The effects of infusion of perineural pregabalin in the experimentally created sciatic nerve anastomosis in rats

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INTRODUCTION AND OBJECT: *The aim of our study was to assess the effect of perineural pregabalin administration on the success of coaptation in experimental rat sciatic nerve anastomosis by measuring the expression of anti-inflammatory cytokine TGF-β. It is thus to provide alternative solutions to this problem which we often see in clinical practice and whose results are not satisfactory.*
METHODS: *In our study, 40 adult, male, Sprague-Dawley rats; 5 groups were randomly assigned. Group 1: This group's sciatic nerves were dissected and the surgical site was sutured. Group 2: Rats whose sciatic nerves are sectioned transversely through the full-thickness and end-to-end anastomosis is performed and no additional procedure is performed. Group 3: Intraperitoneal administration of 30 mg / kg pregabalin for 7 days with anastomosis. Group 4: 30 mg/kg pregabalin given orally for 7 days with anastomosis. Group 5: Given 10 microliters / h pregabalin subcutaneous perineural infusion for 7 days with anastomosis. After 60 days of surgery, the experiment was terminated with high dose thiopental (50 mg/kg). The right sciatic nerves of all animals were taken and sections obtained were examined immunohistopathologically.*
RESULTS: *Inflammation was significantly less in the 5th group than in the other groups. TGF-β expression in Groups 3, 4, and 5 is significantly higher than Groups 1 and 2, which also supports this situation. Although the expression in group 5 was not statistically significant, the number of TGF-β expression was higher than Groups 3 and 4. In terms of immunohistochemical properties; 1 to 3, 1 to 4, 1 to 5, 2 to 5 groups were statistically significant ($p < 0,05$).*
CONCLUSIONS: *In conclusion, perinural infusion of pregabalin into the anastomotic region has not been previously tried in the literature and it has been found that immunohistochemistry provides positive contributions to healing of anastomosis. More research is needed to demonstrate that this effect is superior to other methods of administration of the drug.*

KEY WORDS: Anastomosis, Peripheral nerve, Perineural, Pregabalin, Sciatic nerve

Introduction

Sciatic nerve damage is very common in peripheral nerve diseases. Bone fractures, skin lacerations, nerve cuts, mechanical factors such as ischemic and chemical factors may occur [1,2]. When the peripheral nerve damage occurs, the blood-nerve barrier deteriorates. Also; increased circulating macrophages, schwann cell proliferation, cytokine production and increased production of extracellular matrix components occurs [3-7]. Following tissue damage, oxidative stress is increased and the peripheral nerve damage is worsened by inflammatory mediators [8,9]. TGF-B, which is a prototype of multifunctional growth factors; it plays an important role in the nervous sys-

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tem, its continuity and the repair process [10-13]. TGF-B plays a critical role in the regulation of immune cell function by limiting the production of inflammatory mediators. Neuronal progenitor cells have neuroprotective activity. Based on previous studies, it is thought that TGF-B1 is a useful cytokine for neurons in the process following nerve injury. In this regard, neuroprotective character of TGF-B1 expression emerges [17,18].

Nerve regeneration is a complex biological phenomenon. Nerve injuries in the peripheral nervous system; they can heal spontaneously without treatment unless the integrity of the nerves is disturbed (axonotmesis). In the case of many serious traumas, end-to-end anastomosis of damaged nerve endings (ETE anastomosis) is necessary. Unfortunately; the functional consequences of nerve repair are not as desired. Therefore, research focuses on healing modalities in nerve regeneration [19-22]. Pregabalin is gamma-aminobutyric acid analog. Pregabalin inhibits the release of neurotransmitters in the central nervous system. Pregabalin has anti-convulsive, neuropathic pain associated with diabetic peripheral neuropathy, postherpetic neuralgia and fibromyalgia activity. Pregabalin also has efficacy in incisional injury and inflammatory injury, and is a drug that comes forward with its high tolerability by its ease of use [25,26].

When the relevant literature was searched, studies on enteral and parenteral use and effects of pregabalin were found in various experimental models. However, there was no other study comparing the pregabalin effect of sciatic nerve anastomosis or the classic use of enteral and parenteral use versus perineural infusion [24,26,27].

In light of this information our hypothesis is that perinoral pregabalin administration in rabbits with sciatic nerve anastomosis will increase the success of coaptation. In this study, it was aimed to evaluate the effect of pregabalin by measuring TGF-β expression which is anti-inflammatory cytokine.

Materials and Methods

Animals

This study was performed at Baskent University School of Medicine Experimental Research Center, and was approved by the local ethics committee. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (USA).

Fourty adult male Sprague-Dawley rats mean weight 300 g were randomly divided into 5 groups (n=8) as follows:

– Group I (Sham operated group): This group's sciatic nerves were dissected under general anesthesia without anastomosis and the surgical site was sutured;

– Group II: Sciatic nerves were sectioned and ETE anastomosis was performed;

– Group III (anastomosis + intraperitoneally 30 mg/kg of pregabalin): Sciatic nerves were sectioned and ETE anastomosis was performed and the animals received 30 mg/kg of pregabalin intraperitoneally every day for seven days;

– Group IV(anastomosis + orally 30 mg/kg of pregabalin): Sciatic nerves were sectioned and ETE anastomosis was performed and the animals received 30 mg/kg of pregabalin orally dissolved in 1 ml of distilled water every day for seven days;

– Group V(anastomosis + 10microliter/h of pregabalin via subcutaneous perineural infusion): Sciatic nerves were sectioned and ETE anastomosis was performed and the animals received 10microliter/h of pregabalin via subcutaneous perineural infusion for seven days.

Surgical procedure

Rats were housed at room temperature and had free access to food and tap water. They were anesthetized by intramuscular injection of 50 mg/kg ketamine (Ketalar, Pfizer) and 5 mg/kg xylazine (Rompun, Bayer). Arterial pressure and heart rate were constantly monitored; breathing was unassisted. After administration of anesthesia, the right hind limbs were prepared for surgery using a sterile technique. Skin incision was followed by dissection of the gluteal muscles in order to expose the sciatic nerve, including the tibial, peroneal, and sural branches, under a surgical microscope (Opmi 9-FC, Carl Zeiss AG, Germany).

ETE anastomosis of the proximal and distal stumps was performed after sectioning the sciatic nerve. Anastomoses were performed using 10-0 nylon sutures.

In animals where perinural pregabalin infusion is to be administered (Group 5); a horizontal incision was made about 2 cm long at the scapula level. A space of 2x2 cm was opened in this region and an osmotic micropump (Alzet, Durect Corp. Cupertino, CA) filled with 2 ml of 1% pregabalin solution was implanted. The gluteal isthmus was reached by advancing the subcutaneous tissue through a micro-bore catheter. The microcatheter was placed adjacent to the anastomotic region with subcutting tissue, gluteal nerve and fascia secured with 4-0 silk suture. The layers were closed by providing hemostasis.

Drug preparation

Pure pregabalin in powder form was obtained through ARIS as a gift. For oral and intraperitoneal administration, the purified powdered pregabalin distilled water was diluted to 10 mg at 1 ml and the solution was agitated before each use. For perineural infusion, 1% solution of pregabalin in the form of purified powder was prepared. The osmotic pump was filled with 1 ml of solu-

tion to be applied at 10 microliters / hour for 7 days. In addition, the efficacy of the osmotic micropump filled with methylene blue and the adequacy of perineural infusion were checked before starting the 2-rat study. The function of the pump was confirmed by measuring the amount of aspirated solution remaining in the pump after rats were killed [28,29].

Histological analysis and Immunohistochemistry

Sciatic nerves dissected from the rats fixed in 10% buffered formaldehide. After routine tissue process, samples were embded in parafin blocks and 4µ thick sections were taken. Sections stained with H&E, evaluted under light microscope. The presence of Schwainan cells, inflammation and axonal degeneration were evaluated and scored semiquantitatively (0: absent, 1: mild, 2: moderate, 3:severe/marked). Four micron-thick sections taken from the parafin blocks were immunhistochemically stained with anti-CACNA2D1 antibody (Abcam cat: 2864) as primary antibody and with rabbit Antimouse IgG (Abcam) as secondary antibody. The results were scored semiquantitaviley (0: absent, 1: mild, 2: moderate, 3:strong).

Molecular Investigations

Total RNA extraction and cDNA synthesis

Total RNA extraction with cDNA synthesis was isolated. The tissues were stabilized in 30 mg of RNA stabilization reagent (RNAlater, Qiagen) and disrupted with the aid of Tissue Lyser II (2 x 2 min for nerve; 2 x 5 min, Qiagen). Total RNA was purified in RNeasy Mini Kit (Qiagen).
The RNA specimens were transcribed into complementary DNA by cDNA reverse transcription kit (Applied Biosystem).
The total RNA in 10 µl was processed with 2 µl of 10xRT random primers, 2 µl of 10xbuffer of RT, 0.8 µl of 25 X dNTPs mix, 4.2 µl of DEPC-H2O and1 µl of multiscribe reverse transcriptase.
Reverse transcription was realized at 25 C for 10 minutes, after that 120 min at 37 C, and lastly 85 C for 5 minutes employing a Veriti 96 Well Thermal Cycler (Applied Biosystem).
The cDNA quality and concentration were assessed using Take 3 Plate (Biotek) and the Epoch Spectrophotometer System.

Relative quantification of gene expression

Relative TGF β1 expression investigation was carried out with Step One Plus Real Time PCR System technology (Applied Biosystem) by cDNA synthesized from rat sciatic nerve RNA.
qPCR was run by a TaqMan Probe-based technology and TaqMan Probe mix (Applied Biosystem).
Real-time PCR was carried out by primers generated for rat TGF-B1 Rn0057201 m1 and rat Bactin (forward) 53TGGTGGGTATGGGTCAGAAG (reverse) 53GACAATGCCGTGTTCAG.
The β-actin expression in tissues was used for as an endogenous control.
Probes and primers for β-actin were conceived by Primer Design, Southampton, UK.
For each tissue, the experiments were carried out in triplicate in a 96-well optical plate for both targets using 1 µl of Primer Perfect Probe mix, 9 µl of cDNA (100 ng), and 10 µl of Quanti Tect Probe PCR Master mix (Qiagen, Hilden, Germany) in each 20 µl reaction.
The plates were warmed for 2 minutes at 50 C and 10 minutes at 95 C, after that 40 cycles of 15 secinds at 94 C and 60 seconds at 60 C.

Statistical Analysis

For calculating the sample size of the control and experimental groups of our study, the power of the test for each variable was determined by taking at least 0,80 and type 1 error at 0,05. Descriptive statistics for the continuous variables obtained from the groups included in my study are expressed as median and range (minimum-maximum) values. Kruskall-Wallis H test was used for continuous (quantitative) variables (schwann H.proliferation, axonal degeneration, inflammation, immunohistochemistry, molecular results) in comparison with each other. Dunn's test was used for post hoc analysis. The statistical significance level (a) in the calculations was taken as 5% and the statistical package programs of SPSS (IBM SPSS for Windows, ver.24) were used for the calculations.

Results

The groups were compared in terms of Schwann cell proliferation, axonal degeneration, inflammation, immunohistochemistry and molecular outcomes. Groups forming the difference can be summarized as follows. For Schwann cell proliferation; the difference between group 1 and all other groups was statistically significant ($p < 0.05$). Contrary to this situation; 2, 3, 4, 5 groups were similar ($p > 0.05$) (Fig. 1).
In terms of axonal degeneration; only 1 and 5 groups were statistically different from each other ($p < 0.05$). The difference between all other groups was not significant ($p > 0.05$).
In terms of inflammation; Groups 1 and 5 were statistically different ($p < 0.05$). Similarly, the differences

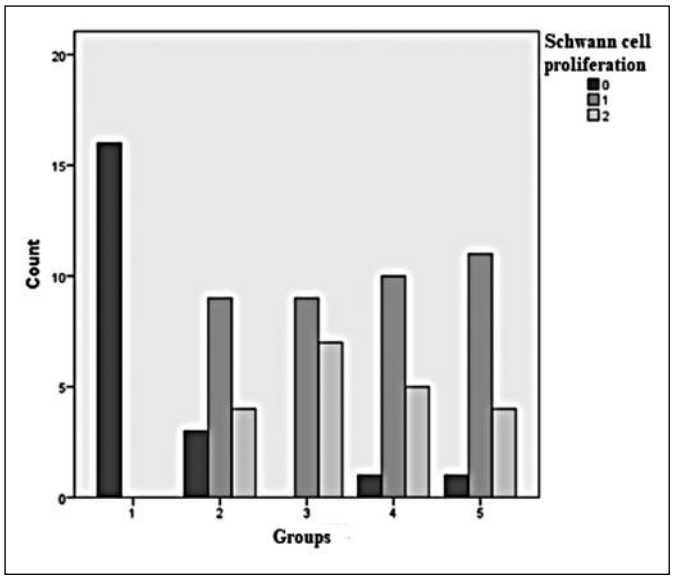

Fig. 1: Groups and overall distribution of Schwann cell proliferation is shown.

between groups 2 and 5, 3 and 5, 4 and 5 were significant ($p < 0.05$) (Fig. 1). These differences between the outside of the remaining pairwise comparisons were not significant ($p > 0.05$).
In terms of immunohistochemistry; The differences between 1 and 3, 1 and 4, 1 and 5, 2 and 5 groups were statistically significant ($p < 0,05$) (Fig. 2).These differences between the outside of the remaining pairwise comparisons were not significant ($p > 0.05$).

Discussion

Despite all the researches on neural healing, nerve healing is still not achieved at the desired level.
The main goal of treatment in peripheral nerve injuries is to restore neural conduction and replace the nerve-related end organ functions with minimal loss.
For successful nerve regeneration, steps such as axonal budding, growth, end organ reinnervation and integration of the central nervous system and regenerated fibers need to be completed.
Rats are a frequently used animal when evaluating this regeneration. The reasons for the inevitability of these nerves in rats are cheap and easy to obtain, the long course of the sciatic nerve permits easy dissection in the mid-thigh region, and has a suitable area for manipulation and nerve trunks similar to those in humans [30]. We also used the rat sciatic nerve for this reason in our study.
Experimental studies have used many drugs and techniques such as nonsteroidal antiinflammatory drugs, steroids, low dose radiotherapy and vitamins. These studies aim to improve the process of nerve regeneration and

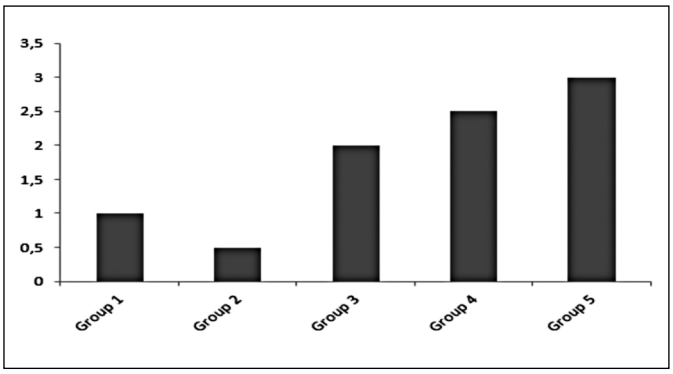

Fig. 2: Distribution of molecular results (TGF-beta1 expression)by group.

functional recovery with the help of therapeutic interventions [28,31,32]. If effective treatment can be developed to minimize the post-traumatic dysfunction, it will revolutionize the treatment of peripheral nerve diseases.
We also aimed to evaluate the effect of pregabalin in sciatic nerve incisions in rats treated with primary surgery. Pregabalin, also known as an anti-epileptic agent, is also used for the treatment of neuropathic pain, postherpetic neuralgia, and trigeminal neuralgia. In many experimental studies; neuroprotective properties of pregabalin have been shown- [24,26,29].
Pregabalin lipid peroxidation represses microglial cells and weakens cellular apoptosis of oligodendrocytes. This demonstrates that the neuroprotective effect is through the antiinflammatory effect [25].
Buys et al. have shown that administration of pregabalin by the perineural route results in more analgesic effect than systemic administration of the same dose. Carlton et al. have shown that the local peripheral injection of gabapentin dramatically reduces formalin-induced nociceptive behavior· [29,33].
We also showed that in our study, especially inflammation was much less in the 5th group than in the other groups ($p < 0.05$). If we consider that the neuroprotective property of pregabalin is through its anti-inflammatory properties; statistically, we support that perinural use of pregabalin is more neuroprotective effect than intraperitoneal and oral use of pregabalin. Another advantage of perineural administration is that the side effect is much less because it acts without reaching the central nervous system [29].
Although sciatic nerve injuries are rare in humans, another reason why this nerve is preferred in animal experiments is that it is a polyphacic mixture type nerve, it contains axons in different sizes and types and provides a comprehensive research opportunity. It allows you to evaluate both sensory and motor functions at the same time [30].
Functional evaluation is a standard metric for motor function assessment that reflects nerve function after peripheral nerve injury [34]. However, functional evaluation was not per-

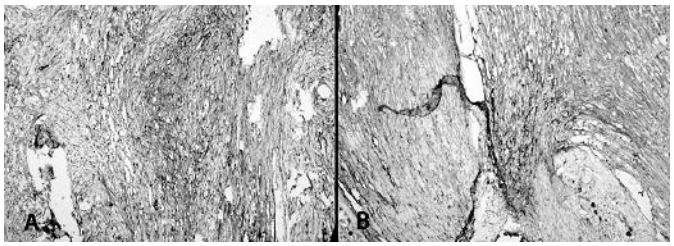

Fig. 3: In the preparations examined by H & E staining; Inflammation was evident in group 2 and 3, whereas inflammation was relatively rare in group 4 and 5. In group 5, very few inflammatory cells are observed (X20 magnification).

formed in our study because nerve regeneration and functional recovery were not completed on the 60th day [35].
Transforming growth factor-β (TGF-β) plays an important role in wound healing and repair of peripheral nerve lesion. TGF -β has three subtypes (1, 2 and 3) that have different biological functions. TGF-B1 stimulates extracellular matrix synthesis and angiogenesis. It is chemotactic to macrophages and fibroblasts [36,37].
In the rat sciatic nerve injury model, Rufer et al. evaluated nerve regeneration via TGF-β mRNA expression [36]. In cases where Schwann cells were unable to contact axons sprouting from the proximal region, an increase in TGF-β mRNA expression was also observed in distal stumps of damaged nerves. The "reactive" Schwann cell phenotype following the peripheral nerve lesion can be said to cause upregulation of TGF -β expression. Similarly, in our study, schwann cell proliferation was significantly higher in the treatment groups (Groups 3, 4, and 5) (Table I). Although there was no statistically significant difference, there were more reactive Schwann cells in the perinural pregabalin group (group 5) than in the intraperitoneal and oral groups.
These rates between the groups also coincided with the TGF expression. Similarly; group 3, 4 and 5 had significantly higher expression than the other groups, but there was no significant difference between them (Table I). However, in the 5th group, more expressions were seen than in the 3rd and 4th groups. In our study, in addition to the study of Rufer et al; It was found that even after 60 days of sciatic nerve injury, expression of TGF- B1 was greater. In previous studies in the literature; TGF-B1 levels after maximum 35 days after injury were evaluated.
More studies should be done to examine the potential of pregabalin perineural use and evaluating TGF-β1 expression in relation with peripheral nerve regeneration.

Conclusion

Perineural use of pregabalin was found to have more neuroprotective effect. If we consider that the dose-dependent side effect will be less for perineural use, the efficacy and possible side effects of higher doses should be assessed in other studies.

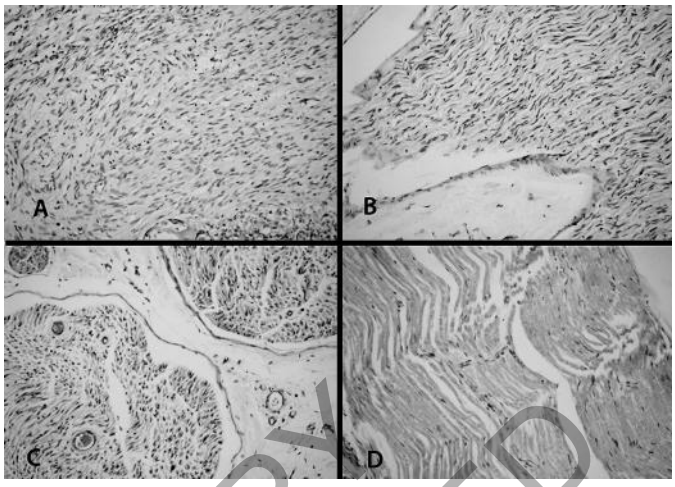

Fig. 4: Immunohistochemically, there was marked difference in staining with anti-CNCA2D1 between group 2 and group 5. (X20 magnification).

Riassunto

Lo scopo di questo nostro studio era quello di valutare l'effetto della somministrazione di pregabalin livello perineurale sul successo sperimentale del combaciamento nelle anastomosi del nervo sciatico del ratto, misurando l'espressione della citochina anti-infiammatoria TGF-β, e di conseguenza di offrire soluzioni alternative a questo problema che si incontra spesso nella pratica clinica e i cui risultati non sono soddisfacenti.
Lo studio è stato condotto su 40 ratti Sprague-Dawley adulti, di sesso maschile, suddivisi in 5 gruppi distribuiti in modo casuale.
Gruppo 1: disssezione dei nervi sciatici e successiva sutura della sede chirurgica.
Gruppo 2: sezione trasversale a tutto spessore dei nervi sciatici e successiva anastomosi termino-terminale senza nessuna procedura aggiunta.
Gruppo 3: somministrazione intraperitoneale di 30 mg / kg di pregabalin per 7 giorni dopo sezione-anastomosi
Gruppo 4: 30 mg / kg di pregabalin somministrato per via orale per 7 giorni dopo sezione-anastomosi.
Gruppo 5: somministrato 10 microlitri / h di infusione perineurale pregabalin per via sottocutanea per 7 giorni dopo sezione-anastomosi.
Dopo 60 giorni dall'intervento l'esperimento è stato interrotto con una dose elevata di tiopentale (50 mg / kg). Sono stati prelevati i nervi sciatico di destra di tutti gli animali e le sezioni istologiche preparate sono state studiate con l'immunoistochimica.
Come risultato si è osservato che l'infiammazione risultava significativamente inferiore nel 5° gruppo rispetto

agli altri gruppi. L'espressione TGF-β nei gruppi 3, 4 e 5 era significativamente più alta dei gruppi 1 e 2, che supporta anche questa situazione.
Sebbene l'espressione nel gruppo 5 non fosse statisticamente significativa, l'espressione del TGF-β era superiore rispetto ai gruppi 3 e 4.
Per quanto riguarda le proprietrà istochimiche è risultato statisticamente significativo il rapporto tra i gruppi: del 1 rispetto a 3, del 1 rispetto a 4, del 1 rispetto a 5, del 2 rispetto al 5i ($p < 0,05$).
In conclusione, l'infusione perinurale di pregabalin nella regione anastomotica, non è stata studiata precedentemente in letteratura e si è verificato con immunoistochimica che fornisce contributi positivi alla guarigione dell'anastomosi. Sono necessarie ulteriori ricerche per dimostrare che questo effetto è superiore ad altri metodi di somministrazione del farmaco.

References

1. Dahlin LB, Lundborg G: *Use of tubes in peripheral nerve repair*. Neurosurg Clin N Am, 2001; 12(2):341-52.

2. Raimondo S, Fornaro M, Tos P, Battiston B, Giacobini-Robecchi MG, Geuna S: *Perspectives in regeneration and tissue engineering of peripheral nerves.* Ann Anatomy-Anatomischer Anzeiger [Internet], 2011; 193(4):334-40. Available from: http://dx.doi.org/10.1016/j.aanat.2011.03.001

3. Olsson Y: S*tudies on Vascular Permeability in Peripheral Nerves*. Acta Neuropathol, 1966; 15(7(1):1-15.

4. Mellick RS, Cavanagh J: *Changes in blood vessel permeability during degeneration and regeneration in peripheral nerves*. Brain, 1968; 91(1):141-60.

5. Stoll G, Griffin J, CY L, Trapp B: *Wallerian degeneration in the peripheral nervous system : Participation of both Schwann cells and macrophages in myelin degradation.* J Neurocytol, 1989; 683, 18(5):671-83.

6. Taskinen H, Roytta M: *The dynamics of macrophage recruitment after nerve transection.* Acta Neuropathol, 1997; 93(3):252-59.

7. Salonen V, Aho H, Peltonen J: *Quantitation of Schwann cells and endoneurial fibroblast-like cells after experimental nerve trauma*. Acta Neuropathol, 1988; 75(4):331-36.

8. Roytta M, Salonen V: *Long-term endoneurial changes after nerve transection*. Acta Neuropathol, 1988; 76(1):35-45.

9. Siironen J, Sandberg M, VuorinenV, Roytta M: *Laminin B1 and collagen type IV gene expression in transected peripheral nerve: reinnervation compared to denervation.* J Neurochem, 1992; 59(6):2184-92.

10. Dunker N, Krieglstein K: *Targeted mutations of transforming growth factor- b genes reveal important roles in mouse development and adult homeostasis*. Eur J Biochem / FEBS, 2000; 6988(267(24)):6982.988.

11. Flanders KC, Ren RF, Lippa CF: *Transforming growth factor-betas in neurodegenerative disease*. Prog Neurobiol, 1998; 54(1):71-85.

12. Schober A, Hertel R, Arumae U, Farkas L, Jaszai J, Krieglstein K, et al.: *Glial cell line-derived neurotrophic factor rescues target-deprived sympathetic spinal cord neurons but requires transforming growth factor-beta as cofactor in vivo*. J Neurosci, 1999; 19(6):2008-15.

13. Wang X, Chen W, Liu W, Wu J, Shao Y, Zhang X: *The role of thrombospondin-1 and transforming growth factor-beta after spinal cord injury in the rat.* J Clin Neurosci Off J Neurosurg Soc Australas, 2009; 16(6):818-21.

14. Benveniste EN, Tang LP, Law RM: *Differential regulation of astrocyte TNF-alpha expression by the cytokines TGF-beta, IL-6 and IL-10*. Int J Dev Neurosci, 1995; 13(3-4):341-49.

15. Letterio JJ, Roberts AB: *TGF-beta: A critical modulator of immune cell function*. Clin Immunol Immunopathol, 1997; 84(3):244-50.

16. Rubio-Perez JM, Morillas-Ruiz JM: *A review: inflammatory process in Alzheimer's disease, role of cytokines.* Scientific World Journal, 2012; 2012:756357.

17. Doyle KP, Cekanaviciute E, Mamer LE, Buckwalter MS: *TGFbeta signaling in the brain increases with aging and signals to astrocytes and innate immune cells in the weeks after stroke.* J Neuroinflammation, 2010; 7:62.

18. Caraci F, Battaglia G, Bruno V, Bosco P, Carbonaro V, Giuffrida ML, et al.: *TGF-beta1 pathway as a new target for neuroprotection in Alzheimer's disease.* CNS Neurosci Ther, 2011; 17(4):237-49.

19. Lundborg G: *Bridging nerve defects-the role of tissue interpositioning*. Sev Trauma defects Up limb, 2004; 153.

20. Siemionow M, Brzezicki G: *Chapter 8: Current techniques and concepts in peripheral nerve repair*. Int Rev Neurobiol, 2009; 87:141-72.

21. Gordon T, Sulaiman OAR, Ladak A: *Chapter 24: Electrical stimulation for improving nerve regeneration: Where do we stand?* Int Rev Neurobiol, 2009; 87:433-44.

22. Zacchigna S, Giacca M: *Chapter 20: Gene therapy perspectives for nerve repair.* Int Rev Neurobiol, 2009; 87:381-92.

23. Goa KL, Ross SR, Chrisp P: *Lamotrigine. A review of its pharmacological properties and clinical efficacy in epilepsy*. Drugs, 1993; 46(1):152-76.

24. Kumar N, Laferriere A, Yu JSC, Leavitt A, Coderre TJ: *Evidence that pregabalin reduces neuropathic pain by inhibiting the spinal release of glutamate.* J Neurochem, 2010; 113(2):55-61.

25. Verma V, Singh N, Jaggi A: *Pregabalin in Neuropathic Pain: Evidences and Possible Mechanisms.* Curr Neuropharmacol [Internet] 2014;12(1):44–56. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1570-159X&volume=12&issue=1&spage=44.

26. Freynhagen R, Strojek K, Griesing T, Whalen E, Balkenohl M: *Efficacy of pregabalin in neuropathic pain evaluated in a 12-week, randomised, double-blind, multicentre, placebo-controlled trial of flexible-and fixed-dose regimens*. Pain, 2005; 115(3):254-63.

27. Verma V, Singh N, Singh Jaggi A: *Pregabalin in neuropathic pain: evidences and possible mechanisms.* Curr Neuropharmacol, 2014; 12(1):44-56.

28. Celik M, Kose A, Kose D, Karakus E, Akpinar E, Calik M, et al: *The double-edged sword: effects of pregabalin on experimentally induced sciatic nerve transection and crush injury in rats*. Int J Neurosci

[Internet], 2015; 125(11):845-54. Available from: http://www.tandfonline.com/doi/full/10.3109/00207454.2014.978976

29. Buys MJ, Alphonso C: *Novel use of perineural pregabalin infusion for analgesia in a rat neuropathic pain model*. Anesth Analg, 2014; 119(2):481-88.

30. Yunus K: *Siyatik sinir kesi modelinde eritropoetin Ile Gelsolin'in Rejenerasyon Üzerine Etkişinin Karila tirilmasi.* 2014

31. Akarca O, Öcal Ö, Bulduk EB, Paşaoglu ÖT, Gündo du AÇ, Kaplano lu GT, et al.: *Protective effects of resveratrol in a rat model of ischemia-reperfusion injury of sciatic nerve*. Gazi Med J, 2017; 28(4).

32. Güdü OB: *Deneysel Spinal Kord Yaralanmasinda Pregabalinın Nöroprotektif Etkisi Kanda Ve Nöral Dokuda Antioksidan Enzimlerin Oksidatif Stres Açısından ncelenmesi.* 2012;

33. Carlton SM, Zhou S: *Attenuation of formalin-induced nociceptive behaviors following local peripheral injection of gabapentin*. Pain, 1998; 76(1-2):201-07.

34. Ozbek Z, Aydin HE, Kocman AE, Ozkara E, Sahin E, Bektur E, et al.: *Neuroprotective effect of genistein in peripheral nerve injury*. Turk Neurosurg, 2017; 27(5):816-22.

35. Özer H: *Deneysel Siyatik Sinir Yaralanmasinda Poli (3-Hidroksibütirat) Ve Chitosan Kaplı Tübüler Grefiler le Birlikte Mezen imal Kök Hücre Kullanımının Aksonal Rejenerasyona Etkisi.* 2013.

36. Rufer M, Flanders K, Unsicker K: *Presence and regulation of transforming growth factor beta mRNA and protein in the normal and lesioned rat sciatic nerve.* J Neurosci Res, 1994; 39(4):412-23.

37. Atkins S, Smith, Loescher KG, Alison R, Boissonade F, Ferguson MWJ, Robinson PP: *The effect of antibodies to TGF β1 and TGF β2 at a site of sciatic nerve repair.* J Peripher Nerv Syst, 2006; 11(4):286-93.