

The Effect of Decompression on The Treatment of Chronic Constriction Injury in Peripheral Nerve ^[1]

Orhan ÖZATİK ¹ Atacan Emre KOÇMAN ² İlknur DAĞ ³
A. Aydan KÖSE ² Ahmet MUSMUL ⁴ Tayfun ŞENGEL ⁵

^[1] This work was supported by a grant from Eskişehir Osmangazi University (Project no. 2014-369)

¹ Department of Histology and Embryology, Medical Faculty, Ahievran University, TR-40100 Kırşehir - TURKEY

² Department of Plastic and Aesthetic Surgery, Medical Faculty, Eskişehir Osmangazi University, TR-26100 Eskişehir - TURKEY

³ Department of Medical Laboratory Techniques Health Service Collage, Eskişehir Osmangazi University, TR-26100 Eskişehir - TURKEY

⁴ Department of Biostatistic, Medical Faculty, Eskişehir Osmangazi University, TR-26100 Eskişehir - TURKEY

⁵ Central Research Laboratory Application and Research Center, Eskişehir Osmangazi University, TR-26100 Eskişehir - TURKEY

Article Code: KVFD-2016-15126 Received: 26.01.2016 Accepted: 05.04.2016 Published Online: 05.04.2016

Abstract

Chronic constriction injury (CCI) is a common clinical entity and characterized by allodynia or spontaneous neuropathic pain. Treatment of neuropathic pain is difficult, because a lack of knowledge about the underlying mechanisms and limited effectiveness of the existing drugs. Surgical decompression enables a more radical treatment by releasing the compressed nerve. Beside the pain behaviour morphological changes occur in CCI. Ultrastructural morphological changes at the injury site of the sciatic nerve and in the dorsal root ganglia (DRG) are believed to play role in the pathogenesis of CCI and in the development of neuropathic pain behaviour in individuals. However, the effects of surgical decompression on the ultrastructure of constricted nerve site as well as in the dorsal root ganglia have not been studied in details. We investigated the effect of nerve decompression on ultrastructure of rat sciatic nerve and DRG by light and transmission electron microscopic methods. For this aim, CCI was established on the rat sciatic nerve with four loose ligatures. Surgical decompression was held at 1st, 3rd and 5th the weeks after CCI by removing the ligatures. Our results suggest that the efficacy of decompression was superior when applied one week after compression. The results of the study verify the need for early surgical decompression to prevent irreversible damage of the peripheral nerve and DRG.

Keywords: Decompression, Sciatic nerve, Chronic constriction injury, TEM

Periferik Sinirde Kronik Konstrüksiyon Hasarı Tedavisi Üzerine Dekompresyonun Etkisi

Özet

Kronik konstrüksiyon hasarı (CCI), yaygın klinik bir oluşumdur ve allodini ya da spontan nöropatik ağrı ile karakterize edilir. Nöropatik ağrının tedavisi, altta yatan mekanizmaların yeterince bilinmemesi ve mevcut ilaçların sınırlı etkisinden dolayı oldukça zordur. Cerrahi dekompresyon uygulaması, sıkışmış sinirin serbestleştirilmesi suretiyle oldukça radikal bir tedavi imkanı sağlar. CCI'da ağrının yanısıra morfolojik değişimler de olur. Siyatik sinirin yaralı bölgesi ve dorsal kök gangliyonundaki (DRG) ultrayapısal morfolojik değişimlerin, CCI patojenezinde ve bireylerdeki nöropatik ağrı davranışı gelişiminde rol oynadığına inanılır. Ancak, sıkışmış sinir bölgesinde ve dorsal kök gangliyonlarındaki ultrayapı üzerine cerrahi dekompresyonun etkileri henüz detaylı olarak çalışılmamıştır. Çalışmamızda rat siyatik siniri ve DRG'nun ultrayapısı üzerine sinir dekompresyonunun etkisini ışık ve geçirimli elektron mikroskopik metodlarla inceledik. Bu amaçla CCI rat siyatik siniri üzerine yapılan dört gevşek bağ ile oluşturulmuştur. Cerrahi dekompresyon, CCI sonrası 1. 3. ve 5. haftalarda bağların uzaklaştırılmasıyla sağlanmıştır. Verilerimiz dekompresyon etkisinin sinir sıkıştırılmasından bir hafta sonra uygulandığında çok daha iyi sonuç verdiğini desteklemektedir. Erken cerrahi dekompresyonun, periferik sinir ve dorsal kök ganglionunun geri dönüşümsüz hasarını önlemeye etkileri üzerine ilave çalışmalara ihtiyaç bulunmaktadır.

Anahtar sözcükler: Dekompresyon, Siyatik sinir, Kronik konstrüksiyon hasarı, TEM



İletişim (Correspondence)



+90 386 2803900



orhanozatik@yahoo.com

INTRODUCTION

Neuropathic pain arises as a consequence of nerve injury either of the peripheral or central nervous system. It tends to be chronic, and less responsive to the administration of analgesic drugs and other conventional medical management [1,2]. Following peripheral nerve injury, a cascade of events in the primary afferents leads to peripheral sensitization, resulting in spontaneous nociceptor activity, decreased threshold, and increased response to supra-threshold stimuli [3]. However, the pathophysiological mechanisms underlying neuropathic pain are poorly understood and the available treatments are unsatisfactory [4].

Animal models of neuropathic pain are available that help to clarify the underlying mechanisms [5]. Especially, compression-related nerve injury is the main model for neuropathic pain, and it includes chronic constriction injury (CCI), partial sciatic nerve ligation, and spinal nerve ligation [6]. CCI is relatively simple to perform, and produces robust and stable pain hypersensitivity for at least one month after injury. It is also commonly used to investigate both the pathophysiology, and potential therapeutic agents for treatment of neuropathic pain [7].

In clinical practice, surgical decompression is frequently used to relieve symptoms of neuropathic pain, e.g. carpal tunnel syndrome, spinal root compression, and trigeminal neuralgia due to vascular compression [8]. Few studies have investigated the molecular mechanisms of CCI and decompression [9] whereas some studies evaluated morphological changes [10-12]. However experimental evidence on neuropathic pain and CCI is still lacking. Further research is required to correlate the effects of decompression on neuropathic pain mechanisms and morphological changes in constricted nerves.

The aim of this study is to investigate changes in the ultrastructural morphology of constricted peripheral nerve and its associated DRGs and whether surgical decompression at given times after established CCI can reverse these changes.

MATERIAL and METHODS

All the experiments were approved by the Local Ethical Committee of Osmangazi University (Protocol No: 376/2014) in Eskisehir (Turkey) and conducted according to the health-care guidelines for the laboratory animals and Universal Declaration on Animal Welfare. The animals used for the experiments were provided by the Center for Medical and Surgical Research (TICAM). The animals were housed in separate cages with day and night cycle. Access to free standard rodent food and water *ad libitum* were allowed for all animals in the course of experiments.

Thirty adult female Sprague-Dawley rats, weighing 250-300 g, were used in this study. Rats were divided randomly

in five groups (n=6). The first group comprised of SHAM operated rats (SHAM). CCI was established in the other groups which were entitled as control (C) and chronic constriction release groups at first, third and fifth weeks after CCI (CCR1w, CCR3w, CCR5w) respectively.

Surgical procedures were performed under Thiopental Sodium (40-50 mg /kg) anesthesia via intraperitoneal injection. After depilation of the right hindlimb the right sciatic nerve was exposed at the mid-thigh level. CCI was induced by four silk ligatures loosely tied around the nerve at 1 mm intervals proximal to the trifurcation. Thereafter the wound was closed by muscle and skin layers and animals were left to recover. The constricting ligatures were remained in control animals for 8 weeks. CCR groups were operated for surgical decompression at given times under the same anesthesia procedure. All four ligatures were carefully removed at first, third and fifth weeks after CCI. All experiments were ended for CCR groups at the end of the 8th week from the CCI operation.

Animals were euthenized by blood exsanguination and intracardiac perfusion of 2.5% gluteraldehyde in 0.1M pH 7.4 Sodium phosphate buffer (PBS). Right Sciatic nerve segments (site of the constriction and its 5mm proximal and distal parts) and associated DRGs were dissected out carefully and stored in the same fixative solution.

Light and Transmission Electron Microscopy

Tissue samples were prepared by fixation in 2.5% gluteraldehyde in 0.1 M phosphate buffer for 24 h at 4°C and then rinsed with phosphate buffer. Specimens postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h at room temperature. All specimens were then dehydrated in graded solutions of ethyl alcohol (30%, 50%, 70%, 90%, 96% and 100%) and embedded in epon-araldite resin. Semithin sections (700 nm) were collected at a variety of depths of the sample and stained with toluidine blue. The sections were examined by light microscope (Olympus BX50) to select appropriate areas for TEM analysis. Degenerated axons were determined by two criteria involving myelin debris formation and finer degeneration in axons. In addition, the number of normal and degenerated nerve fibres was counted and Degenerated/Normal Axons Ratio (Deg/Nor) were calculated. The measurements were done blindly by two investigators (O.O and I.D) to minimize subjectively affected differences in results. Later, ultrathin sections (60 nm) were taken on an ultramicrotome (Leica Ultracut RM, Wetzlar, Germany) and counterstained with uranyl acetate-lead citrate [13,14]. They were examined and photographed using a TEM (JEOL JEM 1220) with digital imaging capabilities.

Data Analysis

All data were obtained and originally analyzed using SPSS v2.2 for Windows (IBM Inc. Istanbul, Turkey). Shapiro-Wilk test was used to assess the normal distribution of

data. Independent samples Kruskal -Wallis test was used for comparing differences between groups. Data were presented both as mean \pm SD and median (25%, 75%) percentiles. $P < 0,05$ was accepted as statistically significant.

RESULTS

Light Microscopic Results

Light microscopic findings showed the normal structural features in the SHAM animals. Myelinated axons of sciatic nerve was normal in appearance and a few unmyelinated axons were observed (Fig. 1a). After CCI, axonal damage and thinly myelin sheaths were increased and common decrease was seen in the number of myelinated axon. Some areas with a depletion of nerve fibers were also shown (Fig. 1b). One week after decompression, significant increase in the number of myelinated axon was observed and only a few degenerated fibres were determined. Remyelinated axons were also abundant (Fig. 1c). After the third week, several degrees of axonal injury were seen and its progression to myelinated fiber degeneration are shown. Degenerated myelin fibres increased from the third to fifth week decompression. Morphological signs of axonal regeneration were reduced remarkably (Fig. 1d-e). DRGs associated both in the control and CCR1w animals showed compact and regularly arranged structures (Fig. 2a-c). After CCI, the major pathological change was that of microvacuolisation of the DRG cells (Fig. 2b). In addition, cell membrane lines were lost and their sizes were larger. These changes were seen in CCR3w and CCR5w groups less frequently (Fig. 2d-e).

In the present study compared to the the SHAM

group (0.07 ± 0.01), CCI group (1.98 ± 0.20) demonstrated a significant increase in Deg/Nor ($P < 0.05$). A significant decrease was found in Deg/Nor of CCR1w (0.20 ± 0.03) compared to CCI ($P < 0.05$). However there were no significant differences between CCI, CCR3w (0.50 ± 0.07) and CCR5w group (1.00 ± 0.22) ($P > 0.05$). In addition, no significant differences were found in Deg/Nor among CCR1w, CCR3w and CCR5w ($P > 0.05$). The data were summarized in Table 1 and Fig. 3.

Electron Microscopic Results

Changes in the ultrastructure of nerve fibres became increasingly noticeable in TEM studies. Axons in SHAM groups exhibited an organized, dark stained filament network. The cytoplasm of the axon appears normal ultrastructural morphology. In CCI group, the most remarkable morphological changes that occurred in myelinated axons were vacuolisation, myelin sheath degeneration loss of axoplasm, and lamellar separation. Also, blebbing lipid-like materials on axonal shrinkages were determined (Fig. 4a-c). In addition, increased numbers of mitochondria and dense neurofilament network were observed in myelinated axon. There were no significant changes in the ultrastructure of unmyelinated fibers in all groups compared to SHAM. Decompression at one week after CCI, no axonal shrinkage or myelin sheath degeneration were observed (Fig. 5a). In particular areas, uncompleted remyelination was also observed (Fig. 5b). In general, a few large vacuoles and several mitochondria were observed in axoplasm. Decompression at third and fifth week, myelin sheath degeneration accompanied with less marked regeneration of related structures. Myelinated axons exhibited axon-myelin separations and numerous intracellular organelles.

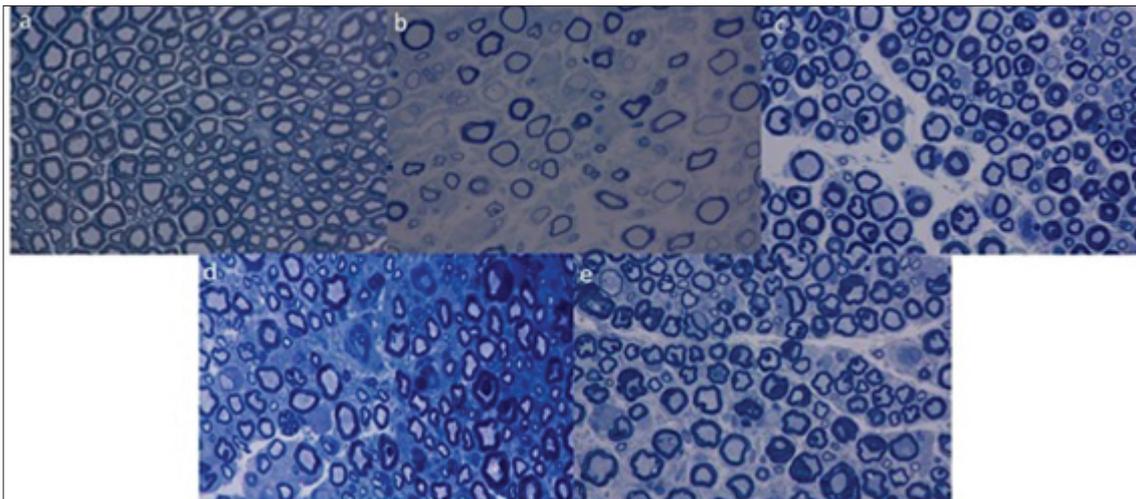


Fig 1. Semithin sections stained with toluidine blue of the sciatic nerve. a- Normal neural morphology in SHAM group, b- Note that the swollen appearance of the axons and the decrease in number of axons with intact myelin sheath in CCI group, c- Marked regeneration of axons of CCR1w group, d,e- Less marked recovery of the neural structures in CCR3w and CCR5w group, respectively (Bar: 10 μ m)

Şekil 1. Siyatik sinirin toluidin mavisi ile boyanmış yarı ince kesitleri. a- SHAM grubunda normal noral morfoloji, b- CCI grupta aksonların şişmiş görünümü ve intakt miyelin kılıflı aksonların sayısında azalma, c- CCR1w grubunda aksonların belirgin rejenerasyonu, d,e- Sırasıyla CCR3w ve CCR5w gruplarında noral yapının daha az belirgin düzelmesi (Bar: 10 μ m)

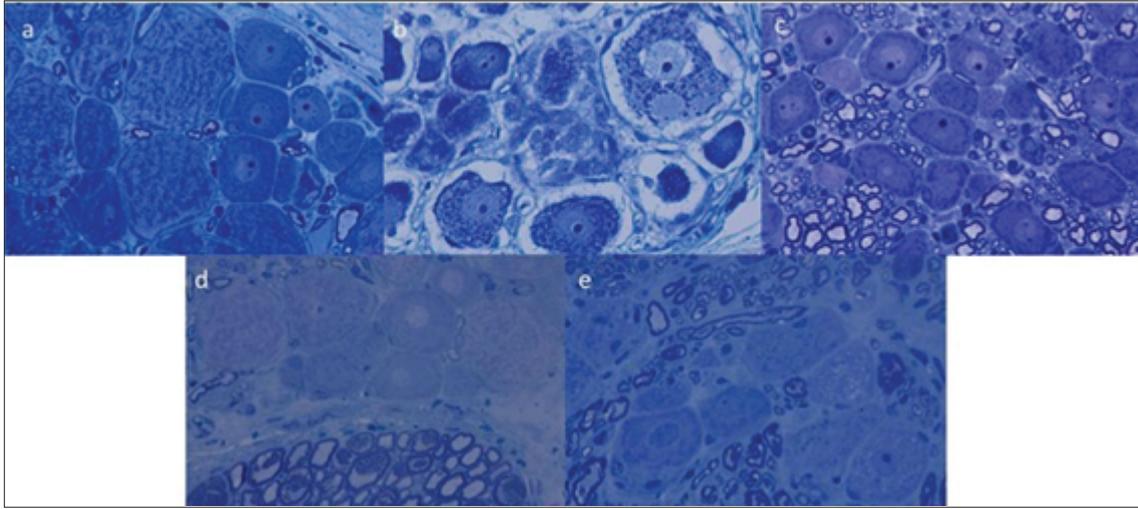


Fig 2. Semi-thin sections of the dorsal root ganglions of same samples. Figures show parallel data with sciatic nerve. a- SHAM, b- CCI group, c- CCR1w, d- CCR3w, e- CCR5w, note that the microvacuolisation of DRG cells in CCI and CCR5w groups (Bar: 10 µm)

Şekil 2. Aynı örneklerin dorsal root ganglionlarının yarı ince kesitleri. Şekiller siyatik sinir ile benzer verileri gösterir. a- SHAM, b- CCI grup, c- CCR1w, d- CCR3w, e- CCR5w, CCI ve CCR5w gruplarında DRG hücrelerinin mikrovakuolizasyonu görülmektedir

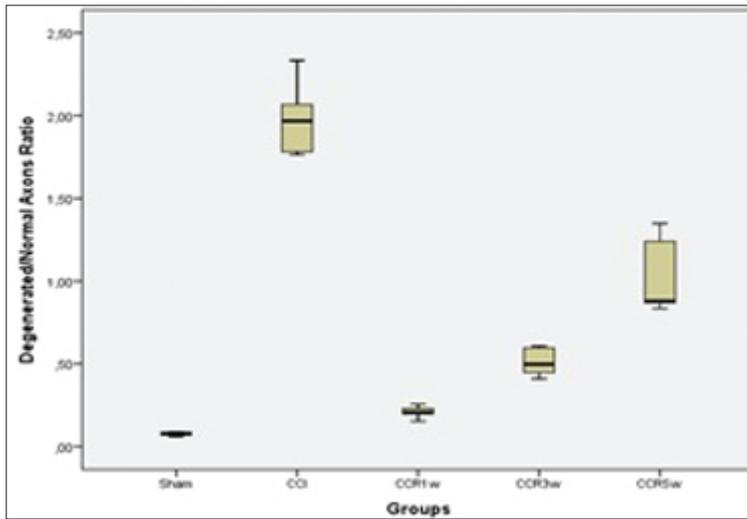


Fig 3. Graphic presentation of the mean numbers of degenerated/normal axon ratios

Şekil 3. Dejenere/normal akson oranlarının ortalama sayılarının grafik gösterimi

Table 1. The number of degenerated/normal axon ratios counted in semithin sections obtained from each study group

Tablo 1. Her çalışma grubunda yarı ince kesitlerin sayımından elde edilen dejenere/normal akson oranları

Groups	Degenerated/Normal Axons Ratio	
	Mean±STD	Median (25%-75%)
SHAM	0.07±0.01	0.07 (0.06-0.08)
CCI	1.98±0.20	1.96 (1.77-2.13)
CCR1w	0.20±0.03	0.20 (0.18-0.23)
CCR3w	0.50±0.07	0.49 (0.43-0.59)
CCR5w	1.00±0.22	0.88 (0.86-1.26)

In some areas, axonal shrinkage occurred (Fig. 6a). Loss of axoplasm was abundantly. Separations between the axoplasm and myelin sheath were prominent. Widened interface was observed between the Schwann cell and the axoplasm (Fig. 6b). In addition, unmyelinated nerve fibres

showed no important morphological changes except a few vacuol formation.

The DRG of SHAM showed normal histologic features. Neurons are surrounded satellite glial cells (SGCs) and nuclei of SGC is visible (Fig. 7a). The DRG morphology was found similar in both CCR1w and CCR3w groups as in SHAM. No degenerative changes were observed in DRGc and Surrounding SGCs. Massive degenerations such as loss of cell membrane and swelling were determined in CCI and CCR5w. However the most striking alteration in these latter groups was vacuolisation in DRGc. These vacuoles appeared as variably sized, but generally they were in clear content. Some dark inclusions were also determined within the cytoplasm (Fig. 7b). Loss of cell membranes of DRGc and cytoplasmic vacuolation were the typical characteristics of apoptosis in some sections of both CCI and CCR5w groups (Fig. 7c). Electron microscopic results were summarized in Table 2.

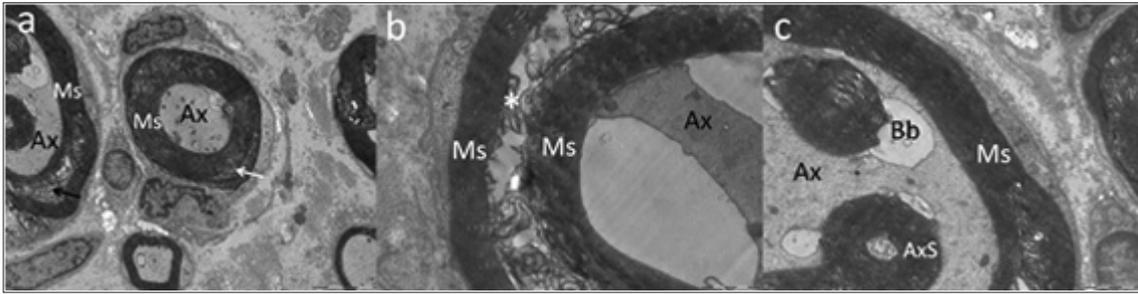


Fig 4. Transmission electron microscopic (TEM) analysis results of rat sciatic nerve after chronic constriction damage (CCI). a- There are some vacuoles (*white arrow*) and myelin separations (*black arrow*) (Ms: myelin sheath; Ax: axon), (Bar: 5 μ m), b- Axon showed the high degree of axoplasm loss. White asterisks indicates the lamellar separation of myelin sheath (Bar: 1 μ m), c- Axonal shrinkage (AxS) is seen in axon. Bb indicates blebbing lipid-like materials on axonal shrinkages (Bar: 2 μ m)

Şekil 4. Rat siyatik sinirine uygulanan Kronik Konstriksiyon hasarından (CCI) sonra elde edilen transmisyon elektron mikroskopik (TEM) sonuçların analizi. a- myelin seperasyonlar (*siyah ok*) ve vakuoller (*beyaz ok*) (Ms: myelin kılıf; Ax: akson), (Bar: 5 μ m), b- aksoplazma kaybının çok olduğu aksonlar görülmektedir. Beyaz yıldızlar myelin kılıftaki lameller seperasyonu göstermektedir (Bar: 1 μ m), c- aksonda aksonal şişme (AxS) görülmektedir. Bb aksonal büzüşmede blebbing lipid benzeri materyali göstermektedir (Bar: 2 μ m)

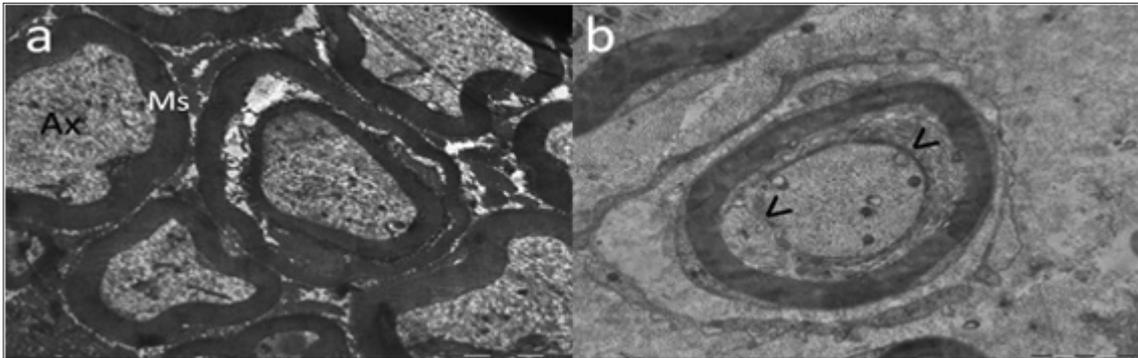


Fig 5. a- Transmission electron microscopic (TEM) analysis results of sciatic nerve from decompression at one week after CCI (CCR1w). Myelinated sheaths (Ms) show normal ultrastructural features. The cytoplasm of the axon (Ax) demonstrates no morphological abnormality (no axonal shrinkage or myelin sheath separation) (Bar: 5 μ m), b- *black arrowheads* indicate uncompleted remyelinated areas (Bar: 2 μ m)

Şekil 5. CCI (CCR1w) den bir hafta sonra serbestleştirilen siyatik sinirin transmisyon elektron mikroskopik (TEM) analiz sonuçları. Miyelin kılıflar normal ultrastrüktürel yapı göstermektedir. Aksonların sitoplazması morfolojik olarak normal görünümündedir (aksonal büzüşme veya miyelin kılıf seperasyonu yok) (Bar: 5 μ m), b- *siyah okbaşları* inkomplet remyelinize alanları göstermektedir (Bar: 2 μ m)

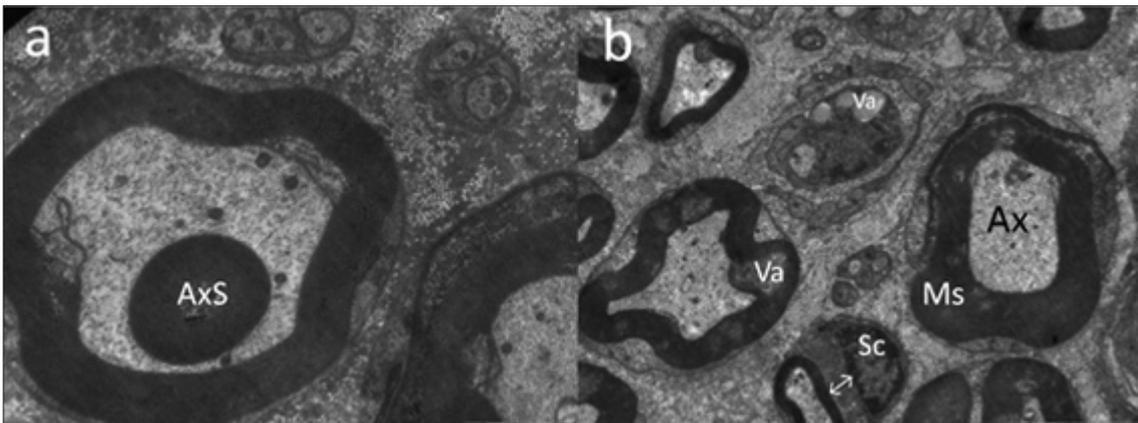


Fig 6. Transmission electron microscopic (TEM) analysis results obtained from decompression at five week after chronic constriction injury (CCR5w). a- on left image, there is an axonal shrinkage (AxS) (Bar: 2 μ m), b- The nerve fibers included in figure on right are vacuolisation areas (Va); *two headed arrow* demonstrate the widened interface was observed between the schwann cell and the axoplasm (Bar: 5 μ m)

Şekil 6. Kronik konstriksiyon hasarı sonrası beşinci haftada serbestleştirilen gruptan (CCR5w) elde edilen Transmisyon elektron mikroskopik (TEM) görüntüleri. a- soldaki görüntüde bir aksonal büzüşme var (AxS) (Bar: 2 μ m), b- sağdaki şekilde sinir lifinde vakuolizasyon alanı (Va); *iki başlı ok* schwann hücresi ve aksoplazma arasındaki genişlemiş arayüzü göstermektedir (Bar: 5 μ m)

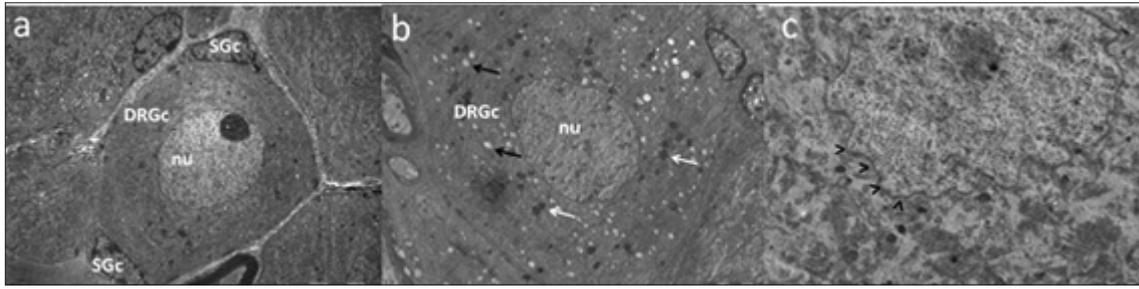


Fig 7. Transmission electron microscopic (TEM) analysis results of normal and damaged DRGc. a- Normal ultrastructural morphology in SHAM. Similar findings were found in CCR1w and CCR3w groups (Bar: 10 μ m), b- loss of cell membrane and swelling in CCI. Clear vacuoles (black arrow) and dark inclusions (white arrow) in the cytoplasm (Bar: 5 μ m), c- Apoptotic changes in cell membranes (arrowhead), DRGc: Dorsal Root Ganglion cells, SGc: Satellite Glial cell, Nu: Nucleus (Bar: 2 μ m)

Şekil 7. Normal ve hasarlı DRG hücrelerinin transmisyon elektron mikroskopik (TEM) analiz sonuçları. a- SHAM grubunda normal ultrastrüktürel yapı. CCR1w ve CCR3w gruplarında benzer bulgular elde edildi (Bar: 10 μ m), b- CCI grubunda hücre membran kaybı ve şişme. Sitoplazmada vakuoller (siyah ok) ve siyah inklüzyonlar (beyaz ok) (Bar: 5 μ m), c- hücre membranında apoptotik değişiklikler (okbaşı), DRGc: Dorsal Root Gangliyon hücreleri, SGc: Satellit Glial hücreler, Nu: Nucleus (Bar: 2 μ m)

Table 2. Summary of the ultrastructural changes in sciatic nerve and its associated DRG

Tablo 2. Siyatik sinir ve ilişkili DRG nin ultrastrüktürel değişikliklerinin özeti

Groups	Sciatic Nerve		Dorsal Root Ganglion	
	LM*	TEM*	LM	TEM
SHAM	Normal myelinated and unmyelinated fibers	Normal myelinated and unmyelinated fibers, typical appearance axoplasm and myelin sheath	Centrally placed nuclei, neurons are completely covered by satellite cells, most of the cell have an angular outline	Satellite glial cells around the neurons, quite evenly distributed organelles throughout the cytoplasm
CCI	Axonal damage, thin myelin sheaths, common decrease in the number of myelinated axon	Vacuolisation, loss of axoplasm, myelin sheath, degeneration lamellar separation. increased numbers of mitochondria, blebbing lipid-like materials on axonal shrinkages	Microvacuolisation of the DRG cells, weakness in cell membrane lines, swollen cells	Microvacuolisation of the DRG cells, weakness in cell membrane lines, swollen cells
CCR1w	Marked increase in the number of myelinated axon, only a few degenerated fibres, Remyelinated axons	No loss of axoplasm, axonal shrinkage or myelin sheath degeneration, uncompleted remyelination areas. a few large vacuoles several mitochondria	Normal morphological findings	SHAM like, normal morphological findings, a few axon-myelin separation
CCR3w	Myelinated fiber degeneration	Less marked regeneration	A few microvacuolisation in DRG cells, weakness in cell membrane lines, swollen cells	Axon-myelin separation, Loss of axoplasm, Increased mitochondria, Lamellar separation, Normal nuclear membrane morphology, Axonal shrinkage
CCR5w	Marked increase in degenerated myelin fibres counts	Axon-myelin separations Increasing electron dense in some unmyelinated axons, axonal shrinkage, Lost of axoplasm	Increasing microvacuolisation in DRG cells, loss of cell membrane	Loss of cell membranes, Many vacuolisation in cytoplasm, increased mitochondria, dark inclusions, axon-myelin separation, neuronal membrane damage

DISCUSSION

CCI of the rat sciatic nerve is an injury model established to investigate neuropathic pain. The injury is characterised behaviorally as mechanical allodynia and thermal hyperalgesia after a few day of the lesion. While some neurotransmitters such Substance P, CGRP and pERK play role in development of neuropathic pain [9,15] morphological changes may occur at the injury site of sciatic nerve [10,16-18], endorgan [8] or dorsal horn [9,15] which is central terminals of for nociceptive information and DRG [11]. Neuropathic pain is treated with drugs symptomatically in clinical practice [19,20]

and also various agents were tested experimentally after CCI established in animals [21,22]. However conservative treatment of neuropathic pain is still limited [19]. Unique radical intervention is decompression which is performed by removing of the ligatures on the sciatic nerve and works for treatment of underlying cause. It is shown that decompression not only relieves neuropathic pain; in addition, it also promotes the regeneration in associated central and peripheral nerves [23].

In the present study the effect of CCI and CCR on morphology of constricted site of nerve and associated DRG have been investigated in a conventional animal

model. CCI caused marked decrease in the number of myelinated axon, loss of axoplasm and vacuolisation at the constricted site of the nerve. deg/reg axon ratio was high in CCI group. In TEM analysis these pathological features were more evident. Axon-myelin separations, vacuolisation and loss of axoplasm were observed exhaustively. Large myelinated axons were found more degenerated compared to other structures. Surprisingly, unmyelinated fibers were not affected from CCI. This findings are somewhat consistent with previous studies. Similar microscopic findings have been reported with CCI injury in the rat, with massive degeneration of large myelinated fibers and less severe changes in small myelinated fibers and unmyelinated fibers [24-26]. Later Prinz et al. [10], have reported the ultrastructural changes of peripheral nerve lesions induced by chronic compression using an experimental model by light and electron microscopy myelinated axons at the compression sites displayed a remarkable increase in the number of small axons up to 60% in comparison with the normal axons. Additionally, remarkable axonal degeneration was observed both in central and in marginal regions of the distal to the constriction. In contrast, we observed significant decrease in the number of both small and large myelinated axons. However, distal and proximal nerve sections were found less damaged, and they were limited with a few lamellar separations in our study (data not shown).

The current scientific information suggests that the DRG is an active participant in the development of neuropathic pain [27]. Although Changes in gene expression of inflammatory cytokines, neuropeptides and ion current of ion channels in response to CCI or other types of nerve injuries have been reported previously [27-29] morphologic changes within the DRG have been less documented [11]. In addition to constricted peripheral nerve segment histopathologic changes were observed within the DRG in the present study. The major morphologic change was formation of microvacuoles within the DRG cells of the CCI group which appeared both in in light and TEM sections. As known, neuronal vacuolisation may be seen in neurons undergoing degeneration and it is a particular concern for neuropathologic significance of DRG. Besides loss of cell membrane and swelling of the DRG cells was also characteristic.

The morphologic changes in CCI injury were clearly demonstrated in the present work and previous studies. decompression can prevent or reverse these pathological conditions, in particular if performed early. In CCR1w group deg/normal axon ratio was found lower than CCI, CCR3w and CCR5w groups. Myelin structures and axoplasms thereby myelin-axon integrity were preserved in CCR1w. Normal schwann cell appearance and less lamellar separation was observed in TEM. Similar to our findings Jancalek and Dupovy [11] showed that decompression after 1-week compression caused a rapid increase in the number of

both small and large myelinated axons within the spinal root including the site of compression. In contrast the degenerative processes with a reduction of the MA number were very distinct when spinal roots were compressed for 5 weeks with subsequently decompressed for 3 weeks similar to the findings of our CCR3w and CCR5w groups.

Decompression also reversed the changes in the sensorial terminals of the peripheral nerves. Attenuation of neuropathic pain by surgical decompression caused normalization of dorsal horn activities [9,15]. The structure of DRG cells were preserved after decompression at first and third weeks whereas degeneration of DRG cells in CCR5w group persisted. However the recovery of peripheral nerve and its central terminals do not guarantee recovery at the endorgan etc skin. Although nerve decompression was accompanied with the disappearance of neuropathic pain behaviors after CCI, morphological studies have shown the evidence of an incomplete skin reinnervation [8,17].

The present study possesses two limiting factors. First the scope was restricted to investigate only morphologic changes of constricted nerve and its associated DRGs during CCI and after decompression. Thus behavioral tests to evaluate the signs of neuropathic pain such as allodynia and hyperalgesia or biochemical analysis to determine neuroinflammatory response were not conducted. Second the pathologic condition is limited to eight weeks period intervened at first, third and fifth week to determine the appropriate timing of decompression for a given disease period. Therefore time course after decompression was not equal between the groups. One can argue that recovery period was shorter for late decompressed groups. Thus degeneration is more apparent in these groups. On the contrary, El-Barrany et al. [30] reported that degenerative changes have been reversed as time course after decompression prolonged. According to their findings, severe degenerative changes in schwann cells and myelinated axons were observed one week after decompression. After the second week of decompression, the endoneurium showed extensive edema with an increase in the regenerating myelinated and unmyelinated nerve fibers. Three weeks after decompression, edema decreased and six weeks after decompression, the endoneurium appeared nearly normal. Nevertheless a further study in our laboratory was planned to investigate how decompression at first, third and fifth weeks affect on morphologic recovery of peripheral nerve and DRG and whether these changes are reversible after a constant follow-up period (8 week after decompression).

Results of our light and electron microscopy studies showed that early decompression preserve the histopathological features of nerve at the constriction site and DRG cells. In the late decompression groups, ultrastructural damages were observed as more intensely similar to the CCI group (Table 2).

Our study data support previous reports of the importance of early surgical intervention in providing optimal treatment for nerve compression conditions. Future quantitative histological studies on regeneration of constricted sciatic nerve will enable us to have better understanding of these mechanisms.

REFERENCES

- Smith HS, Argoff CE:** Pharmacological treatment of diabetic neuropathic pain. *Drugs*, 71, 557-589, 2011. DOI: 10.2165/11588940-000000000-00000
- Kumar K, Taylor RS, Jacques L, Eldabe S, Meglio M, Molet J, Thomson S, O'Callaghan J, Eisenberg E, Milbouw G, Buchser E, Fortini G, Richardson J, North RB:** Spinal cord stimulation vs conventional medical management for neuropathic pain: A multicentre randomised controlled trial in patients with failed back surgery syndrome. *Pain*, 132, 179-188, 2007. DOI: 10.1016/j.pain.2007.07.028
- Jaggi A.S, Singh N:** Role of different brain areas in peripheral nerve injury-induced neuropathic pain. *Brain Res*, 1381, 187-201, 2011. DOI: 10.1016/j.brainres.2011.01.002
- Zieglgansberger W, Berthele A, Tolle TR:** Understanding neuropathic pain. *CNS Spectr*, 10, 298-308, 2005. DOI: 10.1017/S1092852900022628
- Sacerdote P, Franchi S, Moretti S, Castelli M, Procacci P, Magnaghi V, Panerai AE:** Cytokine modulation is necessary for efficacious treatment of experimental neuropathic pain. *J Neuroimmune Pharmacol*, 8, 202-211, 2013. DOI: 10.1007/s11481-012-9428-2
- Kim S, Chung JM:** An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain*, 3, 355-363, 1992. DOI: 10.1016/0304-3959(92)90041-9
- Austin PJ, Kim CF, Perera CJ, Moalem-Taylor G:** Regulatory T cells attenuate neuropathic pain following peripheral nerve injury and experimental autoimmune neuritis. *Pain*, 153, 1916-1931, 2012. DOI: 10.1016/j.pain.2012.06.005
- Tseng TJ, Chen CC, Hsieh YL, Hsieh ST:** Effects of decompression on neuropathic pain behaviors and skin reinnervation in chronic constriction injury. *Exp Neurol*, 204, 574-582, 2007. DOI: 10.1016/j.expneurol.2006.12.018
- Tseng T, Hsieh Y, Hsieh S:** Reversal of ERK activation in the dorsal horn after decompression in chronic constriction injury. *Exp Neurol*, 206, 17-23, 2007. DOI: 10.1016/j.expneurol.2007.04.006
- Prinz RA, Nakamura-Pereira M, De-Ary-Pires B, Fernandes D.S, Fabiao-Gomes BD, Bunn PS, Martinez AM, Pires-Neto MA, Ary-Pires R:** Experimental chronic entrapment of the sciatic nerve in adult hamsters: An ultrastructural and morphometric study. *Braz J Med Biol Res*, 36, 1241-1245, 2003. DOI: 10.1590/S0100-879X2003000900015
- Jancálek R, Dubový P:** An experimental animal model of spinal root compression syndrome: An analysis of morphological changes of myelinated axons during compression radiculopathy and after decompression. *Exp Brain Res*, 179, 111-119, 2007. DOI: 10.1007/s00221-006-0771-5
- Özaydın İ, Ünsaldı E, Aksoy Ö, Yayla S, Kaya M, Ulkay Tunali MB, Aktaş A, Taşdemiroğlu E, Cihan M, Kurt B, Yıldırım HC, Şengöz A, Erdoğan H:** The effect of silicone tube and silicone tube + hyaluronic acid application on adhesion formation in experimental peri- and epineurolysis in a rat model. *Kafkas Univ Vet Fak Derg*, 20, 591-597, 2014. DOI: 10.9775/kvfd.2014.10583
- Reina MA, López A, Villanueva MC, De Andrés JA, Machés F:** The blood-nerve barrier in peripheral nerves. *Rev Esp Anestesiología Reanim*, 50 (2): 80-86, 2003.
- Castejon OJ, Castejon HV, Diaz M, Castellano A:** Consecutive light microscopy, scanning-transmission electron microscopy and transmission electron microscopy of traumatic human brain oedema and ischaemic brain damage. *Histol Histopathol*, 16 (4): 1117-1134, 2001.
- Tseng TJ, Chen CC, Hsieh YL, Hsieh ST:** Influences of surgical decompression on the dorsal horn after chronic constriction injury: Changes in peptidergic and delta-opioid receptor (+) nerve terminals. *Neuroscience*, 156, 758-768, 2008. DOI: 10.1016/j.neuroscience.2008.08.010
- Prinz R.A, Nakamura-Pereira M, De-Ary-Pires B, Fernandes D, Fabião-Gomes BD, Martinez AM, de Ary-Pires R, Pires-Neto MA:** Axonal and extracellular matrix responses to experimental chronic nerve entrapment. *Brain Res*, 1044, 164-175, 2005. DOI: 10.1016/j.brainres.2005.02.085
- Tseng TJ, Hsieh YL, Ko MH, Hsieh ST:** Redistribution of voltage-gated sodium channels after nerve decompression contributes to relieve neuropathic pain in chronic constriction injury. *Brain Res*, 1589, 15-25, 2014. DOI: 10.1016/j.brainres.2014.07.012
- Tseng TJ, Hsiao TH, Hsin T, Hsieh ST, Hsieh YL:** Determinants of nerve conduction recovery after nerve injuries: Compression duration and nerve fiber types. *Muscle Nerve*, 52, 107-112, 2015. DOI: 10.1002/mus.24501
- Finnerup NB, Otto M, Jensen TS, Sindrup SH:** Algorithm for neuropathic pain treatment: An evidence based proposal. *Pain*, 118, 289-305, 2005. DOI: 10.1016/j.pain.2005.08.013
- Vranken JH:** Elucidation of pathophysiology and treatment of neuropathic pain. *Cent Nerv Syst Agents Med Chem*, 12, 304-314, 2012. DOI: 10.2174/187152412803760645
- Hama A, Sagen J:** Altered antinociceptive efficacy of tramadol over time in rats with painful peripheral neuropathy. *Eur J Pharmacol*, 559, 32-37, 2007. DOI: 10.1016/j.ejphar.2006.11.047
- Kukkar A, Singh N, Jaggi AS:** Attenuation of neuropathic pain by sodium butyrate in an experimental model of chronic constriction injury in rats. *J Formos Med Assoc*, 16, pii: S0929-6646(13)00180-0, 2013. DOI: 10.1016/j.jfma.2013.05.013
- Jancálek R, Dubový P, Svíženská I, Klusáková I:** Bilateral changes of TNF-alpha and IL-10 protein in the lumbar and cervical dorsal root ganglia following a unilateral chronic constriction injury of the sciatic nerve. *J Neuroinflammation*, 10, 7-11, 2010. DOI: 10.1186/1742-2094-7-11
- Basbaum AI, Gaulton M, Jazat F, Mayes M, Guilbaud G:** The spectrum of fiber loss in a model of neuropathic pain in the rat: An electron microscopic study. *Pain*, 47, 359-367, 1991. DOI: 10.1016/0304-3959(91)90229-Q
- Shimoyama M, Tanaka K, Hasue F, Shimoyama NA:** Mouse model of neuropathic cancer pain. *Pain*, 99, 167-174, 2002. DOI: 10.1016/S0304-3959(02)00073-8
- Munger BL1, Bennett GJ, Kajander KC:** An experimental painful peripheral neuropathy due to nerve constriction. I. Axonal pathology in the sciatic nerve. *Exp Neurol*, 118, 204-214, 1992. DOI: 10.1016/0014-4886(92)90037-Q
- Krames ES:** The dorsal root ganglion in chronic pain and as a target for neuromodulation: A review. *Neuromodulation*, 18, 24-32, 2015. DOI: 10.1111/ner.12247
- Brázda V, Klusáková I, Hradilová Svíženská I, Dubový P:** Dynamic response to peripheral nerve injury detected by in situ hybridization of IL-6 and its receptor mRNAs in the dorsal root ganglia is not strictly correlated with signs of neuropathic pain. *Mol Pain*, 9, 42, 2013. DOI: 10.1186/1744-8069-9-42
- Dubový P, Jancálek R, Klusáková I, Svíženská I, Pejchalová K:** Intra- and extraneuronal changes of immunofluorescence staining for TNF-alpha and TNFR1 in the dorsal root ganglia of rat peripheral neuropathic pain models. *Cell Mol Neurobiol*. 26, 1205-1217, 2006. DOI: 10.1007/s10571-006-9006-3
- El-Barrany WG, Hamdy RM, Al-Hayani AA, Jalalah SM, Al-Sayyad MJ:** Ultrastructural changes of compressed lumbar ventral nerve roots following decompression. *Saudi Med J*, 27 (7): 955-961, 2006.