Paclitaxel Inhibits Expression of Neuronal Nitric Oxide Synthase and Prevents Mitochondrial Dysfunction in Spinal Ventral Horn in Rats After C7 Spinal Root Avulsion

Paclitaksel Sıçanlarda C7 Spinal Kök Avülsiyonu Sonrasında Nöronal Nitrik Oksit Sentaz Ekspresyonunu İnhibe Eder ve Spinal Ventral Boynuzda Mitokondriyal Disfonksiyonu Önler

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ABSTRACT

AIM: This study evaluated the neuroprotective effect of intrathecally infused paclitaxel in the prevention of motoneuron death and mitochondrial dysfunction following brachial plexus avulsion injury.

MATERIAL and METHODS: Brachial root avulsion injury was induced in Sprague-Dawley rats. The Paclitaxel treatment group (n = 32) received a 5-d intrathecal infusion of paclitaxel (256 ng/d) via a micro infusion pump, whereas the Control group (n = 32) received normal saline. The cervical cord was harvested at survival times of 1, 2, 4, and 6 wk (n = 8 each). The number of surviving and nNOS-positive motoneurons at the injury level in the ventral horn was determined with NADPH-d histochemistry. Mitochondrial function at this location was measured with CcO histochemistry and densitometry. An independent t-test was applied to detect differences between the study groups at specific survival times.

RESULTS: The Paclitaxel treatment group showed a significant relative reduction in nNOS expression at 2, 4, and 6 wk, and significantly improved mitochondrial function at 4 and 6 wk. Motoneuron survival was significantly increased at 2, 4, and 6 wk.

CONCLUSION: Paclitaxel has a significant neuroprotective effect against spinal motoneuron degeneration following brachial plexus avulsion injury, which involves inhibition of nNOS expression and prevention of mitochondrial dysfunction.

KEYWORDS: Root avulsion, Spinal motoneurons, Nitric oxide synthase, Mitochondrial dysfunction, Adult rats

ÖZ

AMAÇ: Çalışma brakiyal pleksus avülsiyon yaralanması sonrası spinal motor nöron ölümünü ve mitochondrial disfonksiyonun önlenmesinde intratekal infüzyonla verilen paklitakselin nöroprotektif etkisi değerlendirilmiştir.

YÖNTEM ve GEREÇLER: Sprague-Dawley sıçanlarında brakiyal kökte avülsiyon hasarı indüklenmiştir. Paklitaksel tedavi grubuna (n = 32) 5 gün boyunca bir mikroinfüzyon pompasya intratekal paklitaksel infüzyonu (256 ng/g) verilirken Kontrol grubuna (n = 32) normal salin verilmiştir. Servikal kord, 1, 2, 4 ve 6 hafta süren sağkalım süreleri sonrasında alınmıştır (her biri n = 8). Sağ kalan ve nNOS-pozitif motor nöronların ventral boynuzda yaralanma seviyesindeki sayışı NADPH-d histokimyası ile belirlenmiştir. Bu konumda mitokondri işlevi CcO histokimyası ve dansitometriyle ölçülmüştür. Belirli sağkalım zamanlarında çalışma grupları arasındaki farkları sapmama ve bağımsız t testi ile uygulanmıştır.

BULGULAR: Paklitaksel tedavi grubu 2, 4 ve 6 haftada nNOS ekspresyonunda önemli bir relatif azalma ve 4 ve 6 haftada mitochondrial işlevde önemli artış göstermiştir. Motonöron sağkalımı 2, 4 ve 6 haftada önemli artış göstermiştir.

SONUC: Paklitakselin brakiyal pleksus avülsiyon yaralanması sonrası spinal motonöron dejenerasyonuna karşı önemli bir nöroprotektif etkisi vardır ve bu etki nNOS ekspresyonunun inhabisyonu ve mitochondrial disfonksiyonun önlenmesiyle ilişkilidir.

ANAHTAR SÖZCÜKLER: Kök avülsiyonu, Spinal motonöronlar, Nitrik oksit sentaz, Motonöron disfonksiyon, Yetişkin sıçanlar

ABBREVIATIONS: ATP: adenosine triphosphate; CcO: cytochrome C oxidase; CNS: central nervous system; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; PNS: peripheral nervous system
INTRODUCTION

Spinal root avulsion injury has the poorest outcome among the different types of brachial plexus injury (5), because it involves the junction between the spinal cord and the spinal nerve. Spinal motoneuron death following spinal root avulsion injury has been identified as the main cause of impaired neuronal regeneration (19).

Factors that associated with motoneurons death following spinal root avulsion injury include: i) Expression of neuronal nitric oxide synthase (nNOS) at the injured ventral horn of the spinal cord resulting in the production of nitric oxide (NO) and the occurrence of secondary oxidative damage (18,19); ii) Mitochondrial dysfunction secondary to inflammatory processes and oxidative damage, which impairs adenosine triphosphate (ATP) production and deprives the source of energy to motoneurons (16). Furthermore, mitochondrial dysfunction itself would lead to excessive production of reactive oxygen species and subsequently an increase in oxidative stress activity in the injured site (6,14); and iii) Neurofibrosis that impairs the retrograde axonal transport of neurotrophic factors which are vital for the motoneurons’ survival, thereby impairing axonal regeneration (10).

Paclitaxel, a diterpene alkaloid, is derived from the bark of the Western yew tree Taxus brevifolia. Paclitaxel is currently used as a chemotherapeutic agent for the treatment of ovarian cancer, breast cancer, non-small cell lung carcinoma, and Kaposi’s sarcoma (13,15). In higher doses paclitaxel causes toxicity, especially peripheral neuropathy (7); however, at a lower dosage it is believed to have a neuroprotective effect after spinal cord injury (10,13). Paclitaxel was chosen in this animal study in view of its effects in stabilizing microtubules, preventing neurofibrosis, and improving axonal regeneration (10). However, the role of low-dose paclitaxel in the prevention of spinal motoneuron death and mitochondrial dysfunction in rats following brachial root avulsion injury has not yet been clarified. The objective of this study was to investigate the neuroprotective effect of intrathecal infused paclitaxel in an animal model with brachial root avulsion injury, specifically in the inhibition of the expression of nNOS, prevention of mitochondrial dysfunction, and improvement of motoneuron survival.

MATERIAL and METHODS

Animals

Sixty-four adult female Sprague-Dawley rats aged 6–8 weeks and weighing 200–250 g were used in this study. The rats were randomly divided into 2 main groups (Control and Paclitaxel treatment) with survival times of 1 week, 2 weeks, 4 weeks, and 6 weeks (8 rats in each survival time subgroup). All rats in this study were supplied by the Animal Research and Service Centre, Universiti Sains, Malaysia. The study was approved by the Animal Ethics Committee, Universiti Sains Malaysia (Reference No. USM/Animal Ethics Approval/2011/73) [346]. The laboratory work was carried out at the Neurosciences Teaching Laboratory and the Central Research Laboratory of the School of Medical Sciences, and the Maxillofacial Science Laboratory of the School of Dental Sciences, Universiti Sains Malaysia.

Intravertebral Spinal Root Avulsion Surgery

Surgical procedures were carried out as described by Wu et al. (19). Each rat was anesthetized with an inraperitoneal injection of ketamine (80 mg/kg, Troy Laboratories, Australia) and xylazine (8 mg/kg, Troy Laboratories, Australia). A dorsal midline skin incision was made at the neck, and the paraspinal muscles separated. The 6th cervical vertebra (C6), which encloses the C7 spinal segment, was identified. The right-sided C6 lamina was removed with bone rongeur. Dura was opened to expose the dorsal root of C7, which was then incised at where it attached to the spinal cord to expose the ventral root. The ventral root was then avulsed with a fine hook under microscope view. Haemostasis secured at the avulsion site. Subcutaneous dissection was performed at the skin incision site with scissors to create a pocket for implantation of the micro infusion pump. The intrathecal catheter was then carefully placed at the ventral-root avulsion site. The micro infusion pump was filled with either normal saline (Control group) or with Paclitaxel (T7402, Sigma-Aldrich, USA) and was programmed to deliver the drug at the rate of 256 ng/d continuously for 5 d to the injury site via the intrathecal catheter. The paraspinal muscles were approximated utilizing polygactin synthetic absorbable sutures (Vicryl 3.0, ETHICON, USA) and skin closure was done using non-absorbable polyamide monofilament sutures (Dafilon 3.0, BRAUN, Tuttlingen). Post-operatively, the rats received adequate analgesia (Meloxicam, intramuscular 1 mg/kg, repeated once after 24 h) and antibiotic (Amoxycillin, intramuscular injection 15 mg/kg daily for 3 d). The well-being of the rats were observed regularly after the surgery using the Otago Animal Welfare Score Sheet.

Spinal Cord Harvesting

The spinal cord was harvested as described by Wu et al. (19). At the end of the survival time (1 week, 2 weeks, 4 weeks, or 6 weeks), the rat was anesthetized with a lethal dose of ketamine (160 mg/kg) and xylazine (16 mg/kg) given intraperitoneally. The thoracic cavity was then opened and the rat perfused transcardially utilizing a 24G cannula needle, with 0.1M PBS (pH 7.4) at a speed of 3 mL/min for 5 min, immediately followed by 4% PFA in 0.1M PBS (pH 7.4) for 40 min. After perfusion, the vertebral column was carefully dissected, and the spinal cord removed. The C7 spinal segment was identified as the region between the uppermost root and lowermost root of the C7 nerve of the contralateral spinal cord. The harvested C7 spinal segment was fixed by immersion in fresh fixative (4% PFA) at 4°C for 3 h. This was followed by fixation in 30% sucrose at room temperature overnight. The following day, the cord was prepared for frozen sectioning. Cross sections of thickness 40 μm were cut on a cryostat and collected in 0.01M PBS. Approximately thirty cross sections of the C7 spinal segment were obtained from each animal, and every third section was used for NADPH-d histochemistry plus neutral
red counterstaining. Every fourth section from the C7 spinal segment was used for the study of cytochrome c oxidase (CcO) activity.

**NADPH-d Histochemistry**

The number of surviving motoneurons and the nNOS expressing motoneurons were quantified using NADPH-d histochemistry as described by Wang et al. (18). Ten sections from the C7 spinal segment of each animal were incubated at 37°C for 1 h in 10 mL of 0.1M Tris-HCl (pH 8.0) containing 0.2% Triton X100, 10 mg NADPH (N6132, Sigma-Aldrich, USA) and 2.5 mg nitroblue tetrazolium (N5514, Sigma-Aldrich, USA); they were then washed with 0.1M PBS three times. The stained sections were then mounted on slides and counterstained with 1% neutral red. These sections were used to count the numbers of both nNOS-positive motoneurons and all surviving motoneurons. Neuronal NOS-positive motoneurons would be stained blue with NADPH-d, whereas the other surviving motoneurons would be stained red with neutral red, which stained the Nissl substance. The sum of the count of blue neurons and red neurons is represented in the all-surviving neurons figure.

**Outcome Evaluation: Surviving and Nnos-Expressing Motoneurons**

Examination and quantification of motoneurons was done under a light microscope with 200× magnification by a double-blinded counting method. For inter-observer agreement, the Kappa coefficient was 0.86, and the proportion of agreement was 93%. Motoneurons could be easily identified by their location in Rexed’s lamina IX in the ventral horn, their large somata, and the granular appearance of the Nissl bodies in their cytoplasm under high magnification. The motoneuron counting methods were based on previous protocol by Wu et al. (18). The number of surviving motoneurons was quantified on both the injured and intact sides of each C7 section. The contralateral intact side was used as an internal control, and the number of surviving ipsilateral motoneurons was expressed as a percent of the number on the contralateral side. Any neutral-red-stained motoneuron with a visible nucleus was counted to obtain the total survivorship data. Finally, the number of surviving motoneurons of each animal was expressed as the mean of the number of surviving motoneurons in the 10 serial C7 sections. Similarly, the number of ipsilateral nNOS-positive motoneurons, represented only by the NADPH-d reactive motoneurons, was expressed as a percentage of the number of surviving motoneurons (neutral-red-stained) on the contralateral side of the same C7 section. The number of nNOS-positive motoneurons of each animal was expressed as the mean of the nNOS-positive motoneurons in the 10 serial C7 sections.

**CcO Histochemistry**

This assay measured CcO activity in tissue sections, and the signal obtained is directly proportional to mitochondrial complex IV activity. Tissues were processed as described in previous studies (11,12). Sections of spinal segment C7 were incubated at 37°C for 20 min in a reaction buffer containing 100mM KH$_2$PO$_4$ (pH 7.4), 4% sucrose, 0.50 mg/mL 3′-diaminobenzidine (D3939, Sigma-Aldrich, USA), 200 mg/mL catalase, and 0.15 mg/mL cytochrome c Oxidase from bovine heart (C5499, Sigma-Aldrich, USA). The reaction was stopped by washing the cord sections three times for one minute in 100mM KH$_2$PO$_4$ (pH 7.4). Cord sections were then rinsed once with distilled water, dried, and mounted on microscope slides.

**Outcome Evaluation: Cytochrome C Oxidase Activity**

Semiqualitative assessment of the intensity of the CcO histochemical reaction was based on the methods described by Hüttemann et al. (11). Images were captured using a microscope eyepiece camera attached to a light microscope. Images of the ventral horn where the motoneurons were located were examined and analysed utilizing a densitometer. The relative intensity (in density per square mm) of the signal was determined by optical densitometry software. The background intensity in the white matter was measured for each individual section and was subtracted from the intensity registered in the areas of interest (the ventral horn) to obtain an absolute value. The intact contralateral side was used as an internal control. The absolute value of CcO activity on the ipsilateral side was expressed as a percentage of the absolute number on the contralateral side of the same C7 section. The CcO activity of each animal was expressed as the mean of the CcO activities in the 10 serial C7 sections.

**Statistical Analysis**

All data for both Control and Paclitaxel treatment groups were analysed using the Statistical Package for Social Sciences (SPSS for Windows) version 20.0 (SPSS Inc, Chicago, IL, USA). All variables were expressed as mean ± standard deviation (SD). The independent t-test was used to determine any significant differences in the variables between Control and Paclitaxel treatment groups at a given time point. The significance level was set at p < 0.05.

**RESULTS**

**Paclitaxel Treatment and Motoneuron Survival Rate**

Quantitative analysis showed that the number of surviving motoneurons at the injured ventral horn in the C7 spinal segment of the Paclitaxel treatment group was higher than in the Control group for all survival times (Table I). At 6 weeks post-injury, the motoneuron loss was less than 40% in the Paclitaxel treatment group compared to 56.6% in the Control group. Statistical analysis showed that the differences in the motoneuron survival rate between the Paclitaxel treatment and Control groups were significant at 2 weeks (p < 0.01, t = -27.71) (Figure 1A). Morphologically, by 6 weeks post-injury many of the surviving motoneurons in the Paclitaxel treatment group appeared reddish (from the neutral red counterstaining) with relatively few nNOS-positive cells, while in the Control group most of the remaining motoneurons...
**Figure 1:** A) Significant improvement in motoneuron survival rate in the Paclitaxel treatment group is observed at 2, 3, and 4 weeks post-injury; B) The expression of nNOS in motoneurons is significantly inhibited in the Paclitaxel treatment group at 2, 3, and 4 weeks post-injury; C) CcO activity is significantly preserved in the Paclitaxel treatment group at 3 and 4 weeks post-injury. nNOS, neuronal nitric oxide synthase; CcO, Cytochrome c oxidase.

**Figure 2:** NADPH-d histochemistry with neutral red counterstaining of the injured C7 ventral horn in the Control group (A) and Paclitaxel treatment group (B) at 6 weeks post injury. There are fewer nNOS-expressing motoneurons (stained blue) and more total surviving motoneurons in the Paclitaxel treatment group. Scale bar = 100 µm.
were stained blue (nNOS-positive) at the same time point (Figure 2A, B). The injured ventral horn also appeared less atrophic in the Paclitaxel treatment group than in the Control group.

**Paclitaxel Treatment and Inhibition of nNOS Expression in Motoneurons**

In the Paclitaxel treatment group, quantitative analysis showed that the percentage of nNOS-positive motoneurons in the injured ventral horn was comparatively lower than in the Control group at all survival times (Table I). The inhibition of nNOS expression with Paclitaxel treatment was statistically significant at post-injury wk 2 (p < 0.01, \( t = 8.379 \)), 4 (p < 0.01, \( t = 9.885 \)), and 6 (p < 0.01, \( t = 10.090 \)) (Figure 1B).

**Paclitaxel Treatment and Prevention of Mitochondrial Dysfunction**

The mitochondrial function after spinal root avulsion injury was assessed by the percentage of the CcO activity remaining in the injured ventral horn in the C7 spinal segment (Table I). The percentage of CcO activity in the Paclitaxel treatment group was higher compared to the Control group at all survival times, and was statistically significant at post-injury wk 4 (p < 0.01, \( t = -34.980 \)) and 6 (p < 0.01, \( t = -23.474 \)) (Figure 1C). The CcO activity in the Paclitaxel treatment group exhibited a declining pattern similar to that in the Control group, where the activity gradually decreased and became static at post-injury wk 4 and 6. Morphologically, by 6 wk post-injury more clusters of dark-brownish product (CcO activity) were observed in the ventral horn of the Paclitaxel treatment group (Figure 3A, B).

**DISCUSSION**

About 65% of adult brachial plexus injuries were roots avulsion (2). It was most often caused by motor vehicle accidents when the arm and shoulder are severely stretched during the collision. The injury involves both the peripheral nervous system (PNS) as well as the central nervous system (CNS) (5, 17). Patients would experience partial or complete sensory and motor functions loss in the affected limb, depending on the severity of the injury. Surgical repair with nerves transfer has been performed (4, 8), however, the functional outcome remained unsatisfactory. Axonal regeneration in the CNS is different from that in the PNS (9). When a peripheral nerve is injured and repaired, axons usually regenerate successfully into the distal stump, eventually leading to a considerable degree of functional recovery. In contrast, axonal regeneration in the CNS of adult mammals, including humans, is usually abortive (9). Laboratory studies (6, 14, 18) showed that failure of axonal regeneration in the CNS was due to spinal motoneurons death as well as to an absence of a permissive environment in the spinal cord secondary to elevated oxidative stress activity and mitochondrial dysfunction.
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Majority of the survivors being nNOS-positive. In contrast, in the Paclitaxel treatment group, many injured motoneurons survived. Although some of these neurons were still NOS-positive, others seemed morphologically normal compared to neurons on the intact side of the spinal cord.

In this study, motoneuron death following brachial root avulsion injury was reduced by paclitaxel, but not completely prevented. This may be because the injury-induced nNOS was not completely inhibited by paclitaxel at the dosage used. Indeed, using continuous intrathecal infusion of paclitaxel for 5 d, at post-injury wk 6 there were still a few NOS-positive motoneurons in the injured ventral horn. Increasing the paclitaxel dose level or prolonging the treatment duration may probably enhance survival of the injured motoneurons. Incomplete prevention of motoneuron death by paclitaxel may also be due to poor absorption, since paclitaxel has poor water solubility. Furthermore, nNOS expression is only one of the many mechanisms involved in post-injury oxidative damage.

Paclitaxel and Spinal Motoneuron Survival Rate

Low dose Paclitaxel has been shown to slow down neurofibrosis and promoting axonal regeneration after spinal root avulsion injury in rats (10). The mechanism involved stabilization of microtubules in the neurons. However, the neuroprotective effect of paclitaxel in preventing motoneuron death and mitochondrial dysfunction following spinal root avulsion is not well documented. Here, we tested the effects of intrathecal infusion of paclitaxel via micro infusion pump at a rate of 256 ng/d continuously for 5 d at the site of injury. It was found that paclitaxel can rescue the majority of injured ventral horn motoneurons from degeneration, and the effect was seen for a relatively long time after avulsion. In our Paclitaxel treatment group, the spinal motoneuron survival rate was significantly higher compared to the Control group at post-injury wk 2 (78.5% vs. 69.7%), 4 (67.4% vs. 54.2%), and 6 (61.2% vs. 43.4%).

Paclitaxel and Oxidative Stress Activity

NO is a free radical gas synthesized from arginine by NOS. In experimental studies, it was found that NOS was induced in CNS neurons of adult mammals after traumatic injury by either spinal root avulsion or spinal cord hemisection (18,19). At early phase, expression of NOS seems to be associated with axonal sprouting and growth, but ultimately the neurons expressing NOS died. The findings that injury-induced NOS within the injured neurons were coincident with the death of these neurons suggested a role of NO in neuronal degeneration. In view NO is responsible for the death of injured neurons, thus inhibition of NOS should prevent their death.

In the Paclitaxel treatment group, the percentage of nNOS-positive motoneurons was significantly lower compared to the Control group at post-injury wk 2 (41.9% vs. 48.7%), 4 (32.6% vs. 41.2%), and 6 (25.2% vs. 32.5%). In the control group at post-injury wk 6, NADPH-d histochemistry showed the number of motoneurons in the injured ventral horn of the C7 spinal segment was markedly reduced, with the majority of the survivors being nNOS-positive. In contrast, in the Paclitaxel treatment group, many injured motoneurons survived. Although some of these neurons were still NOS-positive, others seemed morphologically normal compared to neurons on the intact side of the spinal cord.

In this study, motoneuron death following brachial root avulsion injury was reduced by paclitaxel, but not completely prevented. This may be because the injury-induced nNOS was not completely inhibited by paclitaxel at the dosage used. Indeed, using continuous intrathecal infusion of paclitaxel for 5 d, at post-injury wk 6 there were still a few NOS-positive motoneurons in the injured ventral horn. Increasing the paclitaxel dose level or prolonging the treatment duration may probably enhance survival of the injured motoneurons. Incomplete prevention of motoneuron death by paclitaxel may also be due to poor absorption, since paclitaxel has poor water solubility. Furthermore, nNOS expression is only one of the many mechanisms involved in post-injury oxidative damage.

Paclitaxel and Mitochondrial Dysfunction

Motoneurons are highly differentiated cells that need large amounts of ATP for the maintenance of ionic gradients across the cell membranes and for neurotransmission. The CcO is the key enzyme in the mitochondrial ATP production, where oxygen is the substrate of CcO and NO is the CcO inhibitor (1, 20). Hence, both hypoxic event and NO production following injury will lead to mitochondrial dysfunction (3,16). In a study of spinal cord injury on a rabbit model, there was a significant decreased of CcO activity 24 h after the injury (20). Given the central role of mitochondria in cell function and survival, a therapeutic agent that can maintain mitochondrial homeostasis and bioenergetics appears essential for promoting cell survival following spinal root avulsion injury.

In our Paclitaxel treatment group, the CcO activity was slightly higher at 1 and 2 wk post-injury compared to the Control group, but this was not statistically significant. Whereas at
prevention of spinal motoneuron death following brachial root avulsion injury. Paclitaxel has the potential neuroprotective effect in the root avulsion injury. A quantitative measurement may give more precise activity is a semi quantitative measurement of mitochondrial activity at that time point, and onwards, in both the Control and Treatment groups. A static phase of CcO activity was observed in both Control and Paclitaxel groups after post injury 4 wk, suggests that there might be a common factor influencing CcO activity in that particular time interval. Energy demand for motoneuron survival and axonal regeneration is expected to be increased after spinal root avulsion injury (1). The redistribution of mitochondria to the injury site to meet the local energy demand by an autoregulatory effect may reach a maximal phase at post-injury wk 4, and this may explain the static CcO activity at that time point, and onwards, in both the Control and Treatment groups.

**Suggestion for Future Study Model**

This exploratory study showed encouraging results in which Paclitaxel possesses potential neuroprotective effects in the prevention of spinal motoneuron death following brachial root avulsion injury, by inhibiting the injury-induced nNOS expression, and possibly has a role in preserving mitochondrial function as well. Axonal regeneration in nerve root reimplantation following brachial plexus avulsion injury has been studied in rats (17), and demonstrated a large number of retrograde-marker-labelled neurons in the ventral horn of the spinal cord following reimplantation of an avulsed spinal root, indicating that regeneration succeeded. Based on these findings, a future study with reimplantation of avulsed brachial root and intrathecal infusion of Paclitaxel might be helpful.

**Study Limitations**

The neuroprotective effect of paclitaxel was studied within the survival times of 1, 2, 4, and 6 weeks only. However, the long-term effect of paclitaxel is not known. It is not clear whether a single-bolus dose, a multiple doses with fixed time interval, or a continuous infusion of paclitaxel will give a similar neuroprotective effect in this animal model. The CcO activity is a semi quantitative measurement of mitochondrial function. A quantitative measurement may give more precise information on the mitochondrial activity following brachial root avulsion injury.

**CONCLUSIONS**

Paclitaxel has the potential neuroprotective effect in the prevention of spinal motoneuron death following brachial root avulsion injury by inhibiting nNOS expression and reduction of post-injury oxidative reactions, improved mitochondrial function, and subsequently improved motoneuron survival.

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