ALTERATION OF SCIATIC NERVE CONDUCTION VELOCITY IN RATS SUBJECTED TO IMMobilIZED STRESS

By

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ABSTRACT

Background: Limb immobilization is one of treatments for managing musculoskeletal injury. Although immobilization often benefit the affected part of the body, when prolonged, it often harms the rest of body.

Objective: Studying the morphological and functional changes in the nerves of immobilized muscles.

Materials and Methods: Thirty four adult male albino rats weighing from 140 –160 g were included in the study. The rats were classified into two equal groups: control group included rats not exposed to immobilization, and experimental group included rats exposed to immobilization. After 14 days of immobilization, the rats were sacrificed and the sciatic nerve was dissected and placed in moist nerve chamber, then stimulated by power lab 4/25 stimulator for measuring the nerve conduction velocity (NCV), and the amplitude of action potential.

Results: Immobilized group showed a significant decrease in both the NCV, and amplitude of action potential of sciatic nerve in comparison to the control group. In addition, there were histological findings suggesting degeneration of myelin sheath and nerve axons.

Conclusion: Limb immobilization altered the physiological parameters and histological characters of sciatic nerve.

Key Words: Limb immobilization, sciatic nerve, conduction velocity, action potential amplitude

INTRODUCTION

Spinal cord injury (SCI) is a serious condition that may lead to long term disabilities. Immobilization is one of the methods used to treat traumatological problems. Spinal Immobilization has been considered the standard prehospital care for suspected SCI patients (Oteir et al., 2014). Local immobilization involves casting and splinting, whereas systemic immobilization is accomplished by body casting and bed rest (Millis and Levine, 2014). The physiological and structural modifications in the nerve are proportional to the level of stress and duration of immobilization (Alves et al., 2013). Long term immobilization is known to result in bone loss (Siev?nen, 2010).
After an injury, common signs and symptoms include swelling, redness, pain and decrease functional abilities. Recovering from most injuries requires time to reduce the symptoms and to regain normal function and level of activity through physical therapy. During this recovery period there could be a time immediately following the injury when the area must be immobilized to protect it from re-injury and reduce the acute signs and symptoms (Powers and Jackson, 2008). Immobilization has a dramatic effect on the musculoskeletal system. Immobilization osteoporosis represents a severe complication in hemiplegic patients (HPs), causing fragility fractures, which may occur during rehabilitation reducing functional recovery and survival (Del Peuente et al., 2016).

The aim of the present work was to study the effects of limb immobilization as one of the modalities used in treatment of traumatological problems on the physiological parameters and histological characters of sciatic nerve.

**MATERIALS AND METHODS**

**Animals:** Thirty four adult male albino rats (140-160g) of a local strain were included. All animals were kept in the animal care in Institute of National Ophthalmology, and were provided ordinary rat chow and water ad libitum with a normal light-dark cycles. The experimental protocol and procedures were approved by the Institutional Animal Care and Use Committee of Cairo University. Animals were kept for 10 days prior to the start of study to allow proper acclimatization. Animals were randomly allocated into control group and immobilized group.

**Methods:** Immobilization was done for 14 days via a waterproof tape wrapped around the pelvis, hip, knee, and ankle of the right hind leg in order to achieve full immobilization (Santos-Júnior et al., 2010). All rats were kept in cages with dimensions (45 X 25 X 20 cm), in which one rat in each cage.

At the end of the study (it lasted 8 months), animals were sacrificed (Osanai et al., 2010) in Physiology lab, Faculty of Medicine, Cairo University. Sciatic nerve was identified, dissected, and placed in moist nerve chamber, then stimulated by power lab 4/25 stimulator for recording compound action potential (CAP). Conduction velocity (m/sec) was calculated by dividing distance between electrodes (meter) by time interval (sec) (Ganga et al., 2012 and Alves et al., 2013).

Histological Examination was done at histology lab, Faculty of medicine, Cairo university. After calculations, nerves were removed for histopathological examination. Paraffin blocks were prepared [the thickness of the sections was about 7 – 10 micrometers (μm) for: A-Staining with hematoxylin and eosin (Vazquez, 2014) to give a good picture for the nerve axon only in the middle of a hazy ring - like structure which represented the remains of the myelin sheath that was dissolved by the hematoxylin and eosin stain (not suitable
for staining of lipid). B- Staining with osmic acid (Wei et al., 2007) to observe the myelin sheath surrounding the axon. C- Electron Microscope (Russel and Bozzola, 1999) to give a sharp details about the changes affecting myelin sheath and nerve axons.

**Statistical analysis:** Quantitative data were summarized as means± standard deviations and compared using one-way analysis-of-variance (ANOVA) ,followed by Bonferroni post-hoc test to detect which pairs of groups caused the significant difference. P-values <0.05 were considered statistically significant. Calculations were made on social package of statistical science (SPSS) software 16 (Emsley et al., 2010).

**RESULTS**

The mean values of NCV of control group was $0.38 \pm 0.09$ meter/sec, but of the immobilized group was $0.29 \pm 0.09$ meter/sec. The mean value of NCV of immobilized group showed a significant decline (P value = 0.01) in comparison to control group. The percentage change of the mean values of NCV showed a decline by 23.7%. The difference between the mean values of both groups was 0.09318 that was confirmed by 95% confidence interval test which told us that the mean difference was located by 95% between the lower limit (0.024) and the upper limit (0.162) (Table 1 & Fig 1a).

The true value of the mean difference was between 0.0238 to 0.162.

The mean value of action potential amplitude of the immobilized group showed a significant decline (P value <0.01) in comparison to control group. It was $1.29 \pm 0.24$ volts in immobilized group, while it was $1.6 \pm 0.1$ voltsin control one. The amplitude declined by 19.3%. The difference between the mean values of both groups was 0.371 that was confirmed by 95% confidence interval test. The mean difference was located by 95% between the lower limit (0.229) and the upper limit (0.512) (Table 2 & Fig.1b).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control group (No = 17)</th>
<th>Immobilized group (No = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCV (meter/sec)</td>
<td>0.38 ± 0.09</td>
<td>0.29 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>% of change</td>
<td>-23.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.0931</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td></td>
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<tr>
<td>95% confidence interval of the difference #</td>
<td>0.0238 to 0.162</td>
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</table>

NCV: Nerve Conduction Velocity.
Table (2): Mean values of action potential amplitude of both groups (Mean± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (No. = 17)</th>
<th>Immobilized group (No = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action potential amplitude (volt)</td>
<td>1.6 ± 0.1</td>
<td>1.29 ± 0.24</td>
</tr>
<tr>
<td>% of change</td>
<td>-19.3%</td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.371</td>
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</tr>
<tr>
<td>P-value</td>
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<tr>
<td>95% confidence interval of the difference</td>
<td>0.229 to 0.512</td>
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The true value of the mean difference was between 0.229 to 0.512.

Figure (1a): Effect of immobilization on nerve conduction velocity of both groups.
The results were expressed as mean ± SD.
* Significant change comparing to the control group

Figure (1b): Effect of immobilization on action potential amplitude of both groups.
The results were expressed as mean ± SD.
* Significant change comparing to the control group.

Histological Examination:

Hematoxylin and eosin stain: Control sections revealed normal architecture of nerve bundle surrounded by perineurium. Within each bundle, there was endoneurium. Each nerve bundle was formed of numerous nerve fibers (Fig. 2a). Sections in the nerve tissue in immobilized group showed reduction in diameter of nerve bundle (in comparison to the non-immobilized one) and separation of the nerve bundles from the perineurium (Fig 2b). Hyperplasia of Schwann cell, widening of endoneurium, reduction in the nerve fibers density within the nerve bundle and some of the nerve fibers were divided into multiple compartments (digesting chambers). Aggregation of lymphocytes occurred within the nerve bundle, and some nerve fibers contained no axons others with peripherally located axons (eccentric axons) (Fig 2c).
Osmic Acid stain: In control group, numerous myelin sheath appeared as a black circles surrounding the axon (Fig 3a). In immobilized group, there were decrease in the density of nerve fibers within the nerve bundle, nerve fibers with thinning myelin sheath, and nerve fibers with digesting chambers (Fig3b).
Electron Microscope: Control group showed nerve fiber with its axon surrounding by non-interrupted layer of myelin sheath (Fig. 4a). Immobilized group showed degeneration of myelin sheath (Fig. 4 b), axonal separation from the myelin atrophied myelin, hyperactive Schwann cell nerve fibers with digestive cavities (Fig 4 c).

Figure (3a): Photomicrograph of sections in the nerve bundle of the control non-immobilized rat in (Osmic acid x 400). Showing multiple nerve fibers surrounded by black and thick ring (myelin sheath) (white Arrow).

Figure (3b): Photomicrograph of sections in the nerve bundle of the immobilized rat in (Osmic acid x 400). Showing nerve fibers with multiple digesting chambers (green arrows) and nerve fibers with thinning myelin sheath (white arrows).

Figure (4a): EM picture of the nerve fiber of control non-immobilized rat. Magnification 6000x. showing the axon (green arrow), myelin sheath (white arrow), Schwann cell (brown arrow) and the mitochondria with its cristae (red arrow).

Figure (4b): EM picture of nerve fiber of immobilized rat, magnification 6000x. Showing nerve fiber with degeneration of myelin sheath (black arrows).

Figure (4c): E.M picture of rat sciatic nerve fibers of immobilized rat, magnification 3000 x. showing nerve demyelination “axons without myelin (brown arrow)”, hyperactive nucleus of Schwann cell “pale, large with 2 nucleoli” (white arrow), atrophied myelin (black arrow) and axonal separation from myelin (yellow arrow).

Figure (4d): E.M picture of rat sciatic nerve fibers of immobilized rat, magnification 3000 x. Showing nerve fiber with degeneration of myelin sheath (black arrows).
DISCUSSION

Alves et al. (2013) and Yoshida et al. (2013) state that traumatic injuries and their treatments frequently lead to long-lasting limb immobilization. Traditional approaches to distal radius fractures have included both surgical and nonsurgical treatments. Nonsurgical approaches, include immobilization with or without reduction. It can be the best treatment according to age and other factors like nature of injury and joint movement (Ikpeze et al., 2016). The immobilization may disable the function of the injured limb (Alves et al., 2013).

Under conditions of immobilization, such as casting, splinting, peripheral nerves are exposed to levels of physical stress (Alves et al., 2013). Disuse osteopenia and bone loss have been extensively reported in long duration space mission and long term bed rest. The pathology of the bone loss is similar to osteoporosis, but highly confined to weight bearing bones (Uddin and Qin, 2015).

The results of the present work showed that limb immobilization resulted in significant reduction in the sciatic nerve conduction velocity and in its action potential amplitude. The results coincided with that of Alves et al. (2013) who documented that immobilization had a depressive effect on both the amplitude and the conduction velocity of action potential. Limb immobilization resulted in structural changes in nerves through degeneration of motor end plate (Higuchi et al., 2008 and Paulo et al., 2013). Alves et al. (2013) reported that immobilization carried out by plaster casting of the limb resulted in myelin degeneration and deposition of collagen in the endoneurium. Yoshida et al. (2013) proved that immobilization of the knee joints of rats resulted in characteristic histological changes in the connective tissue around the sciatic nerve. Their study suggested that there was adhesion of the perineurium between the bundle of nerve fibers and the peripheral tissue of the nerve. Canu et al. (2009) reported that hind limb unloading reduced the myelin thickness of the peripheral nerves.

De Lahunta et al. (2009) documented that digesting chamber was a term used for describing the degeneration of myelin with axonal fragments inside it. The changes of digesting chambers were associated with degenerative responses to nerve injury (Whitney et al., 2011). Several studies explained the mechanisms of myelin degeneration. Jessen and Mirsky et al. (2008) reported that demyelination in Schwann cells started mechanically with fragmentation of myelin sheath. Schwann cells were suggested to be responsible for the synthesis and maintenance of the myelin sheath in the peripheral nerve system (Kim et al., 2014). Fontana et al. (2012) and Lee et al. (2014) documented that Schwann cell dedifferentiation is a phenomenon exhibited by Schwann cells in the process of demyelination due to acquired nerve damage. The dedifferentiated Schwann cells suggest that Schwann cells actively participated in demyelinating process (Arthur-Farraj et al., 2012). The protein Krox – 20 is an essential driver of the myelination program and is needed for formation and maintenance of the myelin sheath (Pereira et al., 2012). De Gasperi et al. (2010) documented that pmp 22 was a component of myelin, a
HEMMAT MOHAMED KHLOUSSY et al.

protective substance that covered nerves and promoted the efficient transmission of nerve impulses. The protein was produced primarily by Schwann cells.

The histological results of the present work showed presence of lymphocytes within the nerve bundle suggesting inflammatory changes that were induced by limb immobilization. These results coincided with that of Ohmichi et al. (2012) who documented that cast immobilization in rat induced inflammatory changes in the immobilized hind limb due to ischemia/reperfusion injury. Guo et al. (2014) reported that cast immobilization of rat hind limb induces inflammatory changes in the hind limb due to increased inflammatory mediators release. The histological results of the present work showed presence of hyperactive Schwann cells indicating the try of the nerve to regenerate. These results coincided with that of Kobayashi et al. (2012) who documented that proliferation of Schwann cells played an important role in promoting nerve regeneration. Gonzalez-Perez (2013) reported that proliferation of Schwann cells occurred early after nerve lesion. Svennigsen and Dahlin (2013) documented that Schwann cell proliferation was an important event in the regeneration process. When the axons regenerate, the Schwann cells have started to proliferate and secrete cytokines that recruit immune cells as lymphocytes (Gaudet et al., 2011). The results showed presence of fibroblast helping regeneration of nerve. These results coincided with that of Emanuel and Howard (2009) who documented that fibroblasts have a chief function in producing components of the extra cellular matrix. The present work showed abnormal mitochondrial cristae, indicating defective mitochondrial function. Viader et al. (2011) reported that mitochondria was essential for maintenance of axonal survival and normal peripheral nerve function. Sabatier et al. (2008) reported that physical activity promoted axonal regeneration following peripheral nerve injury through enhancing of axon sprouting. Moderate exercise for 1 hour/day, either active treadmill walking or passive cycling, improves muscle reinnervation, and increases the number of regenerated axons (Udina et al., 2011).

Youshida et al. (2016) suggested that immobility by joint fixation creates different conditions in the perineurium compared to the normal situation, and that ROM (range of motion) exercise helped to maintain the basic environment of the perineurium in the exercised group. The essential functions of laminin are roughly divided into two i.e. interactions with other ECM proteins involved in architectural function such as assembly and stability within the basement membrane; and interactions with cell surface receptors involved in adhesion, migration, and differentiation (Aumailley, 2013). The authors hypothesize that joint immobilization or ROM exercise affects these laminin functions, but the detailed mechanism remains unclear. Their results suggest that immobilization alters the perineurium at a molecular level and the ROM exercise is essential for maintaining the environment of the perineurium.

CONCLUSION

Limb immobilization affected the physiological parameters (nerve conduction velocity and the amplitude of action potential) of the sciatic nerve. These
changes were associated with degeneration of nerve axons and their myelin sheath. The affected nerves tried to overcome these changes by regeneration through proliferation of Schwann cells.

REFERENCES


دراسة التغيير في سرعة إنتقال النبضة العصبية للعصب الوركي في الجرذان المعرضه للشلل الإجهادى

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خلفية البحث: يعتبر عدم الحركة أحد الطرق لعلاج إصابات الهيكل العظمى. وقد يؤدي عدم الحركة لفترات طويلة إلى العديد من المضاعفات بالرغم من فائدته للجزء المصاب.

الهدف من البحث: دراسة التغييرات الشكلية والوظيفية للعصب المعدي للعضلة.

مواد وطرق البحث: تم استخدام أربعة وثلاثين جرذًا ذكرأً أليطاً أليطاً بالغاً، تزن حوالي 140 - 160 جرام في هذه الدراسة، حيث تم تقسيم الجرذان إلى مجموعتين متساويتين: المجموعة الأولى (الضابطة) لم تتعرض إلى الشلل الإجهادى والمجموعة الثانية (مجموعة الشلل الإجهادى) (الضابطة) لم تتعرض إلى الشلل الإجهادى والمجموعة الثانية (مجموعة الشلل الإجهادى)

وبعد 14 يومًا من التثبيت، تم قتل الجرذان وتشريحة الجرذان الخلقي الأيمن للحصول على العصب الوركي ووضعه في حجيرة مصممة لوضع العصب عليها لتشتيته بمؤثر، ثم قياس سرعة استجابة العصب لهذا المؤثر.

النتائج: هناك نقصًا ذا دلالة إحصائيًا في كل من سرعة إنقلال النبضة العصبية وشدتها في مجموعة الشلل الإجهادى بالمقارنة بالمجموعة الضابطة، هذا بالإضافة إلى تغيرات تم ملاحظتها ميكروسكوبيا توضح حدوث تلف في الألياف العصبية وكذاك كساء ميالين المحيط بهذه الألياف العصبية المكونة للعصب الوركي.

الاستنتاج: أثبتت الدراسة وجود علاقة قوية بين تعرض الجرذان للشلل الإجهادى لفترات طويلة والخصائص الوظيفية التركيبية للعصب الوركي.