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Full Research Article

ELECTROMYOGRAPHIC EVALUATION OF THE EFFECTIVENESS OF ELECTROACUPUNCTURE IN EXPERIMENTAL DAMAGE OF NERVUS RADIALIS AND NERVUS ULNARIS IN RABBITS

Eren POLAT[1]*, Cihan GUNAY[1], Ahmet KAVAKLI[2], Murat GONEN[3]

[1]Fırat University, Faculty of Veterinary Medicine, Department of Surgery, Elazig, Turkey

[2]Fırat University, Faculty of Medicine, Department of Anatomy, Elazig, Turkey

[3]Fırat University, Faculty of Medicine, Department of Neurology, Elazig, Turkey

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Abstract

Many different acupuncture points and techniques have been used in the treatment of peripheral nerve injuries. It is known that alternative and complementary medicine techniques have become popular in the treatment of nerve damage in recent years. In this study, it was aimed to investigate the effectiveness of electroacupuncture in acute and chronic radial and ulnar nerve injuries. Electromyographic measurements were made to evaluate the effectiveness of electroacupuncture.

In the study, the rabbits were divided into four groups namely acute nerve injury (ANI) group, chronic nerve injury (CNI) group, positive control (PC) group and negative control (NC) group. In the ANI, CNI and PC groups, damage was created on the nervus radialis and nervus ulnaris by applying pressure for 60 seconds using a hemostatic forceps under anesthesia. No damage was created in the NC group. Fifteen sessions of electroacupuncture were applied to the rabbits in the ANI, CNI, and NC groups every other day using LI-4, LI-10, LR-3, and ST-36 electroacupuncture points. Electroacupuncture was not applied to the rabbits in the PC group. Electromyographic examinations of both forelimbs of rabbits were performed during electroacupuncture applications.

*Corresponding author – e-mail: erenpolat@firat.edu.tr

In the study, rabbits in the ANI group were measured at first amplitudes of 9.78 ± 1.66, and final amplitudes of 18.94 ± 1.83 ($p < 0.01$). Rabbits in the CNI group were measured at first amplitudes of 11.34 ± 1.52, and final amplitudes of 16.03 ± 1.35 ($p < 0.01$). However, no statistically significant difference was found between the first and last latency values for both groups of rabbits. As a result, it was determined that electroacupuncture is an effective treatment method for both acute and chronic nerve injuries, as well as being more effective in acute cases than in chronic cases.

Key Words: electroacupuncture, electromyography, rabbit, nerve, injury

INTRODUCTION

Acupuncture is a treatment method based on the stimulation of specific points on the body in various ways in order to eliminate pathological conditions such as pain and dysfunction that cause disease or to diagnose some functional disorders (Loken, 2001; Horasanlı et al., 2008; Zhao, 2008; Kavakli, 2010; Cheng, 2011). Acupuncture, one of the oldest medical sciences in the world, was mentioned for the first time in the book titled 'Huang Di Nei Jing' which was written in China's Yellow Kingdom period 4700 years ago. The first literature written in the field of veterinary medicine is the book titled " Veterinary Acupuncture Principles of Baile " written by Baile between 659-621 BC. (Imrie et al., 2001; Lindley and Cummings, 2006; Horasanlı et al., 2008; Kavakli, 2010; Cheng, 2011).

Many different techniques have been developed to stimulate acupuncture points. The technique in which acupuncture points are stimulated by electric current is electroacupuncture. The effects of electroacupuncture to improve neuronal functions, to differentiate nerve stem cells by proliferation and to inhibit neuronal apoptosis constituted the basis of this study (Llorent and Hayhoe, 2005; Panagiotus et al., 2017; Cave, 2018). The effect of electroacupuncture applications on stopping neuronal apoptosis and proliferating neural stem cells formed the basis of this study. While histopathological examinations were especially used to evaluate neuronal apoptosis, immunohistochemical examinations were used to evaluate the status of neural stem cells.

Peripheral nerve injuries are among the most frequently encountered problems in human and veterinary medicine. Although nerve injuries can mostly be shaped by traumatic and mechanical effects of the surrounding tissues; sometimes it may take shape due to ischemia, metabolic diseases, toxic problems, tumoral cases, physical phenomena (hot, cold, etc.), chemical events and radiotherapy (Shyu et al., 1993; Kahn and Line, 2007; Lorenz et al., 2011; Kavlak, 2012).

N.radialis and n.ulnaris are the most important peripheral nerves innervating the front legs. While N.radialis innervates the extensor muscles of the foreleg; n.ulnaris innervates the flexor muscles of the foreleg. In radial nerve injuries, the foot hangs downward significantly, while the elbow and wrist joints are in the flexion position and

dragging the upper face of the foot on the ground; carpal hyperextension is observed in ulnar nerve injuries (Aslanbey and Unsaldi, 1991; Yucel, 1998).

The causes of peripheral nerve injuries, the degree of damage to the nerves, and the distance between the area where the damage occurred and the cell body or effector organ are factors that affect recovery, recovery time, and the treatment procedure. In peripheral nerve injuries, medical treatment methods are used in cases where the nerve tissue maintains its integrity. In cases where the nervous tissue preserves its integrity, massage applications with alcoholic and vesicant ointments, vitamin B applications to compensate for myelin loss, and strychnine due to its stimulating effect on the nerves. It is used in drugs containing nonsteroidal anti-inflammatory and cortisone for symptomatic treatment (Yucel, 1998; Samsar and Akın, 2003; Kahn and Line, 2007). In cases where the nerve tissue loses its integrity, primarily operative treatment methods are used. In cases where the integrity of the nerve tissue is impaired, treatment options such as neuroraphy, arthrodesis, tendon transposition, nerve transplantation and amputation are preferred depending on the location and condition of the injury (Aslanbey and Unsaldi, 1991; Lorenz et al., 2011; Anatolitou et al., 2012; Armangil, 2014).

Alternative treatment options such as diathermy, faradization and acupuncture, which support both medical and operative treatment options, are also used in the treatment of peripheral nerve injury cases.

The aim of this study was to investigate the effect of electroacupuncture on the healing of acute and chronic period radial and ulnar nerve injuries. Electromyographic measurements were made to evaluate the effectiveness of electroacupuncture.

MATERIALS AND METHODS

Ethical approval. The research related to animal use complied with all the relevant national regulations and institutional policies for the care and use of animals (Firat University Animal Experiments and Ethics Committee; Protocol no: 2016/43, Decision no: 65, Dated: 23.03.2016)

Study design and creation of groups. As a material, 28 adult New Zealand rabbits (8-12 months old, both genders) obtained from Firat University Experimental Research Center were used. Rabbits were divided into 4 equal groups, 7 animals in each group. The rabbits were divided into four equal groups namely acute nerve injury (ANI) group, chronic nerve injury (CNI) group, positive control (PC) group and negative control (NC) group, each containing seven animals. Experimental damage was created on the radial and ulnar nerves of rabbits in the ANI and CNI groups and then, electroacupuncture therapy was applied. The radial and ulnar nerves of rabbits in the PC group were damaged, but electroacupuncture was not performed. No radial and ulnar nerve damages were created in rabbits in the NC group, but electroacupuncture was performed.

Damage to the radial and ulnar nerve. While creating experimental nerve injury, the rabbits in ANI, CNI, and PC groups were given 5 mg/kg xylazine hydrochloride (Rompun®; 23.32mg/mL, Bayer, Istanbul, Turkey) and 35 mg/kg ketamine hydrochloride (Ketalar®; 50 mg/mL, Eczacıbaşı, Istanbul, Turkey) intramuscularly for anesthesia. Necessary asepsis procedures were applied to the right anterior legs of the rabbits in the groups where experimental damage would be created and they were, then, placed on the operation table in the lateral position. A skin incision was made from the distal part of the articulatio humeri to the distal part of the articulatio cubiti by approaching from the medial part of the leg. The fascia was excised and anterior and medius muscles of the musculus scalenus were excluded to reveal the root of plexus brachialis. The nerves were exposed to pressure for 60 seconds using a hemostatic forceps 1 cm below the area where plexus brachialis was branching as nervus radialis and nervus ulnaris(Inoue et al.,2003; Sencar, 2007) (Figure 1). When the ANI group was formed, treatment was started immediately after the nerve damage was established. When creating the CNI group, treatment was started 30 days after the nerve damage was created. Then, hemostatic forceps were removed and the area was closed via an appropriate surgical method in accordance with the asepsis and antisepsis rules. Parenteral cephalexin therapy (Bavet Sefaleksin; 150 mg/ml; Bavet, Istanbul, Turkey) (15 mg/kg) was applied for five days and dressing was performed for the surgical wound for a week to prevent the risk of postoperative infection.

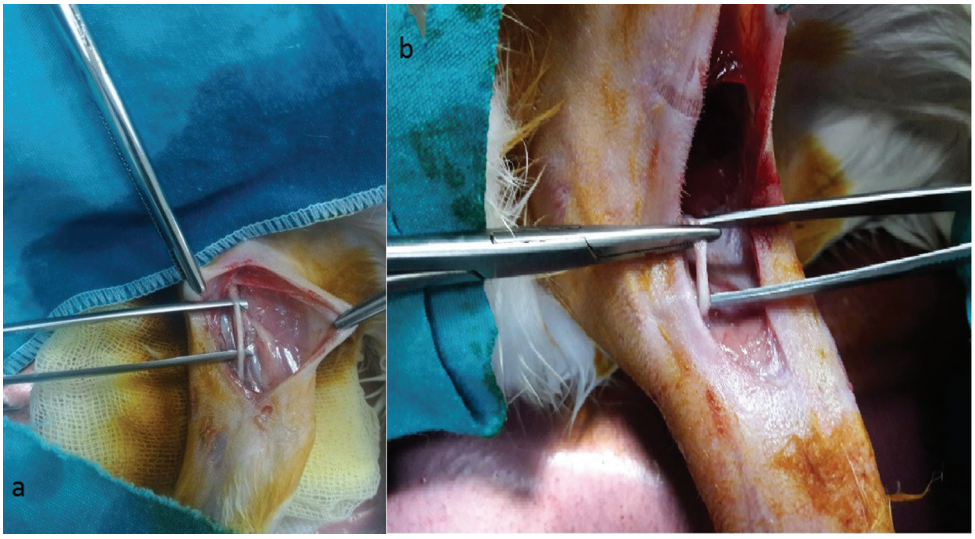

Figure 1. Nervous dissection **(a)**, Creation of nerve damage **(b)**

Electroacupuncture application. Following the experimental damages, electroacupuncture applications were started on the same day for rabbits in the ANI group and after 30

days for rabbits in the CNI group. Animals in the ANI, CNI, and NC groups received 15 sessions (30 days) of electroacupuncture every two days. For the application, LI-4, LI-10, LR-3, and ST-36 acupuncture points were used (Figure 2). Electroacupuncture was performed at 50 Hz mA and 400 μs constant current for 30 minutes (Figure 3). Rabbits were given 5 mg/kg xylazine hydrochloride intramuscularly for each session (Unsaldi, 2011).

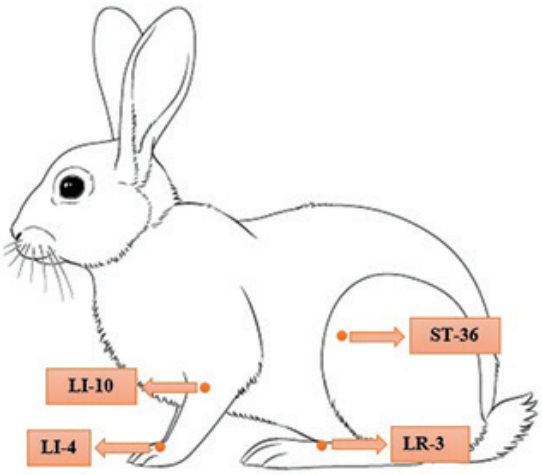

Figure 2. Electroacupuncture application points

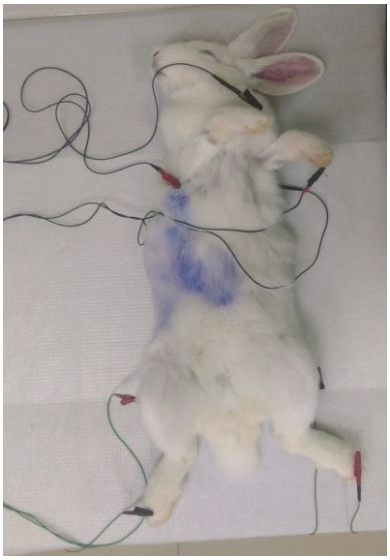

Figure 3. Electroacupuncture application

Electromyographic evaluation. After every four electroacupuncture sessions, animals in each group underwent electromyography. Before this application, rabbits were administered 5 mg/kg xylazine hydrochloride intramuscularly for sedation. In electromyographic

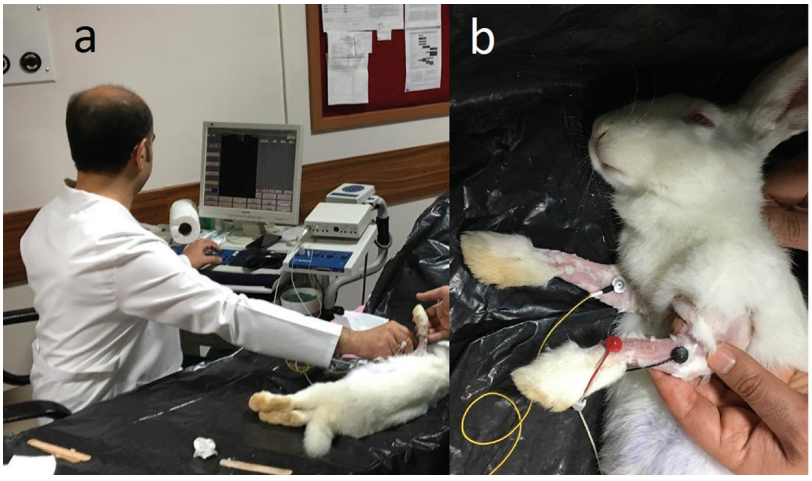

Figure 4. Electromyography application **(a,b)**

examinations, latency and amplitudes of the musculus extensor digitorum communis were measured using the specific tendon method. For this procedure, the active electrode was placed where the muscle was the most bulging, and the reference electrode was placed on the tendon of the muscle. Later, amplitudes and latencies were recorded from the medial part of both subjects (around the articulatio humeri) by giving supramaximal stimulation (Shyu et al., 1993) (Figure 4).

Statistical analysis. SPSS Ms Windows Release 21.0 program was used in statistical analysis. While evaluating electromyographic findings, one-way analysis of variance (ANOVA) was used for differences between groups. Binary comparisons between groups were determined by the Tukey post hoc test.

RESULTS

During the study, clinical examinations of the rabbits' legs with nerve damage were performed. In clinical examinations, it was observed that the rabbits in the ANI and CNI groups (treatment groups) used their nerve injured feet better than the rabbits in the PC group. The rabbits in the treatment groups were found to be in a better condition than the rabbits in the PC groups in terms of pain sensation, removing their nails, and grasping an object with their paws. When the ANI and CNI groups were compared in terms of clinical evaluations, it was found that the rabbits in the ANI group were better than the rabbits in the CNI group.

Latency and amplitude values were obtained as a result of electromyographic examinations. It was determined that there was no statistically significant difference in initial and final latencies neither within groups nor between groups. However, it was found that the average latency of the rabbits in the ANI and NC groups were more similar to each other (Table 1).

Table 1. Analysis of EMG amplitude and latency in the Outcome Assessment Schedule

	Groups				P<[1]
	ANI	**CNI**	**PC**	**NC**	
The first latency	1,44±0.10	1,41±0.08	1,58±0.08	1,30±0.05	>0.05
The last latency	1,19±0.06	1,45±0.06	1,42±0.09	1,28±0.06	>0.05
P<[2]	>0.05	>0.05	>0.05	>0.05	--
First Amplitude	9,78±1,66[b]	11,34±1,52[b]	9,43±1,99[b]	22,48±0,70[a]	0.001
Last Amplitude	18,94±1,83[ab]	16,03±1,35[b]	13,41±2,16[b]	22,56±0,74[a]	0.01
P<[2]	0.05	0.05	0.05	>0.05	--

The data are presented as mean and standard error.

[1] The differences between the groups were determined by one-way ANOVA, and binary comparisons between the groups were tested with the Tukey post hoc test.

[2] Binary comparisons were made between groups with the Wilcoxon test. (P <0.05).

[a-b:] The difference between groups carrying different letters in the same line is statistically significant (P<0.05).

When the amplitude values were examined, no statistically significant difference was found between the initial amplitudes treatment groups (ANI and CNI groups) and the PC group. The amplitudes also increased over the course of the study in both the CNI and PC groups. In addition, it was determined that the NC group had a statistically significant difference from other groups. When the final amplitude values were examined, it was found that there was an increase in the amplitudes of the rabbits in the ANI group and that there was a statistically significant difference according to the average amplitude of the rabbits in the CNI and PC groups. It was found that there was no statistically significant difference between the PC and CNI groups. Nonetheless, the mean final amplitude of the CNI group was numerically higher than that of the PC group (Table 1).

DISCUSSION

Peripheral nerve injuries are among the most common health problems in both human and veterinary medicine. When examined etiologically, it is seen that peripheral nerve injuries mostly occur due to traumas and mechanical effects of the surrounding tissues on the nerves (Yucel, 1998; Dursun, 2006; Kahn and Line, 2007; Albornoz et al., 2011; Lorenz et al., 2011; Kavlak, 2012; Nedeljkovic et al., 2017). Radial and ulnar nerve injuries were the subject of this study because it is one of the most common peripheral nerve problems among all animals, especially cats and dogs.

Although there are generally similar treatment protocols in the treatment of peripheral nerve injuries, there are differences at some points. In cases where the nerve tissue loses its integrity, first of all, operative treatment options (neuroraphy, arthrodesis, tendo transposition etc.) are applied. In the injuries that nerve tissue does not lose its integrity, medical (vesicant pomade, B vitamin application etc.) and alternative treatment options (faradization, acupuncture, diathermy etc.) are used (Aslanbey and Unsaldi, 1991; Yucel, 1998; Samsar and Akin, 2003; Kahn and Line, 2007; Anatolitou et al., 2012; Gorgul et al., 2012; Armangil, 2014; Nedeljkovic et al., 2017). Electroacupuncture is used as a supportive treatment method along with medical and operative treatments in peripheral nerve injuries due to its effects on improving neuronal functions, inhibiting neuronal apoptosis, and enhancing the proliferation and differentiation of nerve stem cells (Llorent and Hayhoe, 2005; Panagiotus et al., 2017; Cave, 2018). In this study, it was aimed to use only the electroacupuncture method in the treatment of radial and ulnar nerve tissue damage and to evaluate the effectiveness of electroacupuncture in terms of electromyographic results.

Electromyographic findings are valuable in evaluating the treatments of peripheral nerve injuries. In the study where Ho et al. (2013), performed needle and electroacupuncture using PC-3 and PC-7 acupuncture points after the rats damaged the median nerves; found that there was a positive difference in the amplitude averages obtained as a result of the electromyographic measurements of the rats in the treatment groups compared to the amplitude averages of the rats in the control group. In their study,

Ho et al. (2013) reported that there was no statistically significant difference between the groups in terms of latency averages. Zhang et al. (2013) reported that rats in the treatment group increased axonal regeneration rate in the study where rats damaged the sciatic nerves and investigated the efficacy of electrical stimuli on nerve healing. In their study, they reported that the rats in the treatment groups showed a significant improvement electromyographically within three to four weeks. In this study, in the electromyographic examination performed in the musculus extensor digitorum communis muscle, it was found that the difference between the averages of the first and last amplitude values of the rabbits in the ANI group was positively more significant than the averages of the first and last amplitude values of the rabbits in the CNI group. There was no statistically significant difference between the mean values of the last amplitude values of the rabbits in the ANI group and the mean values of the last amplitude values of the rabbits in the NC group. This shows the importance of the effectiveness of electroacupuncture on acute nerve injuries. The fact that the average of the amplitude values in the rabbits in the CNI group showed positive progress compared to the average of the amplitude values of the rabbits in the PC group, shows that electroacupuncture is also effective in chronic nerve injury cases. A similarity between this study and Ho et al. (2013), study is that the difference in latency between groups is not statistically significant. We think that the reason for this is that the leg lengths of rats and rabbits are not long enough for electromyography measurements.

CONCLUSION

In conclusion, the electromyographic results obtained in this study show that electroacupuncture is more effective in acute peripheral nerve injuries. When the mean amplitude and clinical results are evaluated, it is seen that electroacupuncture is also effective in chronic peripheral nerve injuries; however, it was found to be not as effective as in acute injuries.

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Authors' contributions

EP creation of nerve injuries, acupuncture applications, gathering data and evaluation, CG creation of nerve damage, AK consultant in electroacupuncture practices, MG electromyography applications.

Competing interests

The authors declare that they have no competing interests.

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ELEKTROMIOGRAFSKA PROCENA EFIKASNOSTI ELEKTROAKUPUNKTURE PRI EKSPERIMANTALNOM OŠTEĆENJU *NERVUS RADIALIS* I *NERVUS ULNARIS* KOD ZEČEVA

Eren POLAT, Cihan GUNAY, Ahmet KAVAKLI, Murat GONEN

Kratak sadržaj

Pri lečenju povreda perifernih nerava korišćene su različite akupunkturne tehnike i tačke. Poznato je da su tehnike alternativne i komplementarne medicine poslednjih godina postale popularne u lečenju oštećenja nerava. Cilj ovog istraživanja je bio da se ispita efikasnost elektroakupunkture kod akutnih i hroničnih povreda radijalnog i ulnarnog nerva. Efikasnost akupunkture je određena elektromiografskim merenjem.

U studiji su kunići podeljeni u četiri grupe, a to su: grupa sa akutnom povredom nerava (ANI), grupa sa hroničnom povredom nerava (CNI), grupa sa pozitivnom kontrolom (PC) i grupa sa negativnom kontrolom (NC). U grupama ANI, CNI i PC oštećenje je nastalo na *nervus radialis* i *nervus ulnaris* primenom pritiska hemostatskim forcepsom u trajanju od 60 sekundi. U NC grupi nije načinjeno oštećenje na nervima. Kunići iz ANI, CNI i NC grupe su primili svaki drugi dan ukupno 15 terapija elektroakupunktu-re na LI-4, LI-10, LR-3 i ST-36 elektroakupunkturnim tačkama. Elektroakupunktura

nije primenjivana na zečevima u PC grupi. Oba prednja uda su pregledana elektromiografski tokom terapije elektroakupunkturom.

Prve izmerene amplitude kod kunića u ANI grupi su bile 9,78 ± 1,66, a krajnje 18,94 ± 1,83 ($p < 0,01$). Kod kunića u CNI grupi prve izmerene amplitude su bile 11,34 ± 1,52, a krajnje 16,03 ± 1,35 ($p < 0,01$). Međutim, nije pronađena statistički značajna razlika između prve i poslednje vrednosti latencije za obe grupe kunića. Kao rezultat, zaključeno je da je elektroakupunktura efikasan metod lečenja i akutnih i hroničnih povreda nerava, kao i da je efikasnija u akutnim nego u hroničnim slučajevima.

Ključne reči: elektroakupunktura, elektromiografija, kunić, nerv, povrede