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Comparison of the Effects of Local and Systemic Dexamethasone on the Rat Traumatic Sciatic Nerve Model

Rat Travmatik Siyatik Sinir Modelinde Lokal ve Sistemik Deksametazonun Etkisinin Araştırılması

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ABSTRACT

AIM: The aim of the study was to evaluate the effect of peroperatively locally administered dexamethasone on nerve recovery after induced nerve crush injury.

MATERIAL and METHODS: 4 groups of 8 animals were formed. The sciatic nerves of 32 rats were exposed at midthigh level and those of 24 rats were crushed for 30 seconds with a pair of jeweler's forceps.

RESULTS: Sciatic functional index (SFI) measurements of all the animals included at days 7, 14, 21 and 28 was statistically significant ($p<0.05$). Pinch tests that were done on the first 13 days gave negative results in all the groups. There was a difference between the test results of Group II and those of Group III and IV at days 14, 21 and 28. When the duration of the experiment was taken as a whole, no difference was observed between the test results of Group III and IV.

CONCLUSION: Recovery in the group treated with local dexamethasone was more remarkable than that in the group treated with systemic dexamethasone in our study. As the difference was statistically significant, we recommend the perioperative use of local dexamethasone in procedures that might induce nerve injury.

KEYWORDS: Dexamethasone, Nerve injury, Sciatic nerve

ÖZ

AMAÇ: Çalışmanın amacı, sinir ezilme yaralanması sonrası sinir iyileşmesinde peroperatif lokal deksometazon uygulamasının etkilerini incelemektir.

YÖNTEM ve GEREÇLER: 8 hayvan içeren 4 grup hazırlandı. 32 ratta siyatik sinirler orta seviyede açığa kondu ve bunların 24 tanesinde forseps kullanılarak 30 saniye ezilme oluşturuldu.

BULGULAR: Siyatik fonksiyonel indeks (SFI) tüm hayvanlarda 7,14,21 ve 28'inci günlerde istatiksel olarak anlamlı idi ($p<0.05$). İlk 13 günde yapılan Pinch testi tüm gruplarda negatif olarak bulundu. 14., 21. ve 28. günlerinde Group II grubu ile Group III-IV arasında yüzde olarak fark gözlendi. Deney süresi bir bütün olarak alındığında Group III ve IV arasında fark gözlenmedi.

SONUÇ: Çalışmamızda lokal deksometazon ile tedavi edilen grupta düzelme, sistemik deksometazon ile tedavi edilen gruba göre daha belirgin idi. İstatiksel olarak önemli bir fark olup biz sinir hasarı olabilecek prosedürlerde perioperatif lokal deksometazon uygulamayı önermekteyiz.

ANAHTAR SÖZCÜKLER: Deksametazon, Sinir hasarı, Siyatik sinir

INTRODUCTION

Surgical procedures in orthopedic, peripheral nerve and spinal surgery require nerve dissection, nerve transposition and/or induced traumatic injuries to nerve branches. It is a fact that crushing or sectioning may lead to significant functional impairments and/or sequelae. In the case of nerve injury, scarring is uncontrollable and unavoidable**,** and the main phase of wound healing is the scar tissue formation (7,11).

Experimentally, many medications and techniques have been used in rat crush injury models, such as steroids, nonsteroidal anti-inflammatory drugs low dose radiotherapy and vitamins (2,15,19,21,27,29). These experiments are aimed at analyzing the process of nerve regeneration and functional recovery with the aid of therapeutic interventions. If effective treatment could be developed to minimize postoperative dysfunction, this would have important clinical application. Ideally, such a treatment could be instituted at the time of surgery. The aim of this study was to evaluate clinical and histopathological

effects of local dexamethasone therapy on the prevention of intraneural scar formation in peripheral nerve injury in the rat sciatic nerve model.

MATERIAL and METHODS

The experimental protocols were reviewed and approved by the Animal Care and Ethic Committee of Maltepe University. 32 adult Sprague-Dawley rats weigthing 350–400 gr underwent unilateral sciatic nerve crush injury. Before the surgical procedures were performed, the animals were housed in soft wood-chip–lined plastic cages for three days for adaptation purposes. During this period standard laboratory conditions were provided (a 12 hour light, 12 hour dark cycle, a room temperature of 20-22°C). The animals were given as much food and water as they could consume.

The animals were operated, after shaving and preparing the skin with 10% povidone-iodine. The sciatic nerve was exposed by opening the fascial plane between the gluteal and femoral musculature via a longitudinal incision. Following the dissection of the gluteus maximus, 1 cm of the sciatic nerve was exposed among the muscles, 10 mm above the trifurcation of the sural, tibial and perineal nerves. Under intraperitoneal kethamine anesthesia, the sciatic nerves of 32 rats were exposed at midthigh level and those of 24 rats were crushed for 30 seconds with a pair of jeweler's forceps. The wound was sutured in layers and the animals were allowed to recover. For local application, Gelfoam was saturated with 2 mg/kg dexamethasone. For intraperitoneal (systemic) application, 2 mg/kg dexamethasone was drawn into an insulin injector.

4 groups of 8 animals were formed. **Group I (Control group or normal saline group):** In this group left sciatic nerve of each rat was exposed. The aim was to determine whether the rats' walking would be affected by this procedure. **Group II (Local normal saline group):** Left sciatic nerve of each rat was exposed and injured. The nerve was then wrapped with Gelfoam saturated with normal saline. **Group III (Local dexamethasone group):** Left sciatic nerve of each rat was exposed and injured. The nerve was then wrapped with Gelfoam saturated with dexamethasone. **Group IV (Systemic dexamethasone group):** Left sciatic nerve of each rat was exposed and injured. Immediately after the surgical procedure, 2 mg/kg dexamethasone was administered intraperitoneally.

EVALUATION

Walking-track analysis – At 12 weeks after nerve repair, the animals were submitted to walking-track analysis and measurement of the sciatic functional index (SFI) using a method similar to that described by de Medinaceli et al (9). The animals were then made to walk on a walking track of 80 cm x 7 cm, with a slope of 10 degrees. Sheets of paper were placed on the base of the walking track so that foot prints would appear on them. Analysis was performed on day 0 (prior to the operation), and on postoperative days 1, 7, 14, 21, and 28. The difference between the measurement of the operated left leg and that of the normal leg was divided by that of the normal leg. An index value of 0 represents normal functioning, whereas that of -100 indicates that theoretically there is total loss of functioning**.**

Sensory function test was analyzed by "pinch" test. Each animal was seized and the skin of its sole was pinched with a pair of fine forceps used in inducing nerve trauma. The instrument used is known to apply a standard pressure. The number of rats which responded to the pinch with a withdrawal reflex was recorded on day 0 (prior to the operation), and on postoperative days 1, 7, 14, 21, and 28.

Following the completion of tests, rats were sacrificed on the 28th day under anasthesia by means of cervical dislocation. Sciatic nerve of each animal was examined under the light microscope by a pathologist who was uninformed about the medical treatment performed by the authors. 1 cm of the sciatic nerve where injury was induced (10 mm above the trifurcation of the sural, tibial and perineal nerves) was taken as sample. The next step was the staining of these samples with hemotoxylin-eosin stain**,** Toludine blue and Mason trikrom**.** Hemotoxylin-eosin stain brings to light all the elements of a tissue on histopathological and cytological levels. Toludine blue makes it possible to better detect and describe elements of mast, histiocyte, and inflammation. Cross-sections stained with Mason trichrome make the structures of myelin and axon visible. 0 represented absence of connective tissue. The numbers and the amount of connective tissue increase they correspond to are given below: +1 slight, +2 medium, +3 high.

Data recorded in forms were transferred to a computer and analyzed by SPSS (version 11.5). As the study was based on a small sample, non-parametric tests were used in performing statistical analyses. The Kruskal-Wallis test was made use of in comparing the SFI of the three experimental groups. The Mann-Whitney U test was employed in comparing the SFI of the two groups of experimental and control. A p value of <0.05 was considered as statistically significant.

RESULTS

SFI measurements of all the animals included in this study were homogeneous at day 0 and 1, and there was no statistical significance between the groups ($p>0.05$). However, the difference at days 7, 14, 21 and 28 was statistically significant ($p<0.05$) (Table I). Comparisons were made in order to find out the reason for the statistical significance between the groups as regards average SFI (Table II, III). It was found out that at day 7, average SFI of neither Group II and III, nor Group III and IV had a statistically significant correlation ($p>0.05$). However, Group II and IV had this correlation ($p<0.05$) (Table IV). At day 14, average SFI of Group II and III did not have a statistically significant correlation ($p>0.05$). However, on the same day the difference between Group II and IV, as well as Group III and IV was statistically significant ($p<0.05$). At day 21, the difference between Group II and III, as well as Group II and IV was also statistically significant ($p<0.05$). However, on the same day, no statistically significant correlation was observed between the

SFI of Group III and IV (p>0.05). At day 28 SFI of the below-mentioned groups had a statistically significant correlation (p<0.05): Group II and Group III; Group II and Group IV; Group III and IV.

Pinch tests that were done on the first 13 days gave negative results in all the groups (Table V). However, there was a difference between the test results of Group II and those of Group III and IV at days 14, 21 and 28. When the duration of the

Table I: Comparison of SFI in the Groups at Days 0, 1, 7, 14, 21, and 28 (Kruskal Wallis Test)

Day	Group	n	Mean Rank	Mean	χ^2	p
0. day	Group II	8	12.50	0.00	.000	1.000
	Group III	8	12.50	0.00		
	Group IV	8	12.50	0.00		
1. day	Group II	8	10.25	-72.54	1.340	.512
	Group III	8	14.25	-69.39		
	Group IV	8	13.00	-71.39		
7. day	Group II	8	7.25	-72.62	8.235	**.016**
	Group III	8	12.88	-68.52		
	Group IV	8	17.38	-69.67		
14. day	Group II	8	7.50	-71.86	9.645	**.008**
	Group III	8	11.63	-68.54		
	Group IV	8	18.38	-64.37		
21. day	Group II	8	4.50	-69.86	15.965	**.000**
	Group III	8	17.88	-48.54		
	Group IV	8	15.13	-49.45		
28. day	Group II	8	4.50	-69.89	19.280	**.000**
	Group III	8	20.00	-36.19		
	Group IV	8	13.00	-46.45		

Table II: Comparison of SFI within the Members of Each Group on Different Days (Mann-Whitney U Test)

Comparison of groups		U	Z	P
Group II-Group III	7. day	18.000	-1.470	.141
Group II- Group IV		4.000	-2.941	**.003**
Group III- Group IV		21.000	-1.155	.248
Group II-Group III	14. day	20.000	-1.260	.208
Group II- Group IV		4.000	-2.941	**.003**
Group III- Group IV		13.000	-1.995	**.046**
Group II-Group III	21. day	.000	-3.361	**.001**
Group II- Group IV		.000	-3.361	**.001**
Group III- Group IV		21.000	-1.155	.248
Group II-Group III	28.day	.000	-3.361	**.001**
Group II- Group IV		.000	-3.361	**.001**
Group III- Group IV		4.000	-2.941	**.003**

Table III: Average SFI of the Groups on Days of Follow-up

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28
Group II	0	-72.54	-72.62	-71.86	-69.86	-69.89
Group III	0	-69.39	-68.52	-68.54	-48.54	-36.19
Group IV	0	-71.39	-69.67	-64.37	-49.45	-46.45

Table IV: Comparison of SFI within the Members of Each Group on Seventh Day (Mann-Whitney U Test)

Compared to groups	U	Z	P
Group II-Group III	18.000	-1.470	.141
Group II-Group IV	4.000	-2.941	**.003**
Group III-Group IV	21.000	-1.155	.248

Table V: Number of Pinch Test Positive Rats in Sham and Experimental Groups (n, %)

Day	Sham	Group II	Group III	Group IV
0	8	8	8	8
1	8	0	0	0
7	8	0	0	0
14	8	0	3 (%37)	4 (%50)
21	8	1 (%12)	5 (%62)	6 (%75)
28	8	1 (%12)	7 (%87)	7 (%87)

experiment was taken as a whole, no difference was observed between the test results of Group III and IV. It was noticed that in all the groups in which nerve injury was induced, withdrawal reflex was lost, starting from the first day; and that gradual recovery was observed in Group III and IV, to which local or systemic dexamethasone was applied. Contractions were not observed in any of the animals included in this study.

At histological evaluation, myelin loss and degeneration in Group II and III were less than those in Group IV. In Group II, cross-sections with severe degeneration and myelin loss had aelatively few myelinated axons. Vacuolar degeneration was also widespread. Cross-sections of sciatic nerve were stained with Mason trichrome, but this did not serve as strong evidence to suggest an increase in connective tissue (Figure 1A,B). Vacuolization and bleeding were not observed in Group III. +1 vacuolization occurred in two of the rats in Group IV. In Group II, however, +1 vacuolization was detected in five rats, and +3 vacuolization was seen in three rats. One of +3 vacuolization with rats had bleeding and extravasate erythrocytes (Figure 2A,B; 3A,B).

DISCUSSION

The peripheral nerve responds to trauma by an inflammatory reaction with increased vascular permeability and intraneural edema (14,18). Steroids act as antiinflammatory agents by inhibiting phospholipase A2, by preventing degranulation of granulocytes, mast cells, or macrophages; and by suppressing macrophage inhibition factor and stabilizing lysosomal and other membranes. Different steroid forms can be used in damaged peripheral neurotheraphy (1,13,24,26,32,33). As methylprednisolone contains %40 polythene glycol and hydrocortisone benzyl alcohol, it is neurotoxic and not appropriate for local use (24,26). Systemic is applied to the damaged nerve in clinical studies with triamcinolone and positive results are observed (32). Yet, since the solution is particled in local practice, homogeneous dispersion cannot be maintained at very low doses. Intrafascicular injection of triamcinolone hexacetanide is pointed to cause irreversible nerve damage, moderate methylprednisolone and mild dexamethasone damages. Dexamethasone was preferred in our study, since there are no neurotoxical side effects in local use and solution with homogeneous dispersion is obtained in local application.

Hiroaki et al. have used dexamethasone to lessen the degree of inflammation due to nerve damage, and reported that neither edema nor axonal myelin degeneration was observed (14). Beaudry et al. have inflicted pain by placing a balloon onto the sciatic nerve of some rats. Following the administration of dexamethasone, pain neuropeptides and inflammatory response were observed to lessen (3). It has been reported in similar studies that dexamethasone gives rise to edema in the nerves (6,16). Galloway et al. reported that a topically applied dexamethasone significantly improved nerve recovery 14 days after sciatic nerve crush injury (12). In the course of this study, the effect of local dexamethasone was more evident after day 21.

In the rat sciatic model, nerve histomorphometry, electrophysiological studies and measurement of functional recovery are the most popular methods to assess neural regeneration (8,22,23). However, histomorphometry and electrophysiological studies do not always correspond to functional recovery. One of the most common tests, the SFI, provides a noninvasive and quantitative method to evaluate functional recovery of walking ability, the ultimate goal in the regeneration of injured rat sciatic nerve (8,30). Walking track analysis is the gold standard for evaluation of nerve recovery after sciatic nerve injury because proper walking requires coordinated function involving sensory input, motor response, and cortical integration (31). It is a fact that the target of peripheral nerve regeneration is the attainment of functional recovery. Authors reported whose studies have also shown normal walking patterns only after the first month

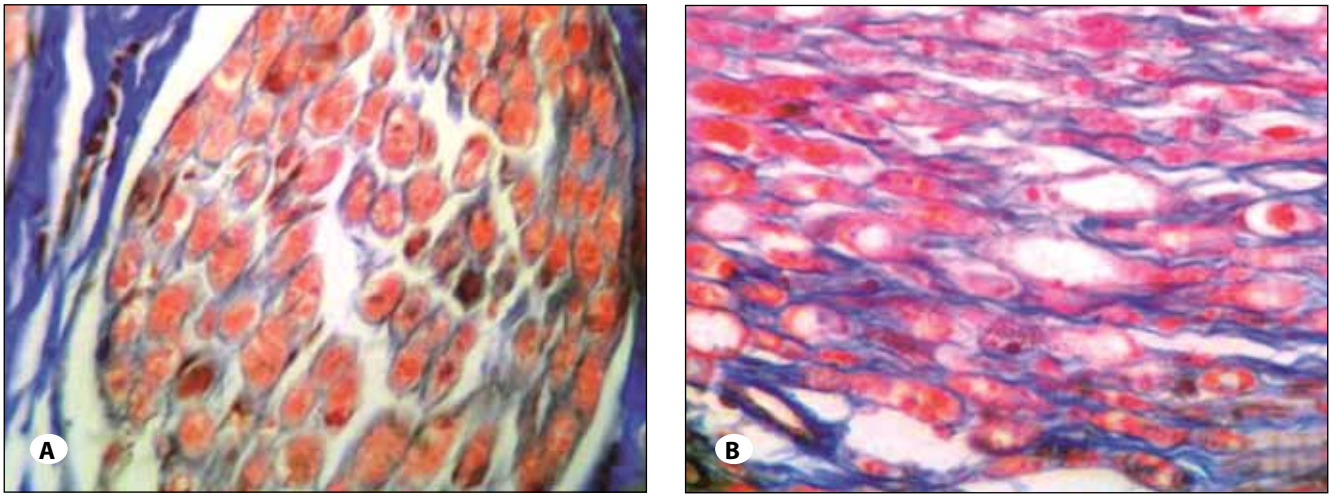

Figure 1: Appearance of a normal axon, and slight degeneration and myelin loss: **A)** appearance of a normally myelinated axon; **B)** mild degeneration and myelin loss (on day 28 in 7 rats in group IV), Mason-Trikrom (x600).

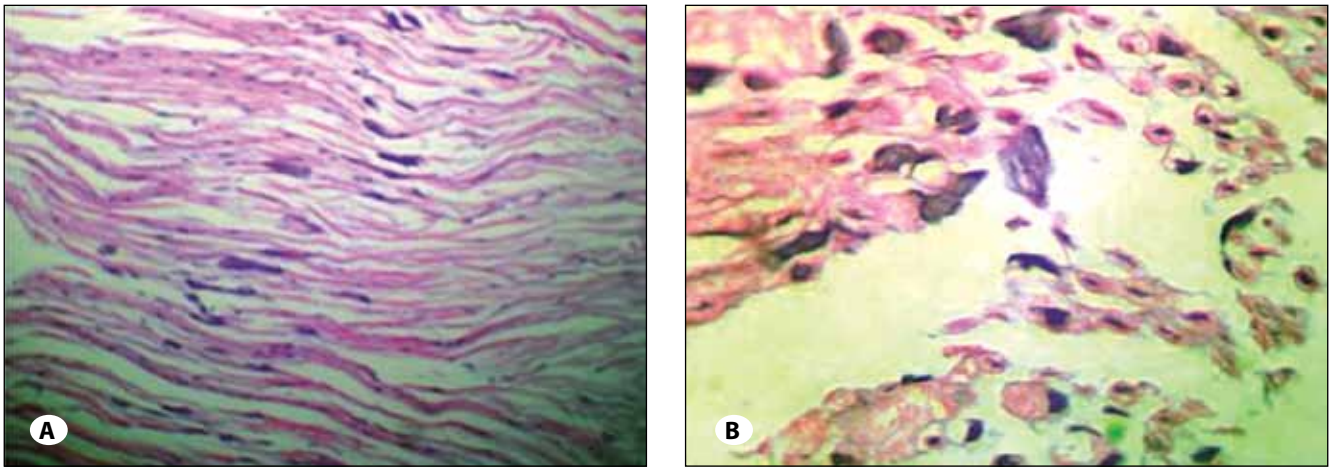

Figure 2: Mild and severe degeneration and myelin loss: **A)** mild degeneration and myelin loss (1 rat in group IV); **B)** severe degeneration and myelin loss (all the rats in group IV), (stained with hematoxylin-eosin stain (x400)).

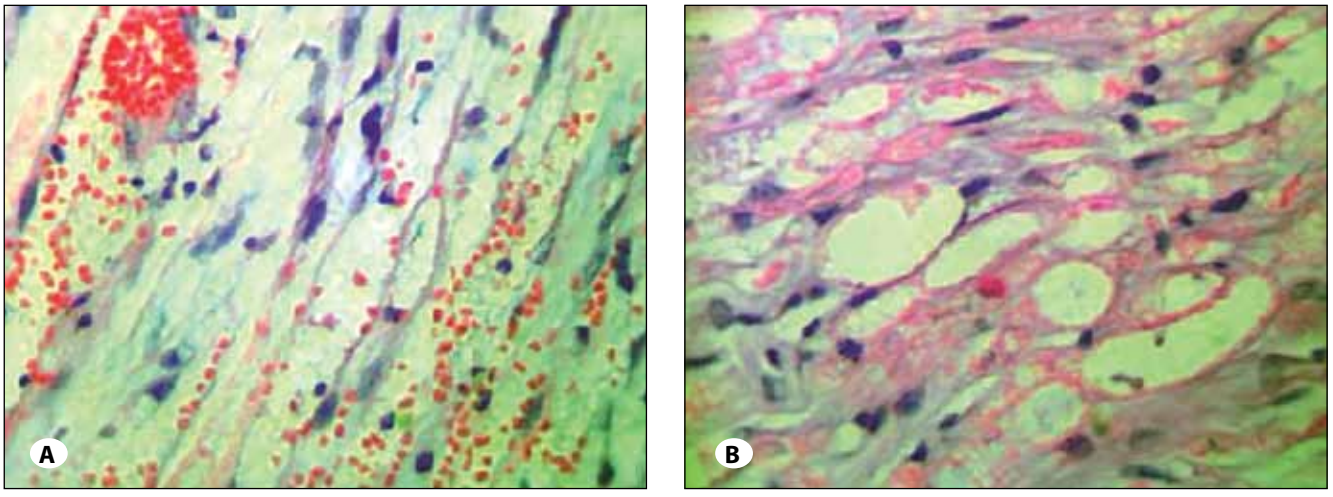

Figure 3: Extravasated erythrocytes and vacuolization: **A)** Bleeding and extravasated erythrocytes (1 rat in Group IV); **B)** severe vacuolization (6 rats in Group IV), (stained with hematoxylin-eosin stain (x400)).

of post crush (20,25). In contrast to these experiments, some authors reported a full recovery at the third and fourth weeks (28). Galloway et al. reported more rapid recovery in topical steroid group at postoperative days 14, 18 and 22 and this reached statistical significance at postoperative day 14 (12). The difference in the rate of motor functional recovery may relate to the pathophysiologic response of peripheral nerves to the magnitude of different crushing loads (10). Walking track analysis is a useful technique if the we found out that starting from day 21, there was a marked difference between the walking track analysis of the rats treated with systemic dexamethasone and that of the rats treated with local dexamethasone. We think that walking track analysis is the most important method that can test functional recovery.

Sciatic and perineal nerve injury gives way to joint contractions, in which case a rat treads on foot. This is because flexor muscles are reinnervated faster than extensor muscles (5,17). Contraction makes it difficult to make walking track analysis and to measure length of imprint (8). It has been reported that for this problem to be eliminated, the injured side of the body should be exercised weekly (17). Contraction was not observed to occur in any of the animals included in this study.

CONCLUSION

This study was specifically aimed at demonstrating that local dexamethasone enables nerve recovery. Motor function tests and histological findings showed that topical dexamethasone has a positive effect on recovery. We, therefore, conclude that local dexamethasone is more effective than systemic dexamethasone. The reason for the local application of dexamethasone was that it has fewer or no adverse effects, such as hyperglycaemia or hypertension.

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