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ARAŞTIRMA / RESEARCH ARTICLE

The effect of taurine on facial nerve regeneration in the New Zealand albino type rabbits

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Yeni Zelanda albino tipi tavşanlarda taurinin fasyal sinir rejenerasyonu üzerine etkisi

Amaç: Tavşanlarda periferik fasyal parsiyel hasarından sonra taurinin fasyal sinir rejenarasyonuna etkisini incelemektir.

Yöntem: 10 erkek Yeni Zelanda tipi tavşan fasyal sinirleri klemple 3 dakika sıkıştırılarak hasar verildi. Tavşanların sağ fasyal sinirleri kontrol grubu olarak oluşturuldu. Sağ fasyal sinir lezyonu tüm tavşanlarda klemp ile yapıldı. Elektrofizyolojik çalışmalar preoperatif, postoperatif 10. ve 45. günlerde yapıldı; amplitüd bileşik kas aksiyon potansiyeli sağ fasyal sinir için bakıldı. İki ay sonra, sol fasyal sinirler aynı yolla hasara uğratıldı ve hasardan hemen sonra günlük 100 mg iv. verildi. Preoperatif ve postoperatif 10. ve 45. günlerde aynı elektrofizyolojik çalışmalarla sol fasyal sinir incelendi.

Bulgular: Zaman ve grup açısından amplitüd ve alan olarak istatistiksel olarak anlamlı bir fark mevcut değildi (p=0.151 and 0.131 Wilks' Lambda sırasıyla amplitüd ve alan için).

Sonuç: Çalışmamızda taurinin aksonal rejenerasyona pozitif bir etkisi tespit edilememiştir.

Anahtar Sözcükler: Taurin, sinir rejenerasyonu, bileşik kas aksiyon potansiyeli.

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Abstract

Objectives: To investigate the effect of taurine on the facial nervous regeneration by applying it to rabbits intravenously after the formation of peripheral partial facial lesion.

Methods: 10 male New Zealand albino type rabbits' facial nerves were injured by grasping with a clamp for 3 minutes. Right facial nerves of rabbits were employed as control group. Right facial nerve lesion was made in all rabbits as with a clamp. Described electrophysiological studies were performed preoperatively as well as postoperatively on the 10th and 45th days, thus amplitude and area of CMAP for right facial nerves were obtained at these time intervals. Two months later, left facial nerves of rabbits were injured in the same way and immediately after the injury taurine was administered with a daily dose 100 mg intravenously. Again, electrophysiological studies were performed preoperatively and postoperatively on the 10th and 45th days and same electrophysiological parameters were obtained for left facial nerves.

Results: There was not a statistically significant interaction between time and group variables for amplitude and area measures (p=0.151 and 0.131 for Wilks' Lambda for amplitude and area respectively).

Conclusion: In our study we could not find any positive effect of taurine on axonal regeneration process after experimental facial nerve lesion.

Key Words: Taurine, nerve regeneration, compound muscle action potentials.

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Introduction

Taurine is an amino acid formally known as 2-aminoethanesulfonic acid with the chemical formula: $C_2H_7NO_3S$. It is present in high concentrations in many vertebrates. Taurine plays important role on the biological functions: including, serving as a trophic factor in the development of the central nervous system, possessing trophic properties in the retina of the goldfish and the rat, protecting the integrity of the cell membrane regulating calcium homeostasis, serving as a neurotransmitter or neuromodulator, having a neuroprotective effect.[1-3]

Taurine is an ubiquitous beta aminoacid and functions as an antioxidant and inhibitory neurotransmitter in the eye, kidney and brain. It promotes neuronal repair and regeneration. Taurine is essential for the maintenance of neuronal integrity and homeostasis.[2]

In recent years it has become clear that taurine is a very important amino acid involved in a large number of metabolic processes and can become essential under certain circumstances. Basically, its function is to facilitate the passage of sodium, potassium and possibly calcium and magnesium ions into and out of cells and to electrically stabilize the cell membranes.[1-4] In this study, the effect of taurine on the facial nervous regeneration has been investigated by applying it to rabbits intravenously after the formation of peripheral partial facial lesion.

Materials ands Methods

Preparation of taurine solution

0.2 $mol.L^{-1}$ Tris buffer (pH=7.4) was prepared as described in Geiyg.[5] Taurine solution was prepared at 100 $mg.kg^{-1}$ for i.v. injection to the rabbits. For this purpose , the certain amount of Taurine was weighed and dissolved in Tris buffer (pH=7.4) continuously stirring by magnetic stirrer at room temperature. The vials were filled with the solution under laminar flow cabinet and the Taurine solution was autoclaved at 121°C under 1 atm pressure.

Experimental animals and study design

10 male New Zealand albino type rabbits weighing 2.5-3 kg were used. For the formation of facial nerve lesion, rabbits were anesthetized with the mixture of xylazinechloride (10 $mg.kg^{-1}$; i.m) and ketamine hydrochloride (8.5 $mg.kg^{-1}$; i.m). Facial nerve was injured by grasping with a clamp for 3 minutes (Figure 1). Facial nerve of the rabbit was then released and the incision is closed with a vicryl suture. The ethic committee permission was obtained for using experimental animals.

Rabbits were anesthetized during electrophysiological studies. Facial nerves were electrically stimulated at stylomastoid foramen proximal to injury side by using surface stimulator and compound muscle action potentials (CMAP) were obtained from orbicularis oris mus-

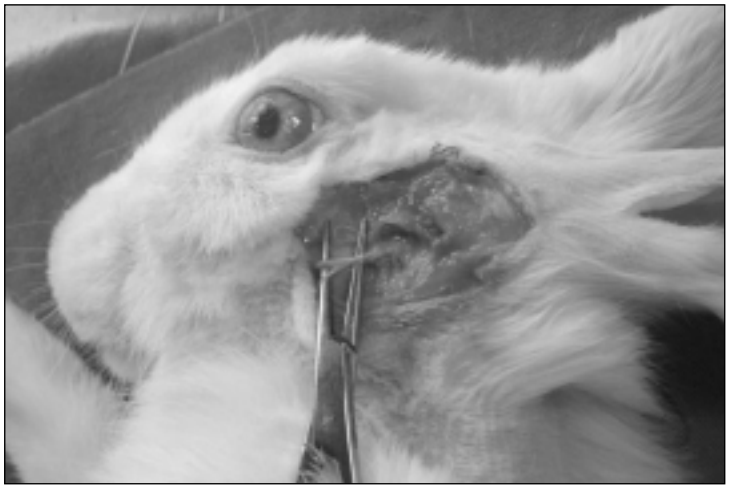

Figure 1. Rabbit's facial nerve intraoperatively.

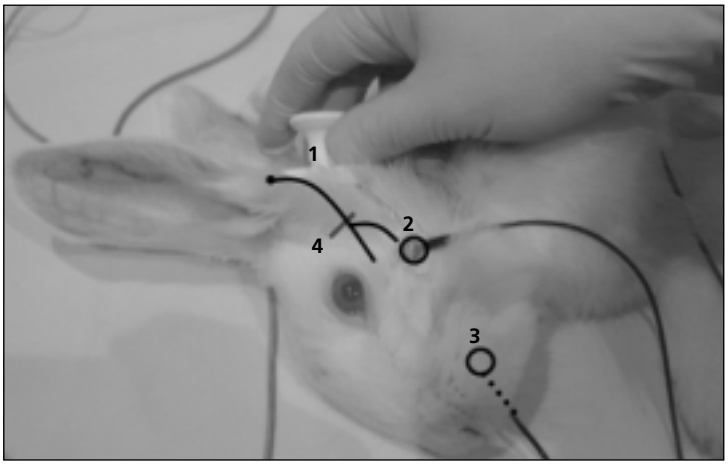

Figure 2. Electrophysiological study of facial nerve.
1. Electrical stimulator, **2.** Active recording electrode, **3.** Reference recording electrode, **4.** Line drawing of facial nerve and injury side

cle by using surface recording electrodes. Active recording electrode was placed on the midportion of the muscle belly and reference electrode on the corner of the mouth at the same side, where ground electrode was placed on the back (Figure 2). In order to achieve the appropriate impedance for surface electrodes, fur under the electrodes was shaved. Facial nerves were stimulated supramaximally by giving attention not to stimulate the neighboring nerves. Baseline-to-negative peak amplitude and area under the negative spike of the CMAP were measured. Electrophysiological studies were performed at room temperature.

In this study, right facial nerves of rabbits were served as control group. Right facial nerve lesion was made in all rabbits as described above. Described electrophysiological studies were performed preoperatively

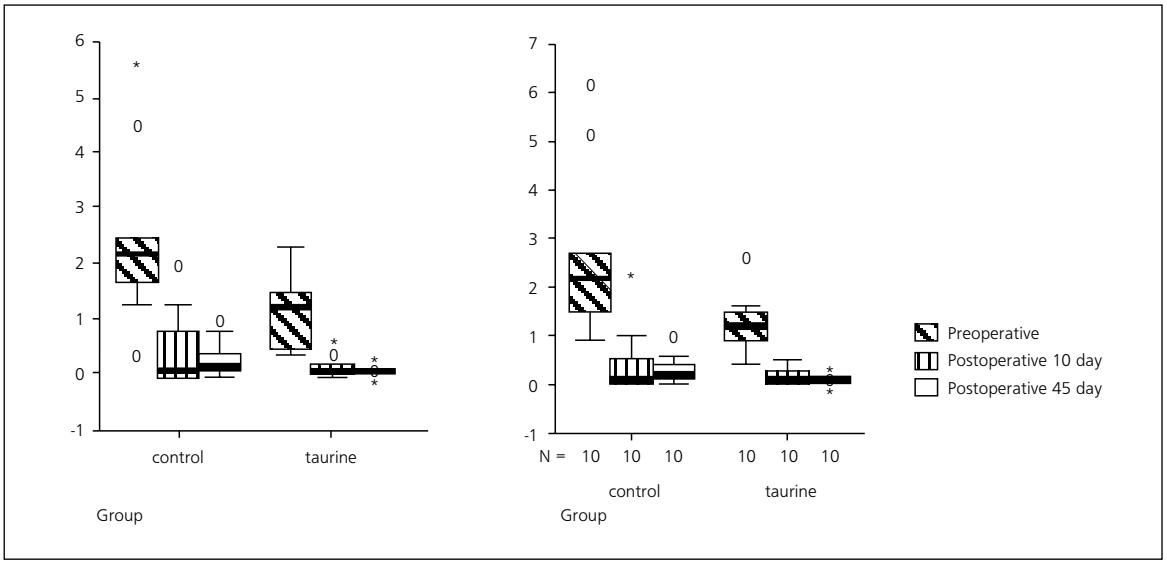

Figure 3. Boxplots for **a**) CMAP amplitudes **b**) area under the CMAPs at peroperative and postoperative 10th and 45th days.
CMAP: Compound muscle action potential.

as well as postoperatively on the 10th and 45th days, thus amplitude and area of CMAP for right facial nerves were obtained at these time points. Two months later, left facial nerves of rabbits were injured in the same way and immediately after the injury, taurine was administered with a daily dose of 100 mg intravenously. Again, electrophysiological studies were performed preoperatively and postoperatively on the 10th and 45th days and same electrophysiological parameters were obtained for left facial nerves.

Results

Both in control and taurine groups, although the amplitude and area of the CMAP obtained at 45th day were smaller than those obtained at the 10th day (Figure 3), effects of time on electrophysiological parameters at 10th and 45th days were not found statistically significant by "two-way ANOVA test for repeated measures tests" ($p=0.374$ and 0.296 for Wilks' Lambda for amplitude and area, respectively). Effects of taurine on axonal nerve regeneration process was also evaluated by comparing electrophysiological parameters of control and taurine groups (i.e., right and left facial nerves of rabbits) at postoperative the 10th and 45th days. For this purpose by using "two-way ANOVA test for repeated measures", interaction between time and group (control and taurine groups) variables were tested. There was not a statistically significant interaction between time and group variables for amplitude and area measures ($p=0.151$ and 0.131 for Wilks' Lambda for amplitude and area respectively) .

Discussion

In CNS, taurine has a protective impact against excitotoxicity of glutamate.[1,6] In the presence of 25 mM taurine alone in the medium, GES and β-alanine did not affected cultured neuronal cells and LDH release secondary to glutamate was also diminished. Briefly, the neuroprotective effect of taurine on excitotoxicity of glutamate ensources from its regulatory role on Ca. It inhibits Ca influx via reversing the mode of Na / Ca exchange channel.[1,6]

Addition of taurine to external medium results in exchange of myo-inositol levels of nerve without influencing nerve conduction velocity in diabetic animals. Diabetic neuropathy may be related to maladaptive osmoregulation, nerve damage and instability aggravated by taurine depletion.[2,6-10]

It functions as an anti-oxidant and inhibitory neurotransmitter and promotes neural repair and regeneration.[2] Sorbitol accumulation in peripheral nerves cause decrease in taurine levels.[2] Sorbitol, taurine, and myoinositol have functions as interdependent osmolytes within the peripheral nerve.

Levels of free aminoacids, eg. taurine, methylamines, betaine, and glycerophosphospatidyl choline, are interdependent and they are reciprocally depleted or accumulated in response to osmotic challenge.[2,11-13]

Taurine is determined in high amounts in brain, both in neurons and glial cells, and in peripheral nerves as well.[2] In nervous system, taurine is found as a neuromodulator and inhibitory neurotransmitter.[2] It hyperpolarizes pyramidal cells by increasing chloride influx in hippocampus.[2,14,15] It acts as a modulator of neuronal hyperexcitability, via both its hyperpolarizing effect, and inhibitory impact on calcium calmodulin dependent protein kinases.[16] It can be also said that taurine functions as an anti-oxidant.[2,8,9]

Taurine was found to be higher in fetal tissues and by aging; its levels tend to decrease. This may be an evidence due to its role in neuronal growth and development.[2,17] It could have positive role in neuronal regeneration, It was shown that in accelerated diabetes taurine level reduce in neurons and decrease regenerative capacity of diabetic peripheral nerve in diabetes.[2,18,19]

In a previous study, it was also demonstrated that duration of neuritis in isolated ganglion cells of the postcrush goldfish retina cultured for 5 days was not affected by addition of taurine.[3,20]

In diabetic rats neural taurine level decrease versus control and this condition could be prevented by dietary taurine supplementation.[7] Supplementation of taurine by diet also prevents the decrease of total and free amino acids, but not that of gluthation.[7]

Even though, anti-oxidant effect of taurine in kidney 86, lens 61, retina 67, liver and pancreas is evident, its exact mode of action could not fully be understood. Taurine depletion increases the lipid peroxidation and other manifestations of oxidative stress in the diabetic

peripheral nerve and it could be prevented with taurine supplementation due to ascorbate system of antioxidative defense.[7,21,22]

In a previous study, it was also demonstrated that duration of neuritis in isolated ganglion cells of the postcrush goldfish retina cultured for 5 days was not affected by addition of taurine.[20]

In our study we could not find any positive effect of taurine on axonal regeneration process after experimental facial nerve lesion. For electrophysiological parameters that are related to axonal regeneration, there were not any significance differences between control and taurine treated groups at postoperative 10th and 45th days. This negative result can be due to insensitivity of our electrophysiological method in detection of small changes in electrophysiological parameters (amplitude and area of CMAP) during time intervals of measurement.

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