THE EFFECTS OF MINOCYCLINE ON SPINAL ROOT AVULSION INJURY IN RODENT MODEL: A HISTOLOGICAL STUDY

By

DR. TAN YEW CHIN
MSurg Neurosurgery (Universiti Sains Malaysia)

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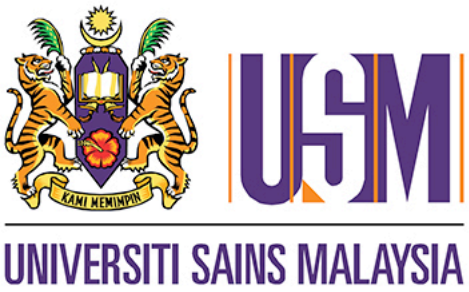

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Abstrak

Pengenalan: Kecederaan saraf brakial adalah sesuatu kecederaan yang memudaratkan. Kecederaan tersebut melibatkan 5% kes-kes politrauma yang diakibatkan oleh kemalangan trafik. Matlamat utama rawatan kecederaan saraf brakial adalah pemulihan tenaga motor. Perawatan kecederaan saraf brakial secara pemindahan graf saraf boleh memberikan perlindungan kepada saraf motor. Namun begitu kesan rawatan pembedahan adalah sangat terhad. Rawatan secara perubatan perlu diberikan untuk mengekalkan fungsi yang wujud pada saraf motor. Minocycline sudah terbukti untuk membekalkan perlindungan kepada sel-sel saraf dalam penyakit angin ahmar dan penyakit pengelupasan selaput saraf. Minocycline adalah antibiotik yang mudah didapati, ekonomik, dan berupaya untuk merawat radangan dan kerosakan sel sel saraf. Literasi saintifik telah menunjukkan bahawa Minocycline berupaya untuk menghasilkan kesan perlindungan kepada sel-sel saraf motor dengan prencatan sel microglia dan Nitric Oxide Synthase. Kedua-dua kesan terapiutik ini dapat mengurangkan radangan dan degenerasi saraf setelah mendapat kecederaan ‘avulsion’ saraf brakial.

Objektif: Kajian ini adalah untuk mengkaji kesan Minocycline yang selama ini digunakan sebagai antibiotik kepada hayat sel saraf motor dan juga penghasilan sel Microglia.

Metodologi: Kajian eksperimental ini menggunakan tikus dewasa Sprague Dawley di mana urat saraf segmen C7 tikus ditarik sehingga putus daripada saraf tunjang. Transeksi fizikal ini akan dipastikan untuk berlaku sebelum ganglion saraf. Untuk mendapatkan validasi saintifik, saraf yang terputus telah diperhatikan di bawah

Mikroskop untuk memastikan akar-akar saraf dicabut bersama-sama dengan tangkainya. Tikus yang mana transeksi saraf berlaku selepas ganglion telah dikecualikan daripada kajian ini. Tikus-tikus yang mempunyai kecederaan saraf brakial telah dirawat dengan ‘intraperitoneal’ dan ‘intrathecal’ Minocycline 50mg/kg untuk seminggu dan 25mg/kg untuk seminggu yang selanjutnya untuk pemulihan sel saraf motor. Selepas enam minggu, saraf tunjang tikus tersebut akan diambil dan ujikaji histokimia dijalankan untuk mengira sel saraf motor dan sel Microglia.

Keputusan: Kajian ini menunjukkan bahawa hayat sel saraf motor telah berkurang dan penghasilan sel Microglia bertambah selepas tikus Sprague Dawley mendapat kecederaan saraf brakial. Kajian ini juga mendapati bahawa Minocycline berupaya untuk mengurangkan bilangan sel Microglia. Rawatan secara ‘intraperitoneal’ telah mendatangkan kesan yang bermanfaat dari segi perlindungan sel saraf motor melalui perencatan aktiviti sel microglia. Namun begitu apabila ubat tersebut telah disuntik secara ‘intrathecal’, bukan sahaja ia mengancam kehidupan sel saraf motor, malah ia juga mendatangkan kesan negatif lain yang memudaratkan.

Kesimpulan: Penggunaan Minocycline mempunyai dua implikasi. Pengurangan sel Microglia dengan menggunakan Minocyline dalam dos yang optimal dapat mengurangkan kematian sel saraf selepas kecederaan saraf brakial. Namun, apabila keberkesanan dan konsentrasi ubat Minocycline ditingkatkan, ubat ini boleh meracuni sel-sel saraf motor melalui perencatan tindak balas sel glia dan degenerasi walerian.

Abstract

Introduction: Brachial plexus injuries are debilitating injury affecting young population, which comprise 5 % of all polytraumas caused by road traffic accidents. Currently the primary aim for the management of root avulsion of the brachial plexus is motor recovery. However, immediate repair or nerve grafting offers some degree of protection to the motoneurons but is clinically limited, so there remains a need for medical approaches to maintain the viability of the injured motor neurons. Minocycline has been proven to show its neuroprotective effect in stroke and demyelination disease. It is a widely available, cost effective antibiotic with anti-inflammatory and anti-apoptotic properties. Literatures have shown that Minocycline exert its neuronal protection effect primarily via Migroglial inhibition and Nitric Oxide Synthase downregulation. Both of the effects are key therapeutic means to ameliorate neuroinflammatory and degenerative process following secondary traumatic avulsion injury.

Objective: To study the Neuroprotective effect of Minocycline in adult Sprague Dawley mouse that suffer from Brachial Plexus Injury.

Methods: The C7 nerve root was avulsed via anterior extravertebral approach. The traction force transected the ventral motor nerve roots at the preganglionic level. The avulsed rootles can be seen under the microscope for validation of the avulsion rodent model in which the avulsion is properly done at the preganglionic level. The rodents in which the transection takes place distal to the rootlets were excluded from the study Intraperitoneal and intrathecal Minocycline 50mg/kg for the first week and (25mg/kg

for the seconda week) was administered to promote motor healing. The spinal cord was harvested at 6 weeks after the injury to analyze the structural changes following avulsion injury and the pharmacological intervention.

Results: Motor neuron death and microglial proliferation was observed following traumatic avulsion injury of the ventral nerve root. The administration of Minocycline to treat rodents suffering from avulsion injury was capable to suppress the Microglial proliferation. Intraperitoneal administration of Minocycline shows some degree of beneficial effect to prolong the motor neuron survival by inhibiting the Microglia activation and proliferation, thus hampering apoptosis of the motor neuron. However when Minocycline was administered via intrathecal route to increase the bioavailability of the therapeutic agent, not only that it compromises the motor neuron survival, some other deleterious effect were also demonstrated.

Conclusion: Microglial suppression via Minocycline can have double effect. Moderate dosage of Minocycline may be beneficial towards motor neuron survival. On the other hand, high concentration of Minocycline (similar drug dosage with increased potency via targeted drug delivery) could be neurotoxic by causing impairment of the Glial response and Wallerian degeneration, which is a pre-requisite to regeneration.

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List of Abbreviations

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

Anterior root exit zone (AREZ)

Autoimmune encephalomyelitis (EAE)

β-Amyloid (Aβ)

Choline acetyltransferase (ChAT)

Cluster of differentiation (CD)

Compound Muscle Action Potential (CMAP)

Cyclooxygenase 2 (COX2)

Damage associated molecular patterns (DAMP)

Histocompatibility complex II (MHCII)

Huntington's Disease (HD)

Inducible NO synthase (iNOS), also known as NO synthase 2 (NOS2)

Insulin-like growth factor-1 (IGF-1)

Mitogen activated protein kinase (MAPK)

N-Methyl-D-aspartate (NMDA)

Nerve conduction study (NCS)

Nitric oxide (NOS)

Pathogen associated molecular patterns (PAMP)

Phosphate buffered saline (PBS)

Prostaglandin (PG)

nNOS (neuronal/Type I/NOS-1/bNOS)

iNOS (inducible/Type II/NOS-2)

eNOS (endothelial/Type III/NOS-3)

Sensory Nerve Action Potential (SNAP)

Ventral root avulsion (VRA)

CHAPTER 1

INTRODUCTION

1.1 Background of study

Brachial plexus injuries comprise 5% polytraumas caused by motor vehicle accidents in European country (Radek *et al*., 2014). The exact incidence figure of brachial plexus injury in Asia is not available. It is a mutilating injury as it commonly affects young men in their productive age. It cripples the function of one, or even two limbs at rare occasions, leaving a devastating effect on the unfortunate patient. It results in loss of neurological function, dependence in daily living, employment, depression and even suicidal tendency. Brachial plexus injury has a serious socioeconomic impact since they affect the function and quality of regular life to a great extent. Injuries resulting nerve transection only produce minimal or even undetectable motor neuron degeneration (Thatte *et al*, 2013). In contrast, a ventral root avulsion injury occurred during brachial plexus injury, with the separation of motor axons from the surface of the spinal cord is followed by a progressive and marked loss of the vast majority of axotomized motoneurons over several weeks after the lesion in rat models (Brabizan *et al.*, 2013, Hoang, 2003). Ventral root avulsion injury in the Rhesus Macaque resulted in a marked loss of axotomized motoneurons and an astroglial reaction in the ventral horn (Marcus, 2013).

Non-Astrocyte Glial cells were discovered by a Spanish neuroanatomist called Ramon y Cajal and identified it as the “third element” of the central nervous system in

distinction from the “first element” (neurons) and “second element” (astrocytes) (Del Rio-Hortega, 1919). After a century after his great discovery, recent researches and studies in microglia have centered not only on understanding their basic biological functions but also on their therapeutic potential during pathological conditions. Recent evidence indicates the potential promise of microglia as targets for therapeutic intervention during pathology. Eyo and Dailey (2013) summarized recent evidence suggesting that microglia perform critical functions during development of the nervous system, including roles in angiogenesis, induction of apoptosis, phagocytic clearance of dead cells, and synapse remodeling (Eyo & Dailey, 2013). In cases of Brachial Plexus Injury, the ventral root avulsion injury triggers an activation of microglia within the motor nuclei (Novikov *et al*., 2000). A robust inflammatory response, which involves the activation of glia and neurons as well as cerebral accumulation of leukocytes, following traumatic injury is well known and contributes toward the development of the secondary injury. Microglial cells respond to traumatic injury to the nervous system with proliferation and migration toward the affected area. However, nobody is sure of the microglial response to axonal growth. Several reports suggest that glial cells might act as a double-edged sword being either detrimental or protective depending on the context (Biber, 2007). For instance, activated microglia synthesize potentially neurotoxic molecules such as reactive oxygen species, and inflammatory cytokines. Conversely, microglia release some neuroprotective molecules in specific conditions (Graeber, 2010). They released chemokines that trigger the Glial response, in which the activated astrocytes also release a wide variety of neuroprotective and/or neurotoxic mediators (Sofroniew, 2010). Reactive microglia

may affect axonal regeneration in a variety of ways. They produce a proregenerative factors such as insulin-like growth factor-1 (IGF-1) (Aldskogius, 2001) and a neurotoxic compounds such as free radicals (Boje & Arora, 1992) and glutamate (Giulian *et al*., 1993).

Minocycline hydrochloride is a semi-synthetic derivative of tetracycline. It is a potent neuroprotective agent, act by inhibiting microglial activation (Stirling *et al*., 2004). Minocycline alleviates the degree of neuropathology in animal models in stroke, spinal cord injury, multiple sclerosis, and neurodegenerative diseases (Parkinson's Disease and ALS) (Yrjanheikki *et al*., 1998; Arvin *et al*., 2002). Using a lumbosacral ventral root avulsion model for cauda equina injury, Huong *et al.* (2008) demonstrated significant protection of the drug to axotomized motoneurons against retrograde degeneration and death.

Minocycline may also attenuate the development of hyperesthesia and allodynia after spinal nerve transection injury in the rat (Raghavendra *et al*., 2003). By inhibiting the microglial activation, and also recruitment of T-cell, it was proven to reduce graft rejection & promote survival of neuronal transplants in rat model (Michel-Monigadon *et al*., 2010). Havton and Carlstedt (2009) suggested that minocycline can be used to improve functional recovery after nerve reimplantation following proximal nerve root injury. In some studies, minocycline has failed to show beneficial effect, especially in neurodegenerative diseases (Smith *et al*., 2003; Fernandez-Gomez *et al*., 2005).

Mechanism of Minocycline is not fully understood. However, it crosses blood brain barrier readily. It has a strong anti-inflammatory effect and has a good safety record. It has been approved by FDA to use to suppress apoptotic pathway (Stirling *et al*., 2004), to reduce the activation of microglia after a peripheral nerve injury, in part by inhibiting the expression of p38 mitogen activated protein kinase (MAPK) (Piao *et al*., 2006) and to reduce pro-nerve growth factor in microglia and thereby reduce death of oligodendrocytes after a traumatic spinal cord injury (Yune *et al*., 2007).

1.2 Problem Statement

Previous studies showed controversial effect of migroglial cells towards axonal regeneration. Therefore, we are still not sure of the effect of microglial activation to the axonal growth. Previous studies had shown the beneficial effects of minocycline (Raghavendra *et al*., 2003; Stirling *et al*., 2004; Piao *et al*., 2006; Yune *et al*., 2007; Havton & Carlstedt, 2009; Michael-Monigadon *et al*., 2010). However, there were also few studies that failed to show the beneficial effects of minocycline (Smith *et al*., 2003; Fernandez-Gomez *et al*., 2005). Therefore, the beneficial effects of minocycline on the avulsion injury are still controversial. Study of Chan *et al* (2001) showed that injured neonatal motoneurons can survive and reinnervate peripheral muscle targets following inhibition of caspases which is another effect of Minocycline but it is still unsure whether Minocycline can be used to promote axonal regeneration in the cervical area.

1.3 Research objectives

General objective: To determine the neuroprotective effect of minocycline in rodent spinal root avulsion injury.

Specific objectives:

1. To confirm the microglial cell activation following avulsion injury.
2. To observe the effect of microglial cells activation towards motor neuron (axonal growth).
3. To determine the effect of minocycline towards motor neuron (axonal growth).
4. To determine the effect of minocycline towards microglial cells.
5. To determine the effect of minocycline towards nitric oxide expression.

1.4 Research Questions

1. Are the microglial cells will be activated following avulsion injury?
2. What are the effects of microglial cells activation towards motor neuron (axonal growth)?
3. What are the effects of minocycline towards motor neuron (axonal growth)?
4. What are the effects of minocycline towards microglial cells?
5. What are the effects of minocycline towards nitric oxide expression?

1.5 Research Hypotheses

Hypothesis 1

H_O: The number of motor neurons (axonal growth) are same between the treatment groups (minocycline) and control group (normal saline).

H_A: The number of motor neurons (axonal growth) are different between the treatment groups (minocycline) and control group (normal saline).

Hypothesis 2

H_O: The number of microglial cells are same between the treatment groups (minocycline) and control group (normal saline).

H_A: The number of microglial cells are different between the treatment groups (minocycline) and control group (normal saline).

Hypothesis 3

H_O: The number of nitric oxide expression are same between the treatment groups (minocycline) and control group (normal saline).

H_A: The number of nitric oxide expression are different between the treatment groups (minocycline) and control group (normal saline).

1.6 Significance of study

By knowing the effect of microglial activation to the axonal growth, a therapeutic agent to promote or inhibit microglial cells can be applied to promote axonal growth following avulsion injury. Data from extensive series of researches showed good regenerative result from nerve reimplantation. If the promotional effect of Minocycline towards axonal growth can be determine and assured, then a combination of both Minocycline & nerve reimplantation can be performed to achieve better result. The anti-inflammatory effect of Minocycline can also reduce the rate of graft rejection.

CHAPTER 2

LITERATURE REVIEW

2.1 Brachial plexus anatomy and spinal root avulsion injures

Brachial plexus is a network of nerves formed by the anterior rami of the lower four cervical nerves (C5-8) and the first thoracic nerve (T1). It exits the spine and descend through the cervicoaxillary canal of the neck to reach the armpit, arm and hands. It is divided into roots, trunks, divisions, cords and branches of peripheral nerves. There are 5 terminal branches, and other collateral branches that leave the plexus at various points. Figure 1 show the schematic diagram of the human brachial plexus anatomy. Spinal root avulsion injures are the injuries to the axons of motor and sensory neurons at the junction between the spinal cord and the ventral root. Brachial plexus avulsion can present as two main types: upper and lower (Carlstedt, 2009). Upper avulsions involving C5-C7 occurs from excessive lateral neck flexion away from the shoulder, leading to loss of rotation capacity in the shoulder muscles, arm flexors and hand extensor muscles and results in Erb's palsy. Lower avulsions involving C8 and T1 occurs through sudden traction force on the arm away from the shoulder with subsequent paralysis of intrinsic muscles of the hand and flexors of the wrist and fingers resulting in Klumpke's palsy.

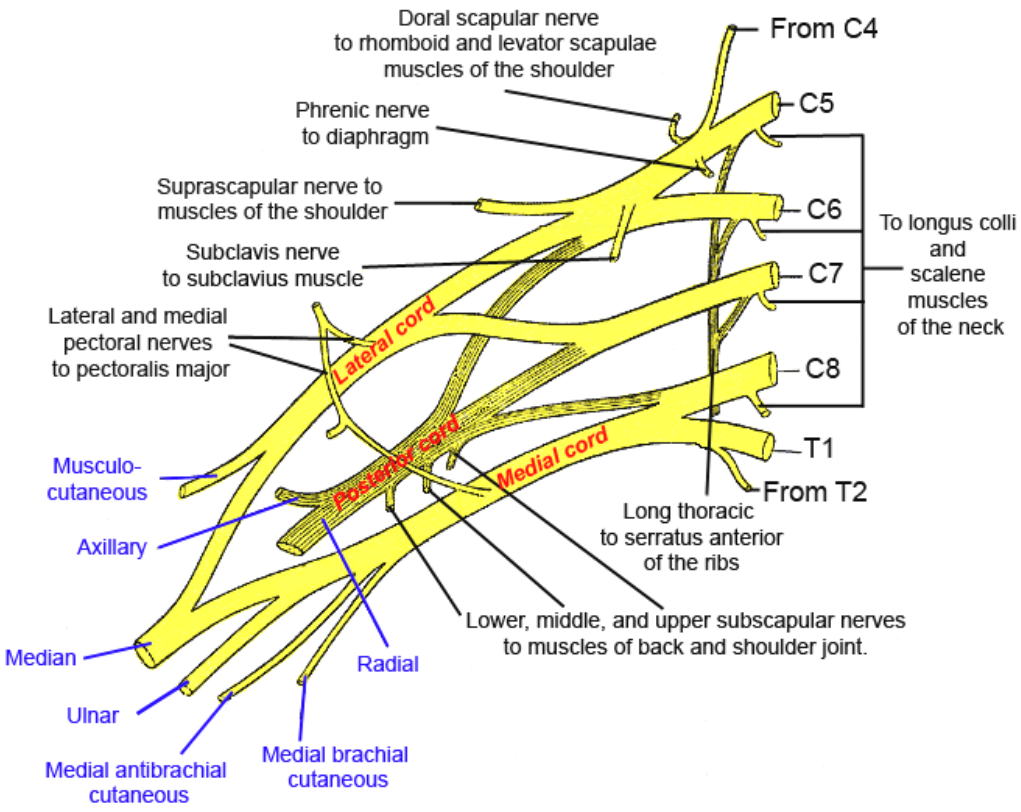

Figure 1. Schematic Diagram of human Brachial Plexus

Figure 1 depicts the anatomical structure of human brachial plexus. It is divided into roots, trunks, divisions, cords and branches of peripheral nerves. There are 5 terminal branches, and other collateral branches that leave the plexus at various points.

(Source: Robert, 2013)

There are 2 types of brachial plexus injuries. In the first type, the nerve may be partially or totally ruptured distal to the intervertebral foramina (postganglionic injury). The second type (avulsion) refers to an injury in which a root or rootlets have been torn from the spinal cord (pre-ganglionic injury). Due to the anatomy, ruptures are more common in the superior part of the plexus (C5-C6) whereas avulsions are more frequent in the inferior part (C7-T1). Because of its proximity to the Ganglion Stellatum, the latter is often associated with Horner's sign. An avulsion is one of the most severe nerve injuries that patients can experience, often with permanent loss of function and a majority of the patients reporting chronic and aggravating pain.

Figure 2a

Figure 2b

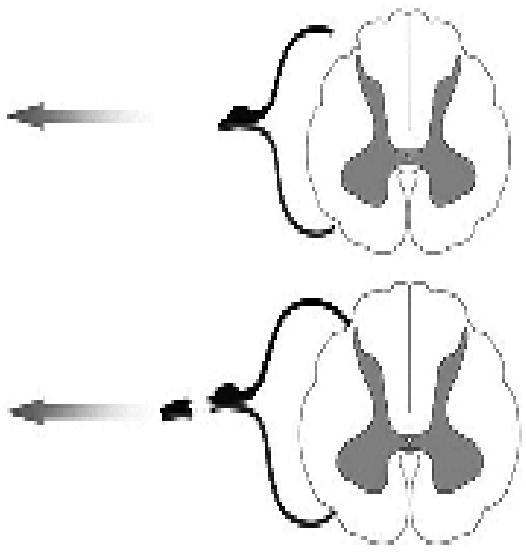

Figure 2. Pre-ganglionic (Avulsion) Injury of the Brachial Plexus Root (Figure 2a on top). Post-ganglionic transection injury of the Brachial Plexus (Figure 2b).
There are two types of injury that can occur: avulsion injuries, as shown in figure 2, When the injury to the nerve is proximal to the DRG, it is called preganglionic, and when it is distal to the DRG, it is called postganglionic. (Adapted from Moran et al. 2005)

Lesions proximal to the Dorsal Root Ganglion (DRG) and on the rootlets from the anterior horn cell on the motor nerve are Pre-ganglionic injuries. The lesions distal to those structures are Post-ganglionic Injuries. Figure 2 illustrated the mechanisms that lead to both types of injuries.

Adult brachial plexus injury remains a dilemma to many surgeons, especially when planning to reconstruct cases of total root avulsion. Different degrees and different levels of injury require different approaches of reconstruction. Chuang (2010) classified brachial plexus injury into 4 levels as shown in Table 1.

Table 1. Chuang's Classification of Brachial Plexus Injury

Type of Injury	Description
Level 1	Preganglionic root injury including spinal cord, rootlets, and root injuries.
Level 2	Postganglionic spinal nerve injury limiting the lesion to the interscalene space and proximal to the suprascapular nerve.
Level 3	Preclavicular and retroclavicular BPI including trunks and divisions.
Level 4	Infraclavicular BPI including cords and terminal branches proximal to the axillary fossa.

Doi et al developed new Magnetic Resonance Imaging classification to demonstratestatus of cervical nerve roots in Brachial Plexus Injuries. He divided Brachial Plexus Injuries into 4 major categories, namely

1. Normal Rootlets
2. Injured Rootlets
3. Avulsion
4. Meningoceles

The radiological classification provides valuable information to assess the nerve rootlets and decide whether or not to proceed for surgical exploration, primary nerve repair and reconstruction (Doi *et al*, 2002).

Electrophysiological tests are essentially important to confirm the diagnosis, site of lesion, and determine the severity of the injury. Both Nerve Conduction Study, and Electromyography (EMG) should be performed three to four weeks after the injury when the impulse transmission is lost owing to Wallerian degeneration. Compound Muscle Action Potential (CMAP) is generally low in Brachial Plexus Injury and it is related to the amount of functional muscles. Sensory Nerve Action Potential (SNAP) is usually normal in Pre-ganglionic lesion simply due to its cell body location at the distally located Dorsal Root Ganglion. Therefore a normal SNAP with an insensate dermatome and low CMAP denotes Preganglionic lesion with less favourable outcome. Denervation (Fibrillation potential) is seen as early as 2 weeks in proximal muscles, and 3-6 weeks in distal muscles. It carries unfavorable prognosis for the patient. Historically, the Seddon classification system has been used to describe the peripheral nerve injuries. The Neurophysiological parameters, i.e. Conduction Velocity, CMAP, SNAP, and Denervation are incorporated into the Sedon's system for prognostication and decision making for surgeon. Three groups of Brachial Plexus Injuries were described, namely Neuropraxia, Axonotmesis and Neurotnomesis. Neuropraxia defined presence of conductional dysfunction of the nerve in the absence of macroscopic lesion. Neuronal transmission is temporarily interrupted at site of lesion. Conduction velocity is normal. CMAP can be normal or reduced. SNAP is

reduced. Denervation evidence in EMG is absent. Axonotmesis described axon transection with intact perineurium and epineurium. Conduction velocity is normal or reduced. CMAP is reduced. SNAP is reduced. Neuronotmesis is the transection of the axon, together with all covering layers of the nerve (Sedon, 1943). All NCS parameters (Conduction velocity, CMAP and SNAP) are absent with presence of spontaneous fibrillation in EMG denoting denervation.

How motor neurons respond to avulsion injury is not fully understood and there is not a cure for such injury. However, previous study showed that spinal root avulsion but not the distal axotomy results dramatic motor neuron degeneration. Both necrosis and apoptosis are involved in the degeneration of injured motor neurons. Axonal injury of motor neurons also results in changes of intracellular molecules as detected by cDNA microarray.

Chai *et al*. (2000) studied the morphological and biochemical changes of motor neurons in response to root avulsion injury and also the regeneration and functional recovery of motor neurons after root avulsion. They had found that root avulsion in adult rats results in dramatic motor neuron loss which is coincident with the de novo expression of neuronal nitric oxide synthase (NOS). Avulsion induced motor neuron death occurs between 2-6 weeks post-injury. Chai *et al.* (2000) had concluded that spinal motor neurons die quickly following root avulsion and re-implantation of avulsed ventral root allowed the regeneration of motor neurons into their axons into the original ventral root after root avulsion. Study conducted by Li *et al.* (1995) also

showed that injuring adult motoneuron axons at the exit point of the nerve from the spinal cord (avulsion) resulted in a 70% loss of motoneurons by 3 weeks following surgery and a complete loss by 6 weeks.

Besides motor neuron degeneration, the ventral root avulsion injury also triggers an activation of microglia within the motor nuclei (Novikov *et al*., 2000). Microglial cells respond to traumatic injury to the nervous system with proliferation and migration toward the affected area. Wu *et al*. (2008) studied the late effects of chronic experimental autoimmune encephalomyelitis in C57BL/6 mice on the spinal cord gray matter. Autoimmune encephalomyelitis (EAE) results in inflammatory white matter lesions in the central nervous system. Their findings showed that EAE induced marked astrocytic, microglial, and macrophage activation in the ventral horn gray matter.

2.2. Brachial Plexus Anatomy in Rat Models

Most of the experimental studies on spinal cord and peripheral nerve injuries were using rats sample. Although there was a clear homology with the elements of the brachial plexus in the rat and in man, the origin of the different terminal and collateral branches were found to be different in these two species (Pais *et al.*, 2010). The rat's spinal cord is made up of 34 segments: 8 cervical (named C1 to C8), 13 thoracic (T1 to T13), 6 lumbar (L1 to L6), 4 sacral (S1 to S4), and 3 coccygeal (Co1 to Co3). A brachial plexus morphology study in 30 rats by Angelica-Almeida *et al*. (2013) demonstrated that brachial plexus was composed of branches originating from the ventral aspect of C4 to C8 and T1. In 57% of cases, the ventral aspect of T2 established an anastomosis with the ventral aspect of T1, thus contributing to the formation of the brachial plexus. This branch from T2, as well as the branch from C4 to the brachial plexus, was smaller than the remaining branches that formed the roots of the plexus. The brachial plexus roots emerged between the anterior and middle scalene muscles, forming a flattened plexus below the clavicle. The lateral, medial and posterior cords of the plexus were not clearly seen compared to those in human. The median nerve was the thickest terminal branch of the brachial plexus in rats, and almost always originated from three different roots. A branch from the second and/or the third intercostal nerve to the medial brachial and medial antebrachial cutaneous nerves was found in 87% of cases. The rat's brachial plexus anatomy and the arterial supply is demonstrated in Figure 3.

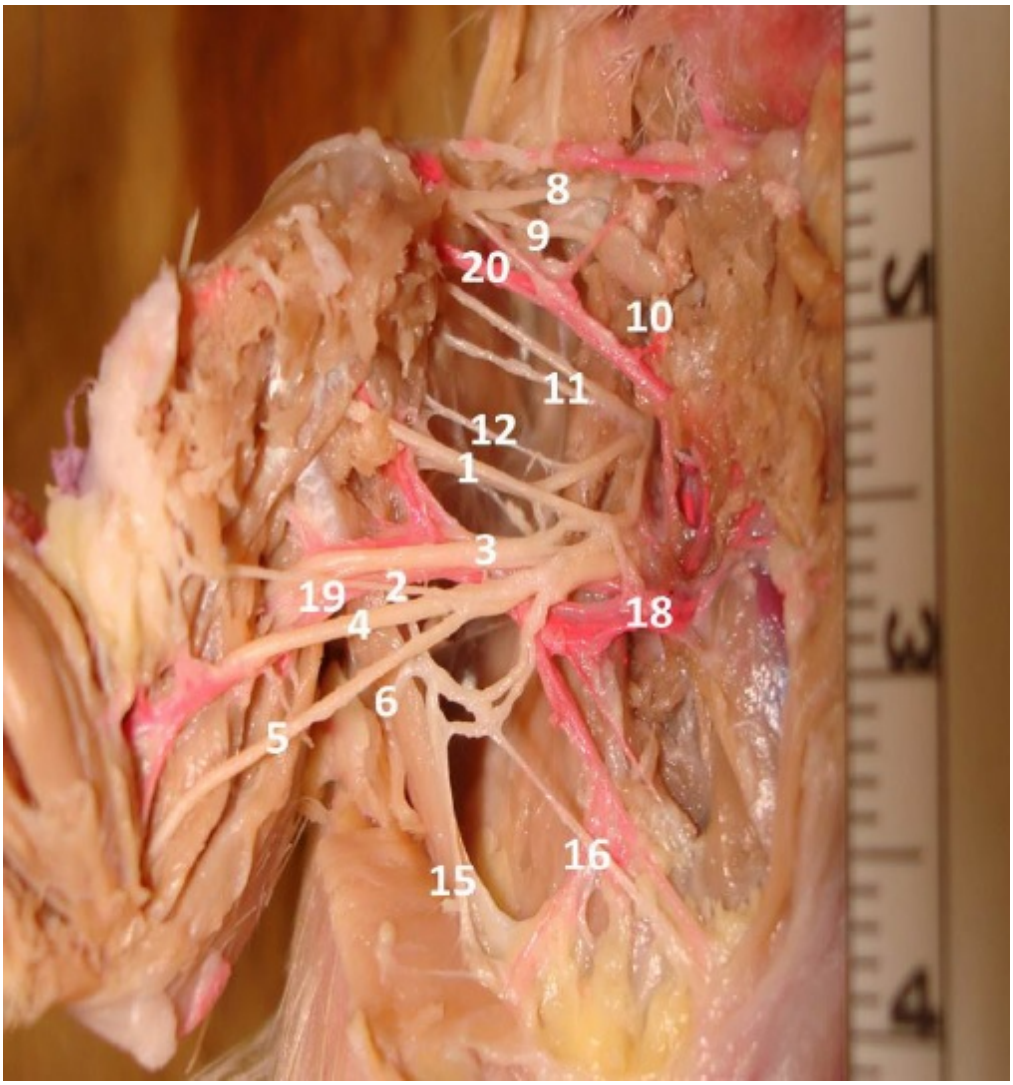

Figure 3. Right Brachial plexus and the arterial supply in rat

Ventral aspect of a right forepaw dissection showing several of the terminal and collateral branches of the brachial plexus, and their association with several major arterial trunks (4X magnification). 1- Axillary nerve; 2- Musculocutaneous nerve; 3- Radial nerve; 4- Median nerve; 5- Ulnar nerve; 6- Medial brachial cutaneous nerve; 8- Dorsal scapular nerve; 9- Suprascapular nerve; 10- Nerve to subclavius muscle; 11- Upper subscapular nerve; 12- Lower subscapular nerve; 15- Lateral pectoral nerve; 16- Medial pectoral nerve; 18- Axillary artery; 19- Brachial artery; 20- Acromial arterial trunk.

(Angelica-Almeida *et al*., 2013)

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The spinal cord is divided into spinal cord segments. Each segment gives rise to paired spinal nerves. Ventra and dorsal spinal roots arise as a series of rootlets (Figure 4). A spinal ganglion is present distally on each dorsal root. Each ventral root (also named the anterior root, radix anterior, radix ventralis, or radix motoria) is attached to the spinal cord by a series of rootles that emerge from the ventrolateral sulcus of the spinal cord. Unlike the dorsal root fibers that are arranged in a neat line at their emergence from the spinal cord, ventral root fibers form an elliptical area named the anterior root exit zone (AREZ). The ventral roots predominantly consist of efferent somatic motor fibers (thick alpha motor axons and medium-sized gamma motor axons derived from nerve cells of the ventral column (Watson *et al.*, 2009).

Each dorsal root (also known as the posterior root, radix posterior, radis dorsalis or radiz sensoria) is attached to the dorsolateral sulcus of the spinal cord by a series of rootlets arranged in a line, the dorsal root entry zone (DREZ). In the experimental

study using rat model, the avulsion surgery was done by separating both the ventral and dorsal roots at the junction between their attachment to the spinal cord, which were the AREZ and DREZ (Watson *et al.*, 2009). Figure 4 demonstrated the cut dissection showing the ventral surface of the spinal cord, as well as the ventral and dorsal rootlets. Figure 5 showed the histological section of the rat's spinal cord. The junction between spinal cord and spinal roots is circled (blue, dorsal root entry zone, DREZ; red = anterior root exit zone, AREZ). Figure 6 showed the relationship between the spine and the brachial plexus in which every spinal nerve root comes from the intervertebral foramen above its synonymous lamina.

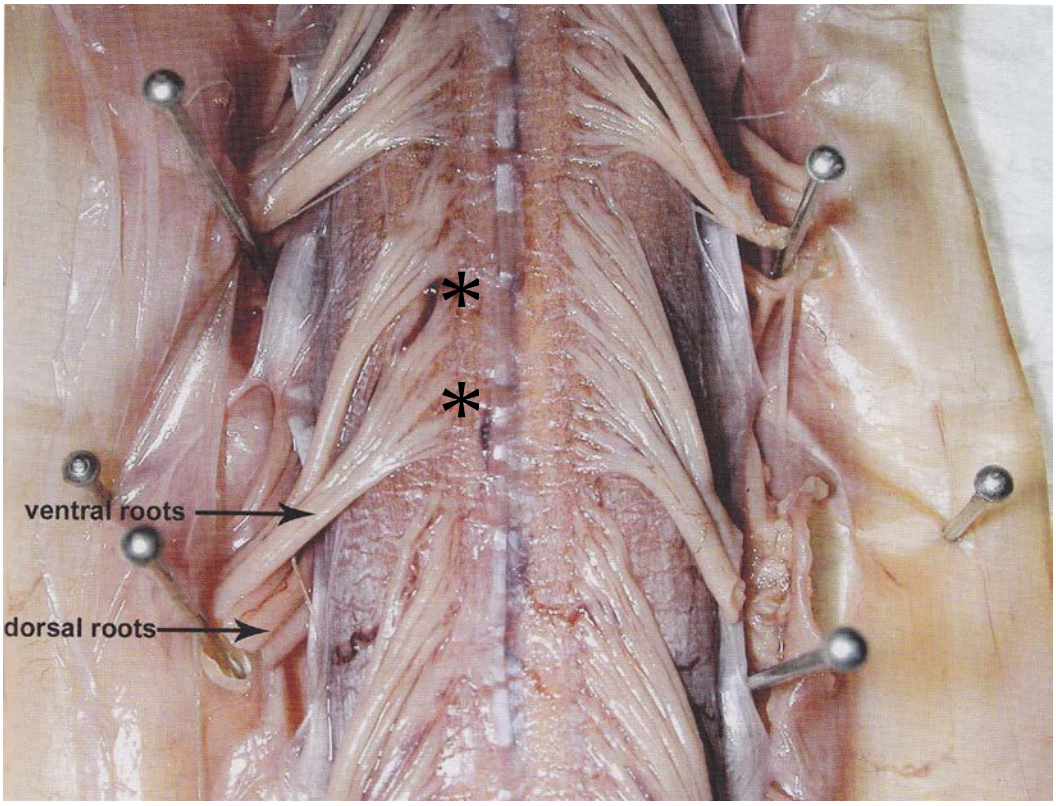

Figure 4. Dorsal and ventral roots of spinal nerves

(Watson *et al.*, 2009)

This is a dissection showing the ventral surface of the spinal cord and the ventral and dorsal rootlets. Groups of rootlets form the dorsal and ventral roots of each spinal nerve. The dura and arachnoid have been removed to expose the spinal cord. The junction between spinal cord and ventral root (anterior root exit zone, AREZ) is labeled **.

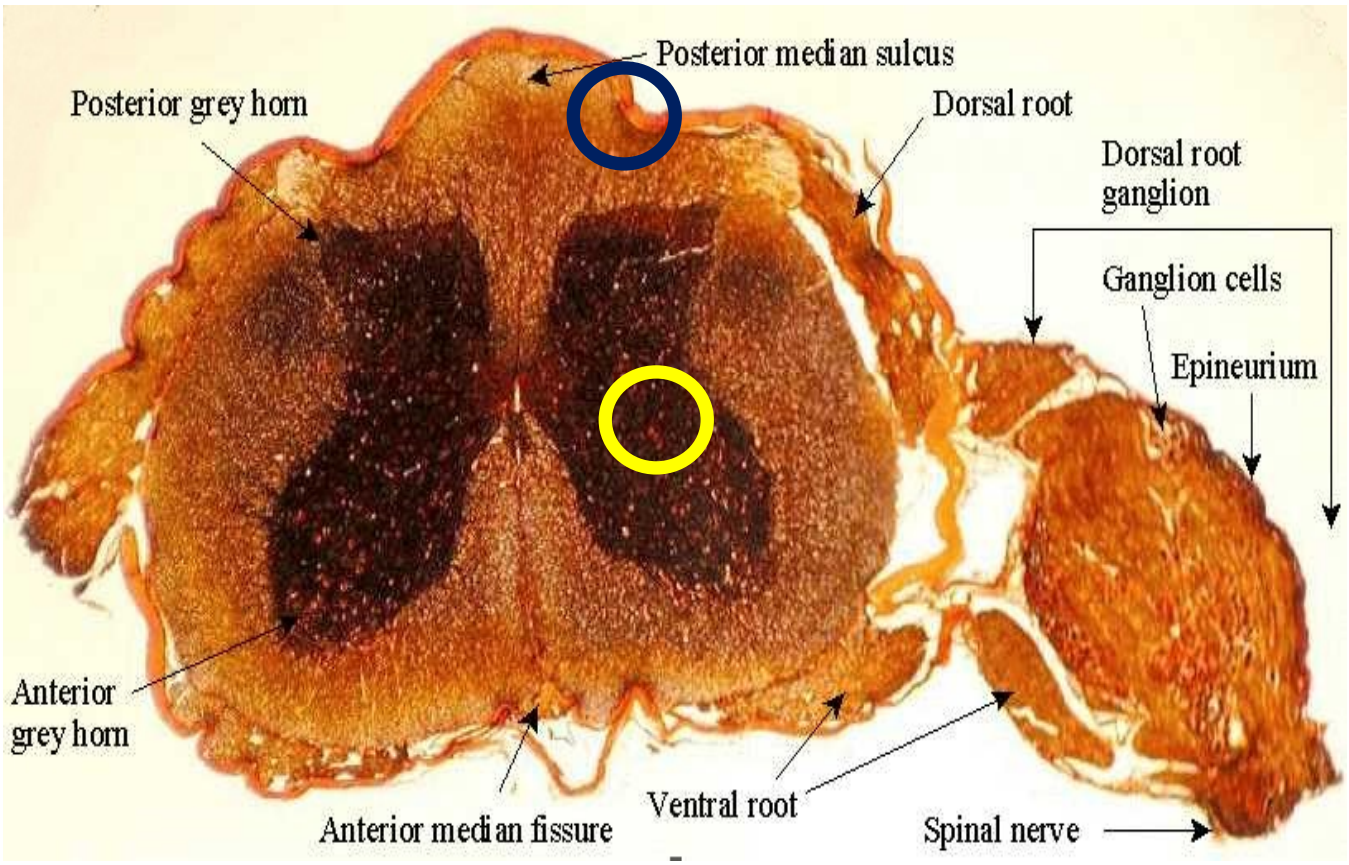

Figure 5. Histological section of a spinal cord in rat

(Watson *et al.*, 2009)

The junction between spinal cord and spinal roots is circled (blue, dorsal root entry zone, DREZ; red = anterior root exit zone, AREZ).

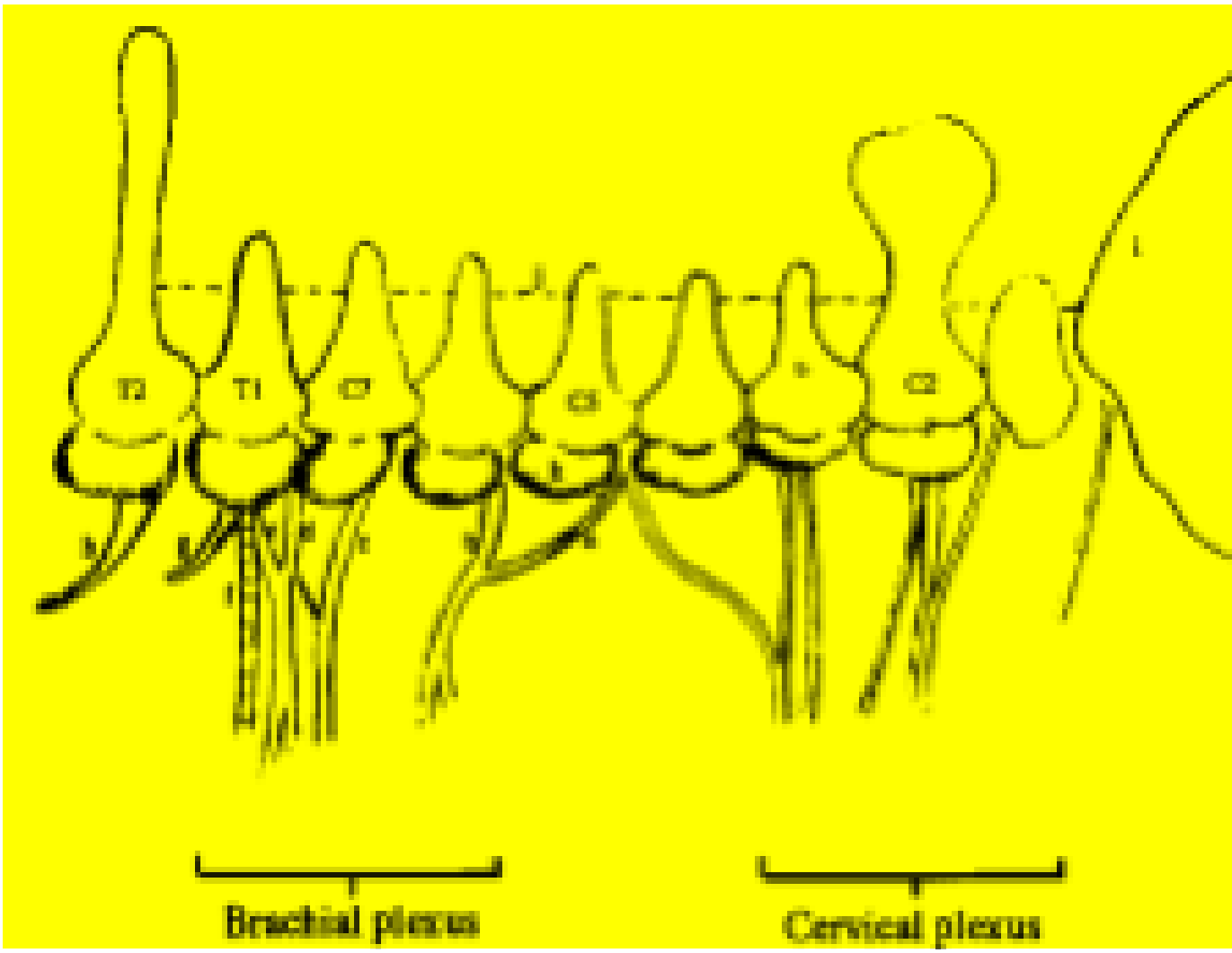

Figure 6. The relationship between the spine and the brachial plexus: Every spinal nerve root comes from the intervertebral foramen above its synonymous lamina. The cervical plexus is also illustrated. *T,* thoracic; *C,* cervical; *a,* fifth cervical nerve root; *b,* sixth cervical nerve root; *c,* seventh cervical nerve root; *d,* eighth cervical nerve root; *e,* first thoracic nerve root; *f,* subclavicular artery; *g,* first rib; *h,* second rib; *i,* cranium; *f,* spinal cord.