ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

TJPS

TIKRIT JOURNAL OF PURE SCIENCE

Journal Homepage: http://main.tu-jo.com/ojs/index.php/TJPS/index

Electron Microscope Evaluation of late changes in the nerve fibers and neurons induced by Sciatic nerve section in rabbits

Ban Ismael Sedeeq[1] , Ayad Hammeed Ibrahim[2] , Abul Kareem Salem Mahood[3]

[1] *College of Dentistry, University of Tikrit, Tikrit, Iraq*
[2] *College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq*
[3] *College of Medicine, Wasit University, Kut, Iraq*

DOI: http://dx.doi.org/10.25130/tjps.23.2018.122

ARTICLE INFO.

Article history:
-Received: 18 / 3 / 2018
-Accepted: 4 / 6 / 2018
-Available online: / / 2018

Keywords: sciatic nerve section, electron microscope, late changes, rabbit.

Corresponding Author:
Name: Ban Ismael Sedeeq
E-mail: drbanasnan@gmail.com
Tel:

Abstract

The aim of the study was to demonstrate late changes occurs after Sciatic nerve section [nerve fibers and their associated neurons]. Five adult rabbits were used in this study. Sciatic nerve was sectioned under general anesthesia. After ninety days, all animals were sacrificed and samples were taken from ventral horn of spinal cord, dorsal root ganglion at L_7 segment level and from sciatic nerve for electron microscope evaluation. The results revealed different changes include: separation of the myelin sheath lamellae within ventral horn, the Nissl bodies were scattered within sensory neuron thoughout the cytoplasm specially at the periphery, the cytoplasm contain numerous closed vesicles of different size associated with endoplasmic tubules and the myelin sheath usually forming a loop, axons seemed relatively smaller than normal. All of which are common features of axonal atrophy.

Introductions

Ultrastructural changes have been described in many organelles in the neurons during axon transection and most of the changes have been interpreted as acellular reorganization in preparation for axonal regeneration. However,yet there is little agreement about the various changes, and part the reports are mutually contradictory [1,2]. One reason for the conflicting findings could be related to species differences or different reactions in various nerve cell types [3]. Jirmanova clarified in his study on young chickens, the ultrastructural axotomized perikarya of spinal cord after peripheral nerves of the brachial plexus transaction. In axotomized neuron of spinal cord and during first post operative week, Nissl bodies are slightly reduced in size but remain scattered throughout the cytoplasm. Mitochondria and dense bodies generally increased in number are also dispersed in the cytoplasm. During second week, chromatolysis occurs in the majority of perikarya. The nuclei are displaced from their central position and in some instances almost touch the cytoplasmic membrane. The number of mitochondria and dense bodies massed in the central region increased during this week [4]. Zelena revealed different results in axotomized perikarya of sensory neurons after section in the nerves of brachial plexus. The majority of the axotomizedperikarya developed central chromatolysis In such neurons, Nissl bodies virtually disappeared from the central area of the neuron and formed more or less continuous zone at the cell circumference. The cytocentrum become filled with large numbers of mitochondria, dense bodies and other organelles. Neurofilaments and microtubules were disarranged and ran at random among the accumulated particles. Microtubules were often more prominent in chromatolytic areas than neurofilaments [5]. Most electron microscopic studies, clarified the acute changes occurs after nerve section, the present study was designated to demonstrate the late changes occurs after sciatic nerve section [at mid thigh region] in the nerve fibers and their associated neurons.

Meterials and methods

Five adult rabbits were used in this study. All were allowed free access to food and water before the experiment. The surgical operations were made under general anesthesia (IM) of [50 mg/kg] Ketamin hydrochloride with xylazine hydrochloride [10 mg/kg]. A longitudinal incision about 2.5 cm was made on the lateral side of the thigh parallel to longitudinal axis of Femur bone, and then separation between muscles continues until reaching the area where the sciatic nerve lies.

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

The nerve was sectioned by small surgical scissor. Then the two edges were retuned back to their original position. The muscles were also retuned back to their original positions and sutured by continuous suture using (chromic catgut) size 3.0. The skin sutured by interrupted suture using (Black silk) size 3.0. Prophylactic antibiotic solution injected (IM) for three days after operation to prevent infection.

Three months later, all animals were sacrificed and samples were taken from ventral horn of spinal cord, dorsal root ganglion at L_7 segment level and from sciatic nerve. The fresh tissues were taken immediately as small blocks about 1 mm^3 and placed in a fixative solution composed of 2.5% glutaraldehyde & 2% paraformaldehyde buffered to pH 6.8 with 0.1 M sodium cacodylate. The tissues were kept in this fixative for at least 2 hours at 4 C°.

Results

Ventral horn: changes could not be observed by electron microscope in the moto-neurons of experimental side, but there was a slight change in some myelinated nerve fibers, this change was loosening or separation of the myelin lamellae (fig 1).

Dorsal root ganglion: Sensory neurons in the dorsal root ganglion showed, that the nuclei of neurons return back to their normal position [center of the cell], the nuclear membrane appeared regular , Nissl bodies scattered throughout the cytoplasm specially at the periphery, and the cytoplasm contained numerous closed vesicles of different size associated with endoplasmic tubules (fig 2&3). Other change was observed in the dorsal root ganglion of the experimental side compared with that of the control side was, the changes in the myelin sheath were observed clearly in the longitudinal section. The myelin usually formed a loop, evaginated to the outside or invaginated to the inside. This loop might be separated from its origin and forming a small round or oval mass of myelin which contained no lumen located either in the Schwann cell cytoplasm or in side axoplasm respectively (fig 4). **Proximal Stump:**As in the dorsal root ganglion, the same changes were observed in the proximal stump ninety days after sciatic nerve section. Myelin sheath made a loop of their full thickness. This loop either evaginated to the outside [outfolded] or invagenated to the inside [infolded] (fig 5). Although, some myelinated nerve fibers showed a severe separation in its lamellae with atrophy in their axons (fig 6), other myelinated nerve fibers clarified, normal axons, normal myelin lamellae but with irregular outline and the fibers seemed relatively smaller than control and the spaces among the nerve fibers filled with fibrotic tissues (fig 7). **Distal stump:**There was a large spaces between the regenerated fibers filled with fibrotic tissues similar to that was found in the proximal stump. In some regions, an old myelin remnants still found and a new process of degeneration was observed (fig 8).

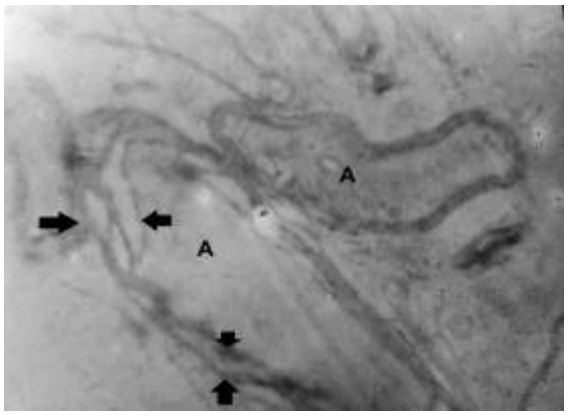

Fig 1: Adult group, spinal cord right side [experimental].90 days after sciatic nerve section. Loosening [separation] in the myelin lamellae of large myelinated fibers [arrows], axons [A].[EM 25000X]

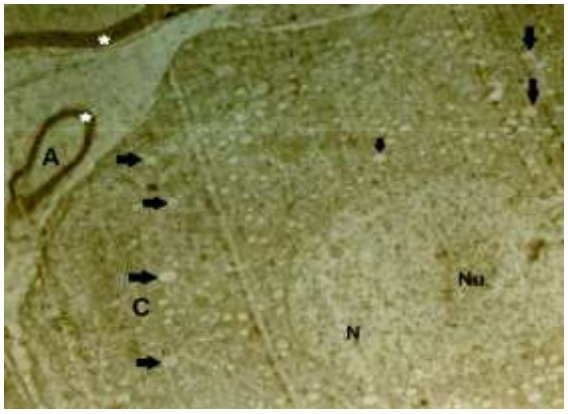

Fig 2: Adult group, Sensory neurons, 90 days after nerve section. Numerous endoplasmic vesicles [arrows], centrally located nucleus [N] with regular nuclear membrane, Nucleolus [Nu], cytoplasm [C], axon [A], myelin [stars]. [EM 4600 X]

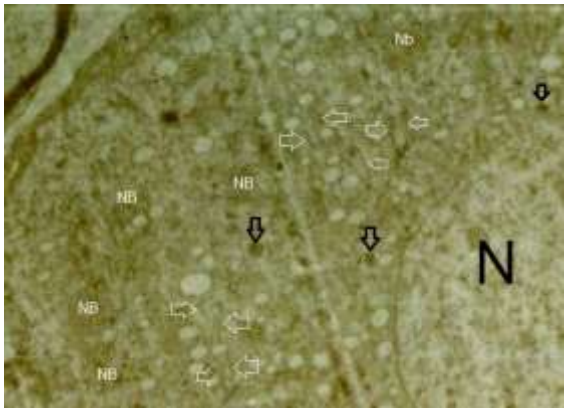

Fig 3: magnified picture of figure [2]. Nissl bodies [NB], endoplasmic tubules [white arrows], mitochondria [black arrows] and nucleus [N]. (computerized zoom)

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

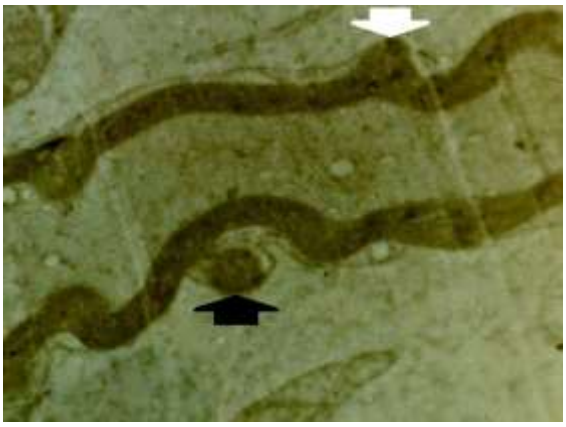

Fig 4: dorsal root ganglion-experimental side. longitudinal section through myelinated nerve fiber, 90 days after nerve section. Out folded of myelin loop [white arrow] and separation of myelin loop in to small mass [black arrow]. [EM 7900 X].

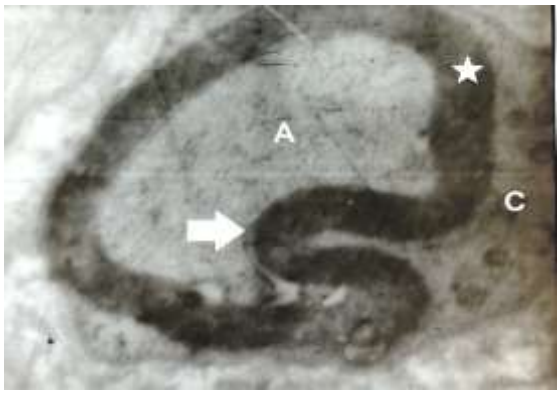

Fig 5: cross section through proximal stump. 90 days after nerve section. Infolded of myelin loop [arrow], axon [A], myelin sheath [star] and Schwann cell cytoplasm [C]. [EM 25000 X].

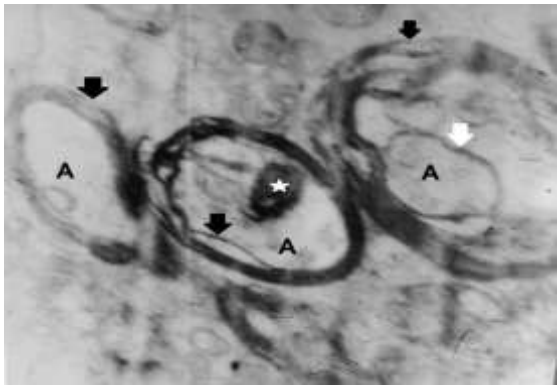

Fig 6: cross section through proximal stump. 90 days after nerve section. Myelin abnormalities: loosening of lamellae [black arrows], very few lamellae [white arrow] remain around atrophied axon [A], myelin loop incorporated inside the axoplasm forming an oval mass [star]. [EM 25000 X].

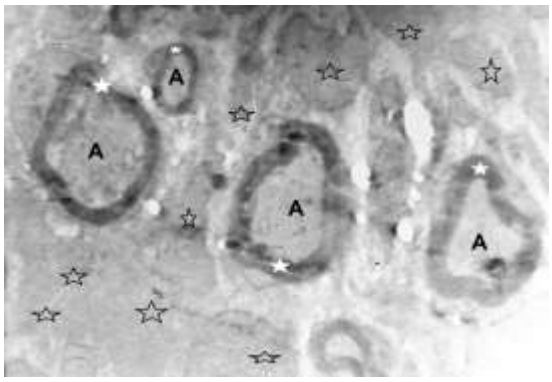

Fig 7: proximal stump of experimental side. Normal appearance of myelin sheath [white stars] and axons [A], fibrosis [black stars]. [EM 5800X].

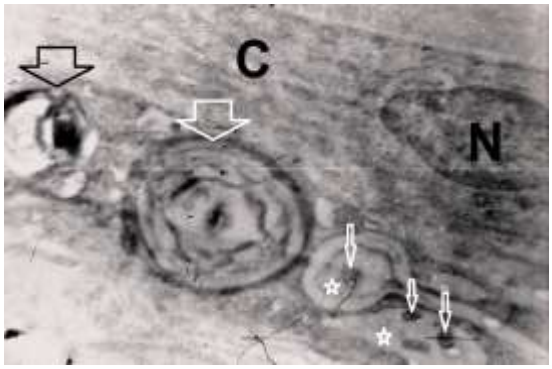

Fig 8: cross section through distal stump. 90 days after nerve section. Myelin remnant [black arrow], demyelintion [large white arrow], newly formed axons [small white arrows] surrounding by Schwann cell cytoplasm [stars], fibroblast nucleus [N] and cytoplasm [C]. [EM 13500 X].

Discussion

Interestingly, results of electron microscope, revealed that, the myelin sheath usually of large fibers in the spinal cord, dorsal root ganglia and proximal stump showed, abnormalities such as separation of their lamellae and formation of myelin loops. all of which are common features of axonal atrophy (Krinke et al [6]). Kerezoudi et al [7] described the changes in the myelin sheath of sectioned sciatic nerve fibers after different survival time by the presence of focal intramyelinicoedema which lead to separation of myelin lamellae on the operated side just proximal to the section. This can be discussed that growth factor deprivation secondary to axotomy is implicated in these changes. Ceballos et al [8] revealed that the process of myelin loop formation. The infolded or the outfolded of myelin sheath resulted from invagination or evagination, with approximation of the lamellae giving rise to free myelin loop which was redundant myelin. It is well known that neurofilaments are major determinants of axonal caliber [9][1]. Failure in the maintaining of cytoskeletal framework, resulting in a decrease of axonal size [10]. In the dorsal root ganglia, the cytoplasm of some sensory neurons showed, numerous closed vesicles or vacuoles of different size associated with endoplasmic tubules. The same results were examined by Tiraihi&Rezaie

[11] in newborn rats following sciatic nerve axotomy which included an early vaculation in Golgi apparatus and endoplasmic reticulum that was associated by mitochondrial and nuclear changes. Edmund & Mary [12] revealed that, abnormal dilation of cisternal elements of granular endoplasmic reticulum leads to the formation of large vacuoles. Structural components of the Golgi complex also may exhibit extensive dilatation. Vacuoles might be formed by other cytoplasmic organelles such as mitochondria.

Recent study was made by Johnson &Sears [13] about the role of the peripheral target in regulating the RER and polyribosomes of Nissl bodies in axotomised adult cat dorsal root ganglion neurons where axonal regeneration and peripheral target reinnervation was either allowed or denied. Indications of polyribosomal dispersal were seen by 6h following axotomy, and by 24h the normal orderly arrangement of lamellae of RER in Nissl bodies had become disorganised. The retrograde response was maximal 8-32 day after axotomy. By 64 day following axotomy with reinnervation, approximately half neurons showed restoration of the orderly arrangement of RER and polyribosomes in their Nissl bodies. This was not seen after axotomy with reinnervation denied. Saggu et al [14] found that, partial or complete loss of axon transformed the myelin into collapsed structures which appeared as 'myelin bodies' in the extracellular space of the distal stump. This observation was in agreement with the current study. In addition to, there was a degenerative process in some fibers while other fibers showed well developed axon surrounding by relatively thick myelin sheath. It has been well documented that axons regeneration may at times successfully traverse long gaps spontaneously, despite the presence of substantial scar tissue. This procedure provides no guarantee of proper fascicle orientation, of course, and regenerating axons may grow into functionally inappropriate endoneurial tubes or even may fail to reenter an endoneurial tube [15].

References

[1] Evans DHL and Gray EG.(1961). Changes in the fine structure of ganglion cells during chromatolysis, In: Cytology of nervous tissue, Proc. Anat. Soc. Gr. Britanian and Ireland. London: *Taylor and Francis Ltd;* P. 71-74.

[2] Hudson G, Lazaro A, Hartman JF. (1961). A quantitative electron microscopic study of mitochondria in motor neurons following axonal section. *Exp, Cell Res.* ;(24):440-56.

[3] Torvik A, Skjörten F. (1971). Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions, *Acta neuropath* (17): 248-64.

[4] Jirmanova I. (1971). Glycogen deposits in Motoneurons of young chickes following peripheral nerve section. *Acta neuropath* ;19:110-20.

[5] Zelena J. (1971). Neurofilaments and microtubules in sensory neurons after peripheral nerve section. *Cell and tissue Research* ;117(2):191-211.

[6] Krinke, FE.; Heumann, M.; Schnider, K., Da Silva, F. and Suter J. (1988). Adjustment of the myelin sheath to axonal atrophy in the rat spinal root by the formation of infolded myelin loops. *Acta Anatomica*; 131: 182-7.

[7] Kerezoudi, E.; King, RH., Muddle, JR.; O'Neill, JA. And Thomas, PK. (1995). Influence of age on the late retrograde effects of sciatic nerve section in the rat. *J. Anat.*;187(Pt 1):27– 35.

[8] Ceballos, D.; Cuadras, J.; Verdu, E. and Navarro X. (1993). Morphometric and ultrastructural changes with aging in mouse peripheral nerve. *J. Anat.*; 195:563-76.

[9] Muma, NA. and Hoffman, PN. (1993). Neurofilaments are intrinsic determinants of axonal caliber. *Micron*; 24:677-83.

[10] Verdu E, Ceballos D, Vilches J, Navarro X. Influence of aging on peripheral nerve function and regeneration.J Peripher NervSyst 2000; 2(4):191-208.

[11] Tiraihi, T. and Rezaie, MJ. (2003). Apoptosis onset and box protein distribution in spinal motoneurons of newborn rats following sciatic nerve axotomy. *Interna J. Neurosci.* ;113(9):1163-75.

[12] Edmund, BM. and, Mary, BB. (1967). Cytological studies of organotypic culture of rat dorsal root ganglia follinf X- Irradiation in vitro. *J. Cell Biol.* 1967;32: 467-95.

[13] Johnson, IP. and Sears, TA. (2013). Target-dependence of sensory neurons: an ultrastructural comparison of axotomised dorsal root ganglion neurons with allowed or denied reinnervation of peripheral targets. *Neuroscience*. 228:163-78.

[14] Saggu, SK.; Chotaliya, HP.; Blumbergs, PC. And Casson, RJ. (2010). Wallerian-like axonal degeneration in the optic nerve after excitotoxic retinal insult: an ultrastructural study. *BMC Neuroscience*;11:97.

[15] Burnett, MG. and Zager, EL. (2014). Pathophysiology of Peripheral Nerve Injury: A Brief Review. *Neurosurg Focus.*;16(5):1-7.

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

تقييم المجهر الالكتروني للتغيرات المتأخرة في الالياف العصبية والعصبونات والمتسبب عن قطع العصب الوركي في الارانب

بان اسماعيل صديق[1] ، أياد حميد ابراهيم[2] ، عبد الكريم سالم ماهود[3]

[1]كلية طب الاسنان ، جامعة تكريت ، تكريت ، العراق

[2]كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق

[3]كلية الطب ، جامعة واسط ، الكوت ، العراق

الملخص

الهدف من هذه الدراسة هو إظهار التغيرات المتأخرة التي تحدث بعد قطع العصب الوركي [الألياف العصبية والخلايا العصبية المرتبطة بها]. استخدمت في هذه الدراسة خمسة أرانب بالغين. قطع العصب الوركي تحت تاثير التخدير العام. بعد تسعين يوما، تم التضحية بجميع الحيوانات وأخذت عينات من القرن البطني للحبل الشوكي، وعقدة الجذر الظهري في مستوى القطعة L7ومن العصب الوركي لتقييمها تحت المجهر الإلكتروني. كشفت النتائج عن تغيرات مختلفة تشمل: أنفصال صفائح غمد النخاعين داخل القرن البطني، وأنتشار أجسام نيسل داخل سيتوبلازم الخلايا العصبية الحسية خاصة في المحيط، و احتواء السيتوبلازم على العديد من الحويصلات المغلقة بأحجام مختلفة مرتبطة بأنابيب الشبكة الإندوبلازمية وغمد المايلين يشكل حلقةعادة، والمحاور العصبية تبدو أصغر نسبيا من الطبيعي. وكلها سمات مشتركة تدل على ضمور المحاور عصبية.