

Acta Oto-Laryngologica

ISSN: 0001-6489 (Print) 1651-2251 (Online) Journal homepage: https://www.tandfonline.com/loi/ioto20

The effects of lipoic acid and methylprednisolone on nerve healing in rats with facial paralysis

Emrah Tekdemir, Arzu Tatlipinar, Dilek Özbeyli, Özge Tekdemir & Emrah Kınal

To cite this article: Emrah Tekdemir, Arzu Tatlipinar, Dilek Özbeyli, Özge Tekdemir & Emrah Kınal (2018) The effects of lipoic acid and methylprednisolone on nerve healing in rats with facial paralysis, Acta Oto-Laryngologica, 138:6, 537-541, DOI: 10.1080/00016489.2017.1420914

To link to this article: https://doi.org/10.1080/00016489.2017.1420914

Published online: 08 Jan 2018.

Submit your article to this journal

Article views: 215

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ioto20

ACTA OTO-LARYNGOLOGICA, 2018
VOL. 138, NO. 6, 537–541
https://doi.org/10.1080/00016489.2017.1420914

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Check for updates

The effects of lipoic acid and methylprednisolone on nerve healing in rats with facial paralysis*

Emrah Tekdemir[a], Arzu Tatlipinar[a], Dilek Özbeyli[b], Özge Tekdemir[c] and Emrah Kınal[a]

[a]ENT Clinic, Fatih Sultan Mehmet Training and Research Hospital, Kozyatagi, Istanbul, Turkey; [b]Experimental Animal Laboratory, Marmara University School of Medicine, Uskudar, Istanbul, Turkey; [c]Internal Medicine Clinic, Haydarpasa Numune Training and Research Hospital, Uskudar, Istanbul, Turkey

ABSTRACT

Objective: To investigate the effects of lipoic acid and methylprednisolone on nerve healing in rats with traumatic facial paralysis.

Materials and methods: The rats were randomly divided into four groups, with six rats in the control group and eight each in the remaining three groups. The buccal branch of the facial nerve in all groups except the control group was traumatized by a vascular clamp for 40 minutes. Group 1 was given lipoic acid (LA), Group 2 was given methylprednisolone (MP), and Group 3 was given lipoic acid and methylprednisolone (LA + MP) for one week. Nerve stimulus thresholds were measured before trauma, after trauma and at the end of the one week treatment period.

Results: When the groups were compared with each other, post-treatment threshold levels of LA + MP were significantly lower than LA. Although post-treatment threshold levels of LA and MP were still higher than the control group, there was no significant difference between LA + MP and control values ($p > .05$).

Conclusion: Lipoic acid has a positive effect on nerve healing and can enhance the effect of methylprednisolone treatment. It is a good alternative in cases where methylprednisolone cannot be used.

ARTICLE HISTORY
Received 31 October 2017
Revised 9 December 2017
Accepted 18 December 2017

KEYWORDS
Facial paralysis; lipoic acid; trauma; methylprednisolone

Introduction

The facial nerve controls the muscles that permit movement (and in particular those muscles which convey expressions) of the face. Facial nerve pathology commonly results in an enhanced asymmetry of the face, a decrease in the patient's ability to communicate emotion, and may result in temporary or permanent paralysis.

The second leading cause of facial paralysis is trauma, following idiopathic facial paralysis (Bell Palsy). The course of the facial nerve is complex with intracranial, intratemporal, and extratemporal segments. The nerve is at risk for injury along any of these segments from external, surgical, or iatrogenic trauma [1,2]. Management of facial nerve trauma is often challenging as spontaneous recovery cannot be predicted and is often unsatisfactory without intervention.

The most commonly employed facial nerve monitoring systems at present are electromyography (EMG) systems. EMG uses facial muscle response as an indicator of facial nerve continuity and activity.

Both pharmacological and surgical treatments are designed to reduce the likelihood of residual facial dysfunction in patients with acute facial paralysis. The most commonly preferred pharmacological treatment is corticosteroids due to its effectiveness. However, in some cases, corticosteroids cannot be used due to their side effects. Therefore, there are many studies investigating the effects of different treatment agents to be used for the treatments of facial nerve paralysis [3].

Lipoic acid (LA) is a natural substance found in certain foods, and it is also synthesized in the human body [4]. Due to its antioxidant properties, it prevents the generation of free oxygen radicals and thus cell death by inhibiting lipid peroxidation and production of reactive oxygen radicals [4]. In the literature, the neuroprotective effect of LA on the central nervous system and peripheral nerves is mentioned, but its effect on nerve healing in traumatic facial nerve paralysis has not been noted [5].

In this study, the effect of LA and methylprednisolone treatment on nerve healing was investigated by using the EMG monitoring system.

Methods

The study was approved by Marmara University Local Ethics Committee for Animal Experiments and performed at the Marmara University School of Medicine, Experimental Animal Laboratory, Istanbul, Turkey.

Thirty healthy male Wistar albino rats were used in the study. The rats were randomly divided into four groups to include six rats in the control group and eight rats in the

CONTACT Emrah Tekdemir dremrahtekdemir@gmail.com ENT Clinic, Fatih Sultan Mehmet Training and Research Hospital, Kozyatagi, Istanbul 34752, Turkey

*Presented at the 37th National Turkish Otolaryngology and Head & Neck Surgery Congress, Antalya, Turkey, October, 2015.

© 2018 Acta Oto-Laryngologica AB (Ltd)

remaining three groups. The same standard surgical procedures were performed in all 30 rats. The rats were anesthetized with intraperitoneal injections of ketamine at a dose of 100 mg/kg and chlorpromazine at a dose of 3 mg/kg.

The regions corresponding to the trace of the left facial nerve were shaved after administration of anesthesia and then painted with povidone–iodine. A 2 cm horizontal skin incision from the anterior part of external auditory meatus toward the level of the first whisker at the corner of the mouth was performed. The buccal branch of the facial nerve was exposed after passing the skin and subcutaneous tissues (Figure 1).

A Nerve Integrity Monitor (Medtronic NIM-Response 3.0 System) was used for electrophysiological monitoring. One of the intramuscular needle electrodes of the nerve integrity monitor was placed in the orbicularis oris muscle, and the grounding electrode was placed in the sternocleidomastoid muscle (Figure 2). Nerve stimulus thresholds of all animals in each group were determined beginning from 0.05 mA by means of nerve integrity monitoring. The intensity of the stimulus was gradually increased until contraction in the facial expression muscles and wave form on the screen of the device were observed. Then, the buccal branch of the facial nerve of the rats in all groups except the control group was traumatized by a vascular clamp for 40 minutes. After trauma, nerve stimulus thresholds were determined in these groups again from the proximal part of the traumatized section by using the same method. The incision site was sutured appropriately after surgery in all groups.

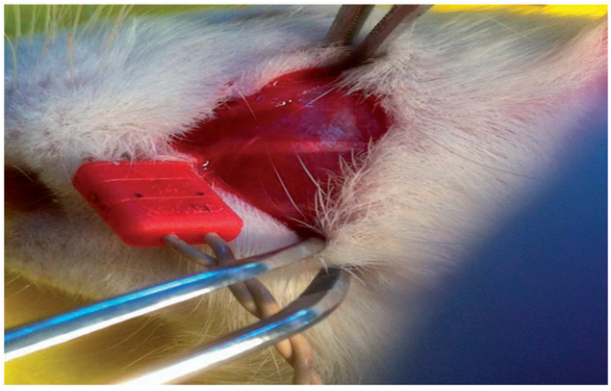


Figure 1. Buccal branch of facial nerve.



Figure 2. Placement of electrodes.

After trauma, groups were given the following agents intraperitoneally for one week: Group 1: LA at a dose of 100 mg/kg; Group 2: methylprednisolone at a dose of 1 mg/kg/day; and Group 3: LA at a dose of 100 mg/kg and methylprednisolone at a dose of 1 mg/kg/day, respectively. At the end of one week, the buccal branch of the facial nerve was found under anesthesia in the rats in all groups, and stimulus thresholds of the facial nerve were determined by means of nerve integrity monitoring using the same method. The animals were euthanized after the procedure, and the experiment was ended.

Statistical analysis

The Number Cruncher Statistical System 2007 Statistical Software program was used for the statistical analysis. A Kruskal–Wallis test was used for the comparisons of descriptive statistical methods (e.g. mean, standard deviation, median, frequency, and ratio) as well as intergroup comparisons of variables without normal distribution due to the number of subjects. The Mann–Whitney U test was used for determination of the group causing difference. A Wilcoxon signed rank test was used for in-group comparisons of the parameters. Significance was evaluated at a level of $p < .05$.

Results

Subjects in each experimental group were evaluated separately. All nerve stimulus threshold findings are shown in Figure 3 and Table 1.

When the groups were compared against each other regarding stimulus thresholds determined before trauma, no statistically significant difference was found ($p > .05$). There was also no statistically significant difference between groups regarding measurements of stimulus thresholds after trauma ($p > .05$).

No statistically significant difference was determined between the measurements of stimulus thresholds after one week compared to baseline measurements in the control group ($p = .157$; $p > .05$). There is significant difference between group 1, 2, and 3 in values before and after trauma ($p < .05$). This result shows experimental trauma caused a significant decrease in nerve response in all groups.

A statistically significant difference was determined between measurements of stimulus thresholds according to the groups after medical treatment for one week ($p < .01$). Although mean threshold levels decreased significantly after all treatment models, there was still a significant difference between pretrauma and post-treatment results ($p = .012$, $p = .011$, $p = .042$, respectively) in all three groups. These results support that LA, LA, and methylprednisolone, or methylprednisolone only had a useful effect on facial nerve

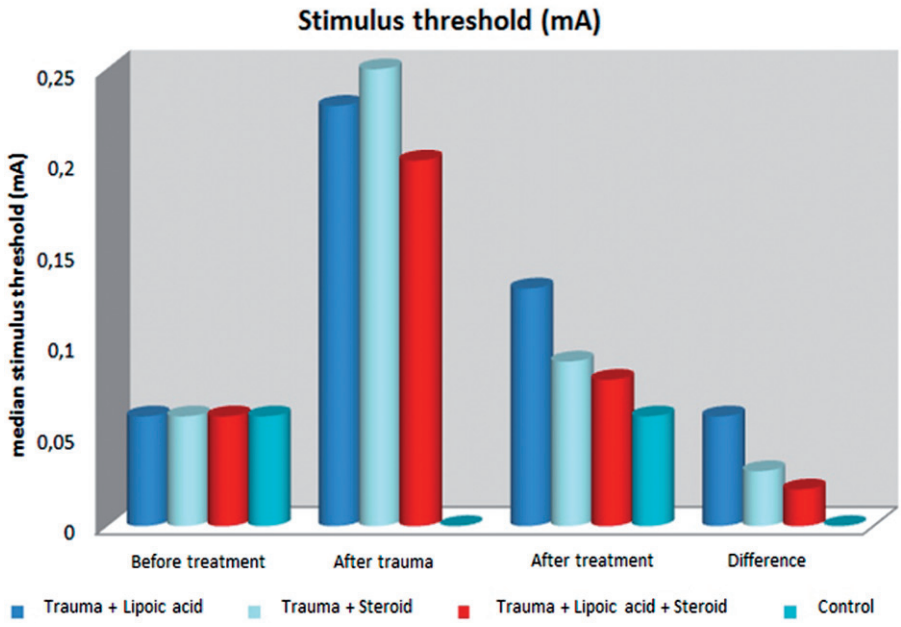


Figure 3. Distributions of measurements of stimulus thresholds according to the groups (BT: Before trauma, AT: After trauma).

Table 1. Evaluations of stimulus threshold measurements according to the groups.

	Stimulus threshold (mA)								
	BT	AT	A.Treat	Difference (A.Treat-BT)	A.Treat-BT; p^a	Difference (AT-BT)	*AT-BT; p*	Difference (AT-A.Treat)[a]	*AT-A.Treat; p*[a]
Lipoic acid									
Mean ± SD	0.06 ± 0.01	0.22 ± 0.05	0.13 ± 0.04	0.06 ± 0.04		0.16 ± 0.05		0.09 ± 0.07	
Median	0.06	0.23	0.13	0.06	***.012****	0.16	***.011****	0.10	***.018****
Min–max	0.05–0.07	0.14–0.30	0.07–0.20	0.02–0.14		0.08–0.25		0–0.23	
Steroid									
Mean ± SD	0.06 ± 0.01	0.23 ± 0.06	0.11 ± 0.05	0.05 ± 0.05		0.17 ± 0.05		0.13 ± 0.06	
Median	0.06	0.25	0.09	0.03	***.011****	0.19	***.011****	0.13	***.012****
Min–max	0.05–0.07	0.11–0.30	0.07–0.20	0.01–0.14		0.06–0.24		0.04–0.22	
Lipoic acid + steroid									
Mean ± SD	0.05 ± 0.02	0.21 ± 0.11	0.07 ± 0.03	0.02 ± 0.02		0.16 ± 0.09		0.14 ± 0.08	
Median	0.06	0.20	0.08	0.02	***.042****	0.15	***.016****	0.12	***.028****
Min–max	0–0.07	0–0.30	0–0.10	0–0.04		0-0.24		0-0.23	
Control									
Mean ± SD	0.06 ± 0.01	–	0.06 ± 0.01	0.01 ± 0.01					
Median	0.06		0.06	0.00	***.157***	–	–	–	–
Min–max	0.05–0.07		0.05–0.07	−0.01-0					
p^b	***.883***	***.947***	***.001*****	***.001*****		***.940***		***.043****	
Post hoc test; p[c]									
G1-G2	*1.000*	*0.533*	*0.365*	*0.260*		*0.505*		*0.442*	
G1-G3	*0.393*	*0.952*	***0.036****	***0.031****		*0.907*		*0.383*	
G1-G4	*0.885*	–	***0.002*****	***0.002*****		–		–	
G2-G3	*0.393*	*0.858*	*0.197*	*0.288*		*0.860*		*0.685*	
G2-G4	*0.885*	–	***0.003*****	***0.002*****		–		–	
G3-G4	*0.389*	–	*0.081*	***0.010****		–		–	

[a]Wilcoxon Signed Rank test.
[b]Kruskal–Wallis test.
[c]Mann–Whitney *U* test.
*$p < .05$.
**$p < .01$.
BT: Before trauma; AT: After trauma; A.Treat: After treatment.

injury. However, no treatment modality could achieve pre-traumatic levels.

On the other hand, while post-treatment threshold levels in G1 and G2 were still higher than control group **(G4)** values ($p < .05$), there was no significant difference between G3 and G4 ($p > .05$). When the groups were compared with each other, post-treatment threshold levels of G3 were significantly lower than G1. These results show an additional beneficial effect of the LA and methylprednisolone combination over other treatment models.

Discussion

The facial nerve is responsible for motor function of the muscles of face, which are important to express emotion.

For this reason, its function is important for facial esthetics. This nerve also has important functions for hearing, taste sensation, and saliva production. However, facial nerve paralysis is very common due to various etiologies [2].

The approach with a patient who has traumatic facial paralysis should be multifaceted and systematic. Estimation of the lesion site and a prognosis should be done first. Indicators for poor prognosis include complete facial palsy and comorbid trauma.

Both pharmacological and surgical treatments are designed to reduce the likelihood of residual facial dysfunction in patients with acute facial paralysis.

Many medical agents are used for the treatment of traumatic facial paralysis [3]. Corticosteroids are the most frequently preferred group among these agents. Many studies note that corticosteroids reduce edema around the facial nerve.

Edema-induced compression on the nerve, reduce axonal degeneration, increase axonal regeneration, prevent lipid peroxidation by increasing capillary permeability; suppress fibrin deposition, cellular migration and phagocytosis, and prevent the development of fibrosis [6].

It has been shown that the rate of complete recovery in facial functions of patients with Bell's palsy at 3 or 9 months with steroid treatment initiated within the first 72 hours after the onset of symptoms was significantly higher compared to placebo [6]. In a similar study, patients given high-dose intravenous prednisolone with vitamin support within the first 72 hours after the onset of symptoms were compared with the placebo group receiving vitamin support alone, and it was reported that facial recovery was better in the group receiving steroids [7].

Yet another study observed that a normal dose of methylprednisolone was efficient in traumatic facial paralysis, but this effect increased with a high dose, and recovery was provided in a short period of time [8].

In addition to these favorable effects of corticosteroids, side effects such as hyperglycemia can occur, especially in diabetic patients. Corticosteroids can also elevate blood pressure in patients with hypertension. There are also known side effects on gastrointestinal system causing difficulty in usage of these agents for patients.

Reactive oxygen radicals generated from free fatty acids in cell membrane due to post-traumatic oxidative stress are believed to have an important role in neuronal cell death. Antioxidant agents like vitamin E prevent formation of free oxygen radicals and thus cell death by inhibiting lipid peroxidation and production of reactive oxygen radicals [9].

Lipoic acid is a natural substance found in certain foods, and it is also synthesized in the human body. It is found abundantly in plant and animal tissues and has many mitochondrial complexes. Spinach, broccoli, and tomatoes are vegetables with the highest concentrations of LA. The heart, kidney, and liver organs are the animal tissues that have the highest concentrations of LA [10].

The antioxidant effects of LA were shown in both *in vitro* and *in vivo* studies [3]. Lipoic acid is effective against hydrogen peroxide and oxygen radicals. Lipoic acid removes the heavy metals in the biological systems by forming stable compounds with transition metals such as Mn^{+2}, Cu^{+2}, Zn^{+2}, and Pb^{+2} [11]. In addition to its own antioxidant effects, LA regenerates other antioxidants and increases their intracellular levels [5].

Besides these effects, LA reduces blood glucose concentration by increasing the translocation of GLUT4 glucose transporters to the plasma membrane and enabling glucose entry into the muscle cells [12]. Furthermore, it has been shown that LA provides neuronal survival by activating the protein kinase B-dependent signaling pathway [13]. Nitric oxide (NO)-mediated vasodilation occurs through this pathway.

Although LA is indicated for diabetic neuropathy in clinical practice [14], LA supplementation is also studied in various human diseases [15]. These diseases include reducing the blood glucose concentration in diabetes, vascular diseases, metabolic syndrome, multiple sclerosis, Alzheimer's disease, and dementia. It has been shown in animal studies that LA was useful against ischemia-reperfusion injury, cataract formation, neurodegeneration, aging, colitis, radiation injury, and miscellaneous drug toxicities (e.g. adriamycin, cyclophosphamide, cyclosporine A) [16]. Again, the neuroprotective effects of LA were shown in different experimental models in the literature [17,18]. There are studies indicating its neuroprotective effects especially on the sciatic nerve after spinal cord injury [18].

Currently, there is no study to our knowledge using LA among alternative treatment for the treatment of facial nerve paralysis. Given the availability of LA in diabetic patients, in whom it has a blood glucose lowering effect, it is important to investigate the efficiency of this agent in the treatment of facial nerve paralysis. To our knowledge, our study is the first one of its kind in the literature. We have shown that LA could be an alternative treatment in patients with facial nerve paralysis in whom steroid use might pose a problem.

Different outcome measures such as behavioral, electrophysiologic, and histomorphometric evaluations can be used to report the effect of interventions on facial nerve recovery [19,20]. In our study, the efficiencies of methylprednisolone and LA on the facial nerve healing in the experimental animals with traumatic facial paralysis were evaluated electrophysiologically. The nerve stimulator can be used to confirm nerve continuity and give prognostic information regarding the functional status of the nerve. Stimulus thresholds of the nerve with injury were found to be higher compared to the nerve without injury ($p < .05$, Table 1). This finding shows that the trauma model is successful. At the end of one week of treatment, mean nerve stimulus thresholds of the animals in the groups receiving treatment (Group 1, 2, 3) were found to be higher compared to the control group. The rate of reduction in stimulus thresholds in G1, 2, and 3 after treatment compared to trauma was found to be statistically significant. Although complete recovery cannot be provided in each of three treatments, these two results reveal that treatments are effective in nerve healing.

Determination of the presence of a statistical difference between the values obtained after treatment with LA compared to the values after trauma showed that LA was effective in the treatment of traumatic facial paralysis. In addition, absence of a difference between stimulus

thresholds of the groups given a steroid and LA obtained in the measurements performed after treatment have found that LA might be successful at a rate that can catch up to the effects of steroid treatment.

Although there was no significant difference between post-treatment stimulus values of G2 and G3; G3 findings approached pretrauma values much more compared to the group receiving the G2. In addition, in contrast to the G2 and G1 groups, there was no difference between the G3 and G4. These results support that a combined treatment model of LA and steroid was the most successful one between groups.

In addition to its antioxidant effects and increasing effects on nerve regeneration, LA lowers blood glucose concentration [5]. Lipoic acid has an indication for use in patients with diabetic polyneuropathy. Since one of the most well-known side effects of steroids is hyperglycemia, our study suggests the idea that the use of LA for the treatment of facial paralysis in patients with diabetes mellitus (DM) may be suggested.

Although follow up period of our study is short, we believe that our results are important to support LA as a potential new treatment modality for facial nerve paralysis. New studies that use more than one outcome measure and longer follow up period may be useful to get further information about LA as a treatment choice.

Conclusions

Although corticosteroids are the most commonly used medical agents in patients with traumatic facial paralysis, it has many known side effects that cause limitation of its use. Due to the blood glucose increasing effect that steroids can have, patients with DM are the leading group that could benefit from LA.

When combined with histopathological methods and future studies, our study may contribute to development of new treatment algorithms, demonstrating there is a chance that lipoic acid could be used both as an alternative treatment in certain cases where methylprednisolone is to be avoided, and as a combination therapy with corticosteroids.

Acknowledgments

This study was conducted at Marmara University School of Medicine Experimental Animal Laboratory, Istanbul, Turkey.

Disclosure statement

None of the authors have a conflict of interest to declare.

References

[1] Cramer HB, Kartush JM. Testing facial nerve function. Otolaryngol Clin North Am. 1991;24:555–570.

[2] May M. Facial nerve paralysis. In: Paparella MM, Shumrick DA, editors. Otolaryngology. Philadelphia (PA): WB Saunders Co; 1991. p. 1097–1136.

[3] Yanilmaz M, Akduman D, Sagun ÖF, et al. The effects of aminoguanidine, methylprednisolone, and melatonin on nerve recovery in peripheral facial nerve neurorraphy. J Craniofac Surg. 2015;26:667–672.

[4] Bast A, Haenen GRMM. Lipoic acid: a multifunctional nutraceutical. In: Kramer K, Hope PP, Packer L, editors. Nutraceuticals in health and disease prevention. New York (NY): Marcel Dekker Inc; 2001. p. 113–128.

[5] Bharat S, Cochran BC, Hsu M, et al. Pre-treatment with R-lipoic acid alleviates the effects of GSH depletion in PC12 cells: implications for Parkinson's disease therapy. Neurotoxicology. 2002;23:479–486.

[6] Sullivan FM, Swan IR, Donnan PT, et al. Early treatment with prednisolone or acyclovir in Bell's palsy. N Engl J Med. 2007;357:1598–1607.

[7] Topaloglu I, Bal M. Electrophyslogic and histopathologic evaluation of normal and high dose corticosteroid treatment on nerve healing in rats with traumatic facial paralysis. Paper presented at 34th Turkish National Otorhinolaryngology Congress Book; 2012.

[8] Hoshida S, Hatano M, Furukawa M, et al. Neuroprotective effects of vitamin E on adult rat motor neurones following facial nerve avulsion. Acta Otolaryngol. 2009;129:330–336.

[9] Al-Bishri A, Dahlin L, Sunzel B, et al. Systemic betamethasone accelerates functional recovery after a crush injury tor at sciatic nerve. J Oral Maxillofac Surg. 2005;63:973–977.

[10] Myckatyn TM, Mackinnon SE. The surgical management of facial nerve injury. Clin Plast Surg. 2003;30:307–318.

[11] Suzuki YJ, Tsuchiya M, Packer L. Antioxidant activities of dihydrolipoic acid and its structural homologues. Free Radic Res Commun. 1993;18:115–122.

[12] Biewenga G, Haenen GR, Bast A. The role of lipoic acid in the treatment of diabetic polyneuropathy. Drug Metab Rev. 1997;29:1025–1054.

[13] Zhang WJ, Wei H, Hagen T, et al. Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. Proc Natl Acad Sci USA. 2007;104:4077–4082.

[14] Foster TS. Efficacy and safety of alpha-lipoic acid supplementation in the treatment of symptomatic diabetic neuropathy. Diabetes Educ. 2007;33:111–117.

[15] Bilska A, Wlodek L. Lipoic acid – the drug of the future? Pharmacol Rep. 2005;57:570–577.

[16] Glantzounis GK, Yang W, Koti RS, et al. The role of thiols in liver ischemia-reperfusion injury. Curr Pharm Des. 2006;12:2891–2901.

[17] Freitas RM. The evaluation of effects of lipoic acid on the lipid peroxidation, nitrite formation and antioxidant enzymes in the hippocampus of rats after pilocarpine-induced seizures. Neurosci Lett. 2009;455:140–144.

[18] Senoğlu M, Nacitarhan V, Kurutas EB, et al. Intraperitoneal alpha-lipoic acid to prevent neural damage after crush injury to the rat sciatic nerve. J Brachial Plex Peripher Nerve Inj. 2009;4:22.

[19] Foecking EM, Fargo KN, Coughlin LM, et al. Single session of brief electrical stimulations immediately following crush injury enhances functional recovery of rat facial nerve. J Rehabil Res Dev. 2012;49:451–458.

[20] Headlock T, Kowaleski J, Lo D, et al. Functional assessments of the rodent facial nerve: a synkinesis model. Laryngoscope. 2008;118:1744–1749.