hyaluronic acid, FK-506: Tacrolimus, TSI: Toe-spreading index

electrophysiological and functional parameters) the ability of HA and FK-506 to prevent scar formation and enhance nerve regeneration. It is also the first to compare the effects of HA and FK-506.

For the electrophysiological analyses, we recorded the MNCV (m/s) as well as the amplitude (mV) and latency of the CMAP (m/s) postoperatively at two different time points (weeks 6 and 12), whereas functional evaluations were performed using the TSI at 4, 8 and 12 weeks after nerve repair. The results of our study are similar to those of other studies that have demonstrated the positive effects of HA^[11,12,22,25,29] and FK-506^[30-32] on peripheral nerve regeneration. For rats at weeks 12, Ozgenel found better mean conduction velocities (MCVs) and faster functional recovery in HA-treated nerves (0.82 ± 0.08 m/s) compared with nerves treated with saline, in which the MCV was 0.76 ± 0.04 m/s ($P < 0.05$).^[11] In addition, Zor *et al.* found significantly less scar formation ($P < 0.001$) and significantly higher peak amplitudes in rats ($P < 0.001$) that received a combination of two treatments (vascular endothelial growth factor gene therapy with HA).^[12] Other authors have demonstrated that a single topical application of HA (without silicone tubes)^[25] or the use of silicone tubes filled with HA after end-to-end cut nerve repair can positively affect CMAP amplitude and latency.^[22] In addition, Wang *et al.* found increased conduction velocities in the HA group compared with control animals ($P = 0.006$).^[29]

Studies using the rat sciatic nerve defect model in which rats were treated with FK-506 via releasing guides demonstrated that more motor functional reinnervation occurred in the treated rats compared to control rats. In addition, the amplitude and velocity of the CMAP reached 60% and 73% of the control values, respectively.^[32] Diaz *et al.* found that topical application of FK-506 at the time of facial nerve repair via entubulation neuroorrhaphy produced superior nerve regeneration results compared to the delivery of a carrier protein via entubulation neuroorrhaphy. However, postoperative spontaneous movement occurred earlier in the FK-506 group than in the other groups, but the electrophysiological results in this group were largely similar to those in the group that received entubulation neuroorrhaphy without pharmacological agents.^[31] Udina *et al.* showed that normal electrophysiological waves were recorded for 12 weeks after local application of the last dose of FK-506; normal electrophysiological responses is a valuable indicator of significant improvement in the onset and degree of reinnervation.^[33]

Other tests for functional evaluation are available. The sciatic function index is one of the best measures for evaluating the functional recovery in rats.^[11,13,24] In rabbits, the toe-spreading reflex has been reported to be a sensitive indicator of motor function during motor recovery in peroneal nerve-dependent muscles.^[18] The PFI was used to assess motor function recovery at 4, 8 and 12 weeks after injury using the TSI described by Gutmann.^[23] In the three groups in our study, motor function recovery was first observed 8 weeks after nerve repair (Grades 2 and 3), but at 12 weeks, Grade 4 was most prominent in Group II (81.25%) and Group III (75%).

TSI has been used to evaluate the functional restoration following nerve repair using other materials (silicone tubes) or other methods (without the application of chemical substances). Yamasaki *et al.* applied a silicone tube around the sciatic nerve lesion site and observed an increase in the TSI score at 6 weeks postinjury, whereas at 8 weeks, Grade 4 TSI scores were observed in 4 of 6 rabbits.^[16] In the study conducted by Beer *et al.*, in which no chemical substances were used at the site of nerve repair, positive TSI was observed in all rabbits 11 weeks after the peroneal nerve was transected and sutured in different locations.^[28] However, several studies have shown that topical application of FK-506 could accelerate the functional recovery of nerves after their repair.^[13,30]

The small number of studies that have electrophysiologically and functionally evaluated peroneal nerve regeneration after nerve repair following topical application of HA or FK-506 limits the interpretation of our results. However, our electrophysiological measurements and functional evaluations indirectly indicated that the effects of HA and FK-506 on nerve regeneration are similar, but these results must be confirmed using macroscopic, histomorphometric and immunohistochemical methods.

CONCLUSION

HA and FK-506 can be successfully applied not only inside conduits but also at the site of nerve repair without nerve defects. Our results demonstrated that topical application of HA and FK-506 equally and effectively enhanced nerve regeneration. Electrophysiological analysis is a suitable method for evaluating the success of nerve regeneration, particularly when it is performed non-invasively, which enables measurements to be repeated over a long period of time. In addition, the TSI is an appropriate, simple and non-invasive indicator of motor function recovery. However, to more comprehensively evaluate the effects of HA and FK-506 on nerve regeneration, additional studies using macroscopic, morphometric, immunohistochemical and biomechanical approaches are needed.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Geuna S, Tos P, Titolo P, Ciclamini D, Beningo T, Battiston B. Update on nerve repair by biological tubulization. *J Brachial Plex Peripher Nerve Inj* 2014;9:3.
2. Robinson LR. Traumatic injury to peripheral nerves. *Muscle Nerve* 2000;23:863-73.
3. Noble J, Munro CA, Prasad VS, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma* 1998;45:116-22.
4. Fex Svenningsen A, Dahlin LB. Repair of the peripheral nerve-remyelination that works. *Brain Sci* 2013;3:1182-97.
5. Sunderland S. Factors influencing the course of regeneration and the quality of the recovery after nerve suture. *Brain* 1952;75:19-54.

6. Woodhall B, Beebe GW. *Peripheral Nerve Regeneration: A Follow-up Study of 3,656 World War II Injuries*. Washington, DC, USA: US Government Printing Office; 1956.
7. Matsuyama T, Mackay M, Midha R. Peripheral nerve repair and grafting techniques: A review. *Neurol Med Chir (Tokyo)* 2000;40:187-99.
8. Reyes O, Sosa I, Kuffler DP. Promoting neurological recovery following a traumatic peripheral nerve injury. *P R Health Sci J* 2005;24:215-23.
9. Mafi P, Hindocha S, Dhital M, Saleh M. Advances of peripheral nerve repair techniques to improve hand function: A systematic review of literature. *Open Orthop J* 2012;6:60-8.
10. Atkins S, Smith KG, Loescher AR, Boissonade FM, O’Kane S, Ferguson MW, *et al.* Scarring impedes regeneration at sites of peripheral nerve repair. *Neuroreport* 2006;17:1245-9.
11. Ozgenel GY. Effects of hyaluronic acid on the peripheral nerve scarring and regeneration in rats. *Microsurgery* 2003;23:575-81.
12. Zor F, Deveci M, Kilic A, Ozdag MF, Kurt B, Sengezer M, *et al.* Effect of VEGF gene therapy and hyaluronic acid film sheath on peripheral nerve regeneration. *Microsurgery* 2014;34:209-16.
13. Azizi S, Mohammadi R, Amini K, Fallah R. Effects of topically administered FK506 on sciatic nerve regeneration and reinnervation after vein graft repair of short nerve gaps. *Neurosurg Focus* 2012;32:E5.
14. Que J, Cao Q, Sui T, Du S, Kong D, Cao X. Effect of FK506 in reducing scar formation by inducing fibroblast apoptosis after sciatic nerve injury in rats. *Cell Death Dis* 2013;4:e526.
15. Mekaj AY, Morina AA, Bytyqi CI, Mekaj YH, Duci SB. Application of topical pharmacological agents at the site of peripheral nerve injury and methods used for evaluating the success of the regenerative process. *J Orthop Surg Res* 2014;9:94.
16. Yamasaki T, Fujiwara H, Oda R, Mikami Y, Ikeda T, Nagae M, *et al.* *In vivo* evaluation of rabbit sciatic nerve regeneration with diffusion tensor imaging (DTI): Correlations with histology and behavior. *Magn Reson Imaging* 2015;33:95-101.
17. Navarro X, Udina E. Chapter 6: Methods and protocols in peripheral nerve regeneration experimental research: Part III-electrophysiological evaluation. *Int Rev Neurobiol* 2009;87:105-26.
18. Schmitz HC, Beer GM. The toe-spreading reflex of the rabbit revisited – Functional evaluation of complete peroneal nerve lesions. *Lab Anim* 2001;35:340-5.
19. Shen J, Zhou CP, Zhong XM, Guo RM, Griffith JF, Cheng LN, *et al.* MR neurography: T1 and T2 measurements in acute peripheral nerve traction injury in rabbits. *Radiology* 2010;254:729-38.
20. Henry FP, Goyal NA, David WS, Wes D, Bujold KE, Randolph MA, *et al.* Improving electrophysiologic and histologic outcomes by photochemically sealing amnion to the peripheral nerve repair site. *Surgery* 2009;145:313-21.
21. Hotson JR. Noninvasive peroneal sensory and motor nerve conduction recordings in the rabbit distal hindlimb: Feasibility, variability and neuropathy measure. *PLoS One* 2014;9:e92694.
22. Huseyinoglu N, Ozayidin I, Yayla S, Yildirim CH, Aksoy O, Kaya M, *et al.* Electrophysiological assessment of the effects of silicone tubes and hyaluronic acid on nerve regeneration in rats with sciatic neurotomy. *Kafkas Univ Vet Fak Derg* 2012;18:917-22.
23. Gutmann E. Factors affecting recovery of motor function after nerve lesions. *J Neurol Psychiatry* 1942;5:81-95.
24. Mohammadi R, Heydarian H, Amini K. Effect of local administration of cyclosporine A on peripheral nerve regeneration in a rat sciatic nerve transection model. *Chin J Traumatol* 2014;17:12-8.
25. Ikeda K, Yamauchi D, Osamura N, Hagiwara N, Tomita K. Hyaluronic acid prevents peripheral nerve adhesion. *Br J Plast Surg* 2003;56:342-7.
26. Konofaos P, Terzis JK. FK506 and nerve regeneration: Past, present, and future. *J Reconstr Microsurg* 2013;29:141-8.
27. Mekaj Y, Mekaj A. Prevention of failed back surgery syndrome with applications of different pharmacological agents: A review article. *Ther Targets Neurol Dis* 2015;2:e507.
28. Beer GM, Burg D, Zehnder A, Seifert B, Steurer M, Grimaldi H, *et al.* Functional, electrophysiologic, and morphometric evaluation of nerve regeneration from coaptation on regenerated nerve fibers: Experimental study in rabbits. *J Reconstr Microsurg* 2004;20:159-66.
29. Wang KK, Nemeth IR, Seckel BR, Chakalis-Haley DP, Swann DA, Kuo JW, *et al.* Hyaluronic acid enhances peripheral nerve regeneration *in vivo*. *Microsurgery* 1998;18:270-5.
30. Konofaos P, Burns J, Terzis JK. Effect of low-dose FK506 after contralateral C7 transfer to the musculocutaneous nerve: A study in rats. *J Reconstr Microsurg* 2010;26:225-33.
31. Diaz LM, Steele MH, Guerra AB, Aubert FE, Sloop GD, Diaz HA, *et al.* The role of topically administered FK506 (tacrolimus) at the time of facial nerve repair using entubulation neurotomy in a rabbit model. *Ann Plast Surg* 2004;52:407-13.
32. Li X, Wang W, Wei G, Wang G, Zhang W, Ma X. Immunophilin FK506 loaded in chitosan guide promotes peripheral nerve regeneration. *Biotechnol Lett* 2010;32:1333-7.
33. Udina E, Rodríguez FJ, Verdú E, Espejo M, Gold BG, Navarro X. FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogeneic Schwann cells. *Glia* 2004;47:120-9.