ABSTRACT

Motor vehicle accidents are the most common cause of injuries involving avulsion of the brachial plexus in humans, resulting in debilitating motor dysfunction. Lack of an established animal model to test drug treatments hinders the introduction of new pharmacological agents. Avulsion injury of cervical ventral roots can be replicated in rats, resulting in a progressive loss of the motoneurons and increase in neurotoxic expression of microglia. This is a report on the effect of prompt nerve implantation and minocycline treatment on the suppression of microglia activation and survival of motoneurons. 20 adult female Sprague-Dawley rats were used for this study, which was approved by the Animal Ethical Committee, USM (approval number /2011/(73)(346)). The animals underwent surgical avulsion of the C6 nerve root, followed by reimplantation with peripheral nerve graft and treatment with intraperitoneal minocycline. At 6 weeks postoperatively, immunohistochemistry using primary antibody Iba1 (microglia) and nicotinamide adenine dinucleotide phosphate diaphorase (NADPH) with neutral-red staining (motoneuron) under fluorescence microscopy was performed at the C6 spinal cord segment and then quantified. This study showed significant reduction of microglia expression in the study group; mean ranks of control and study group were 15.2 and 11.6, respectively; U=9.5, Z=3.02, p<0.05. However, this did not translate into a significant increase of motoneuron survival in the combined group; the mean ranks of control and study group were 40.6 and 41.6, respectively; U=44.5, Z=-.0378, p>0.05. This may be due to the effect of the surgery; the surgery has the potential to cause additional trauma to the cord parenchyma, leading to further motoneuron loss and an increase in scarring around the avulsed region, thus impeding regeneration of the motoneuron.

Keywords: Avulsion; microglia; minocycline; motoneuron; peripheral nerve implantation

ABSTRAK

Kemalangan kenderaan bermotor adalah punca paling biasa kecederaan yang melibatkan avulsi pleksus brakium pada manusia yang mengakibatkan kelemahan fungsi motor. Kekurangan model haiwan yang mantap untuk menguji rawatan ubat menghalangi pengenalan agen baru farmakologi. Kecerdaraan avulsi pangkal rahim ventral akar boleh direplikasi pada tikus yang mengakibatkan kehilangan motoneuron secara progresif dan peningkatan dalam pengekspresan neurotoksik mikroglia. Kertas ini membincangkan tentang keupayaan implantasi saraf pantas dan rawatan minosiklin ke atas penidaman pengaktifan mikroglia dan kemandirian motoneuron. 20 tikus diberi betina Sprague-Dawley digunakan untuk kajian ini dan telah diluluskan oleh Jawatan Kuasa Etika Haiwan, USM (nombor kelulusan/2011/(73)(346)). Tikus tersebut telah menjalani pembedahan avulsi pada akar saraf C6, diikuti implantasi semula dengan cantuman saraf periferi dan rawatan dengan minosiklin intraperitoneum. Selepas 6 minggu pascabedah, imunohistokimia menggunakan antibodi Iba1 utama (mikroglia) dan nikotinamida adenina dinukleotida fosfat diaphorase (NADPH) dengan pewarnaan merah neutral (motoneuron) di bawah mikroskop berpendarflour telah dijalankan pada segmen saraf tunjang C6 dan kemudian dikuantiti. Kajian ini menunjukkan penurunan pengekspresan mikroglia yang ketara dalam kumpulan kajian; pangkat min kumpulan kawalan dan kajian masing-masing adalah 15.2 dan 11.6; U=9.5, Z=3.02, p<0.05. Walau bagaimanapun, ini tidak pula diterjemahkan kepada pertambahan ketara kemandirian motoneuron dalam kumpulan gabungan; pangkat min kumpulan kawalan dan kajian masing-masing adalah sebanyak 40.6 dan 41.6; U=44.5, Z=-.0378, p>0.05. Ini mungkin disebabkan oleh keupayaan pembedahan; pembedahan tersebut berpotensi untuk menyebabkan trauma tambahan kepada parenkima tunjang yang membawa kepada kehilangan motoneuron lebih banyak dan peningkatan parut di sekitar kawasan avulsi, oleh itu menghalangi pertumbuhan semula motoneuron.

Kata kunci: Avulsi; mikroglia; minosiklin; motoneuron; penempelan saraf periferi
INTRODUCTION

Brachial plexus avulsion injuries in adults are commonly caused by self-induced accidents or motor-vehicle accidents. Spinal root avulsions involve a lesion to the peripheral nervous system at the CNS transition zone and can result in trauma to both the central and peripheral nervous systems (CNS and PNS) (Carlstedt 2009) and motor and/or sensory disruption initially and cell death of motor neurons (Bergerot et al. 2004; Bigbee et al. 2007; Koliatsos et al. 1994). The outcome of brachial plexus reconstruction and the restoration of shoulder and elbow function are often poor in spite of the sophistication of the various methods used, such as prompt nerve repair and grafting and neurotization (Holtzer et al. 2002; Songcharoen 2008). There is room for improvement to augment functional recovery after root reimplantation by proposing a combinatorial treatment approach by means of pharmacological agents (Havton & Carlstedt 2009).

The main objective of this study was to investigate the combinatorial use of surgery (prompt peripheral nerve grafting) and a potent pharmacological agent (minocycline) in inhibiting the expression of microglia to prevent the degeneration of spinal motoneurons after brachial plexus root avulsion injury in an animal model. Minocycline is a potent neuroprotective agent that acts 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) (Arvin et al. 2002; Hoang et al. 2008). The rationale for this study is that the most advanced surgical methods for brachial plexus repair still yield poor outcomes and introducing a potent neuroprotective agent may help augment the recovery in patients who have experienced brachial plexus injury.

MATERIALS AND METHODS

ANIMAL SAMPLES

A total of 20 Sprague-Dawley adult rats (weight ranging from 250-280 g) were used in this study (Animal Ethical Committee approval number/2011/(73)(346)). The rats were divided into 2 groups (control and treatment) with a survival time of 6 weeks. The sample size of 10 rats in each group was selected based on previous studies using a similar animal model (Barbizan & Oliveira 2010). The animals were housed in individual cages in a room (12:12-h light/dark cycle with food and water access) with a 12:12-h light/dark cycle with food and water access post-operatively. All animals were in satisfactory health before the end of the study.

At the end of the 6-week survival time, the rats were anesthetized with a lethal dose of 10% chloral hydrate and perfused transcardially with saline, followed by 4% paraformaldehyde in 0.1-M phosphate buffer (pH7.4). After perfusion, the vertebral column was dissected and the spinal cord removed from the spinal canal. The C7 segment of the spinal cord was then excised, fixed by immersion in fresh fixative overnight and cryoprotected in 30% (V/V) phosphate-buffered sucrose overnight. The C7 spinal segment is defined as the region between the upper-most root and lower-most root of the C7 nerve of the contralateral spinal cord. Frozen transverse sections of 40 μm thickness were cut and collected in 0.01-M phosphate buffer. Every third section from each animal was used for nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry plus neutral-red counterstaining (Su et al. 2013). Sections were then incubated at 37°C for 1 h in 10 mL of 0.1-M Tris-HCL(pH8.0) containing 0.2% Triton X100, 10 mg NADPH and 2.5 mg nitroblue tetrazolium and then washed with 0.1-M phosphate buffer for three times. The stained sections were then mounted onto the glass slides and counterstained with 1% neutral red. These sections were used to count the number of surviving motoneurons. Neurons containing nitric oxide synthase (NOS) were stained with nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) (Figure 1).

ADMINISTRATION OF MINOCYCLINE

Minocycline (50 mg/kg; Minocin 100 mg intravenous; Wyeth, Thailand) was administered intraperitoneally (i.p.) daily for 7 days post-operatively in the minocycline treatment group. The first dose was given on the day of surgery in connection with the root injury procedure. The minocycline dose was reduced by half (25 mg/kg i.p. daily) for day 8-14 post-operatively, providing a total of 2 weeks of treatment.

OUTCOME EVALUATIONS

The animals were housed in individual cages in a room with a 12:12-h light/dark cycle with food and water access post-operatively. All animals were in satisfactory health before the end of the study.

The outcome of brachial plexus repair still yield poor outcomes and introducing a potent neuroprotective agent may help augment the recovery in patients who have experienced brachial plexus injury.

INTRAVERTERBAL SPINAL ROOT AVULSION SURGERY AND IMPLANTATION OF PERIPHERAL NERVE GRAFT

The rats were anesthetized with intramuscular injections of ketamine (80 mg/kg) and xylazine (8 mg/kg). A midline incision of 2.5–3 cm was made from T2 upwards (about 4 cm). Using the T2 protrusion as a bony landmark, the sixth cervical vertebra (C6) was identified. The right-side of the C6 lamina and articular process was removed using a bone ronguer and the dorsal root was identified. The dorsal root was then excised after opening the dura mater from where it attaches to the spinal cord to expose the ventral root. A fine-tipped pipette glass hook was used to hook and avulse the ventral root. Together with the ventral root, the dorsal root and its ganglion, about 2-3 mm of the spinal nerve was severed and removed. Then a saphenous nerve graft from the same animal was harvested and implanted to the lateral funiculus of the spinal cord at the avulsed site using an 11-0 monofilament suture.
COUNTING OF MOTONEURONS AND MICROGLIA

Data quantification and analysis were performed by two independent persons trained in the procedure, both of whom were blinded to the treatment groups; the Kappa score for inter-observer bias was 0.7. Approximately 30 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. In total, 10 light microscopic images of the C7 ventral horn of these sections in each animal were captured (20× and 40× lens). In NADPH-d plus neutral red-stained sections, a motoneuron with a visible nucleus in the neutral red stain was counted as a surviving cell. The percentage of surviving motoneurons was quantified on both the intact side and the lesioned side of the C7 section. The percentage of surviving motoneurons on the contralateral intact side was set as 100%. The surviving motoneurons on the lesioned side were then counted. The number of surviving motoneurons ipsilaterally was expressed as a percentage of the number of surviving motoneurons contralaterally in the same C7 section. Immunocytochemical staining of microglial cells was done using rabbit IBA-1 as primary antibody and an anti-rabbit conjugated secondary antibody. For double immunofluorescence, sections were incubated in rabbit Iba1 overnight at room temperature, rinsed and incubated in anti-rabbit Alexa 594 conjugated secondary antibodies for 1 h at room temperature before being mounted on the slides for examination under a fluorescence microscope.

STATISTICAL ANALYSIS

Data were analysed by using computer software SPSS for Windows version 20.0 (IBM; Armonk, New York, USA). The Wilcoxon Mann-Whitney test was applied to determine any significant differences among the study groups. Differences were considered significant at \( P<0.05 \).

RESULTS

EXPRESSION OF MICROGLIA AFTER NERVE ROOT IMPLANTATION AND TREATMENT WITH MINOCYCLIN

In the control group, the expression of microglia was 15.2% (1.8) while in the experiment group the expression of microglia was 11.6% (1.9) (Table 1). There was a significant difference in the expression of microglia between the control group and the experimental group; mean ranks of control and study group were 15.2 and

<table>
<thead>
<tr>
<th>Control group (%)</th>
<th>Treatment group (%)</th>
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<td>18</td>
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</table>

Mean (SD) 15.2 (1.8) 11.6 (1.9)

TABLE 1. Expression of microglia (in mean percentage after 6 weeks) in all the test animals [control versus treatment] (n=20)
11.6, respectively, $U=9.5, Z=3.02, P<0.05$ ($P=0.02$), $r=-0.1$ (Figure 2).

**SURVIVAL OF MOTONEURONS AFTER NERVE ROOT IMPLANTATION AND TREATMENT WITH MINOCYCLINE**

After injury, on the panoramic view deformity of the C6 spinal cord, segment was found on the lesioned side in the intradural avulsion group. There was a significant reduction in the number of neutral red-stained motoneurons on the lesioned C6 segment as compared to the contralateral non-lesioned side in both the control and experimental groups.

On the lesioned site, the number of motoneurons reduced significantly. The mean ranks for the control and study groups were 40.6 and 41.6, respectively; $U=44.5, Z=-0.0378, P>0.05, r=0.77$. There was no significant difference in motoneuron survival between these two groups (Figure 3).

**DISCUSSION**

**EXPRESSION OF MICROGLIA AFTER PERIPHERAL NERVE GRAFT IMPLANTATION AND MINOCYCLINE TREATMENT**

Consistent with previous studies, avulsion injury causes robust activation of microglia expression (Afshari et al. 2009; Block & Hong 2005; Scholz & Woolf 2007). After injury, uptake increases of the Iba-1 staining in the ipsilateral ventral horn as compared to the contralateral

**TABLE 2. Comparison of microglia between control and treatment group (N=20)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (median (IQR))</th>
<th>Treatment group (median (IQR))</th>
<th>$U$</th>
<th>$z$-score</th>
<th>$P$ value*</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microglia</td>
<td>15.2 (4)</td>
<td>11 (3)</td>
<td>9.5</td>
<td>3.02</td>
<td>0.02</td>
<td>-0.1</td>
</tr>
</tbody>
</table>

*Wilcoxon Mann-Whitney test

**FIGURE 2. Iba1 histochemistry showing increased expression of microglia (arrow) in the avulsed ventral horn (40× magnification) (scale bar 300 μm)**

**FIGURE 3. Neutral red-stained motoneuron (arrow) as seen under the fluorescence microscope (40× magnification) (scale bar, 100 nm)**

**TABLE 3. Survival of motoneurons (in mean percentage after 6 weeks) in all the test animals**

<table>
<thead>
<tr>
<th>Control (n=10)</th>
<th>Control group (%)</th>
<th>Treatment group (%)</th>
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<tr>
<td>1</td>
<td>46</td>
<td>43</td>
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<tr>
<td>2</td>
<td>38</td>
<td>45</td>
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<tr>
<td>3</td>
<td>41</td>
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<td>4</td>
<td>32</td>
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<td>8</td>
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<td>9</td>
<td>35</td>
<td>40</td>
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<td>10</td>
<td>40</td>
<td>39</td>
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Mean (SD) 40.6% (5.7) 41.6% (4.3)

(control versus treatment) (n=20)
Minocycline-related studies have been widely performed, primarily in the context of neurodegenerative disease and stroke in animals. These studies showed that inflammation induced by reactive microglia has been identified as a major cause contributing to neuronal dysfunction and death. With the dosages used in this study, minocycline has been proven to reduce excitotoxicity, inhibiting chronic neuroinflammation; particularly of microglial activation and reduction of neuronal and oligodendroglial apoptosis (Sharma et al. 2010).

**SURVIVAL OF MOTONEURONS**

In this study, the control group showed that the surviving motoneuron percentage is 40.6% (5.7). In the treatment group, the percentage is slightly higher at 41.6% (4.3), U = 44.5, Z = -.0378, p>0.05, r = 0.77. However, this result was not significant. It shows that minocycline treatment has no superior effect in ensuring motoneuron survival.

During previous studies, minocycline had been shown to exert a neuroprotective effect by suppressing the expression of microglia, thus reducing the neurotoxic cascade responsible for neuronal death (Plane et al. 2010). However, this result is not reflected in the number of surviving motoneurons in the current study. This divergence in results may be due to several factors. Avulsion injury causes massive destruction of motoneurons (Noguchi et al. 2013); microglia have been shown to undergo significant changes about 2 weeks after injury (Chew et al. 2011). Thus, during the initial 2-week period, the microglia showed no significant effect on the surviving motoneurons and significant motoneuron loss had already occurred during this time even though minocycline did significantly reduce microglia expression. The surviving motoneurons may have only been maintained by the implantation of the nerve root. Furthermore, avulsion injury to the spinal cord causes massive primary loss of cells at the site of injury. Acute spinal cord injury initiates various inflammatory responses that (together with other cytotoxic events) lead to secondary damage and formation of fluid-filled cysts, demyelination and degeneration of axons and motoneuron at the site of injury (Afshar et al. 2009).

Neurons may die through either passive necrosis or via an active metabolic process similar to apoptosis during which intracellular events show a high degree of homology irrespective of the initiating event (Chew et al. 2011). In traumatic injury such as avulsion, necrosis is more associated with direct trauma to the cell body.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>U</th>
<th>z-score</th>
<th>Pvalue*</th>
<th>r</th>
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<tr>
<td></td>
<td>Control median (IQR)</td>
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<tr>
<td>Motoneurons</td>
<td>40.5 (11)</td>
<td>44.5</td>
<td>-0.37</td>
<td>0.7</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Treatment median (IQR)</td>
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*Wilcoxon Mann-Whitney test

(non-avulsed) ventral horn on the same slice of the C6 cervical cord. This study showed that the expression of microglia in the control group was 15.2% (1.8), whereas in the treatment group the expression was statistically lower at 11.6% (1.9), U = 9.5, Z = 3.02, P<0.05 (P = 0.02), r = -0.1 (Table 2).

Reactive gliosis occurred when the microglia become phagocytic, enabling removal of damaged axons, neurons and myelin. Reactive microglia was found to respond within hours to ischemic injury of the central nervous system, affecting axonal regeneration in a variety of ways, for instance by producing neurotoxic compounds such as free radicals. In a spinal cord injury, glutamate (a neurotoxic substance) was released. A fundamental observation from a previous study suggested that a potential role for glia in glutamate-induced neuronal death is that excitotoxicity is associated with activation of astrocytes and microglia (Scholz & Woolf 2008; Tikka & Koistinaho 2001).

Block and Hong (2005) showed that no statistical difference in microglial expression existed between an avulsed animal model and an implanted model, suggesting that implantation alone does not have any influence over the activity of microglia and therefore does not protect the spinal cord from the onset of a neurotoxic environment. However, the current study showed that the expression of microglia was significantly lower in the treatment group. This would suggest that minocycline has a neuroprotective property by means of inhibition of microglial activation, thus preventing aneurotoxic cascade that can culminate in neuronal death.

Minocycline and doxycycline are semisynthetic tetracycline derivatives, which exert neuroprotective effects independently of their anti-microbial action by reducing free-radical formation and inhibition of interleukin-1β synthesis as well as by inhibiting microglial activation and proliferation through inhibition of the p38 MAP kinase (MAPK) pathway. The p38 MAPK pathway is involved in induction of several proinflammatory genes; therefore, inhibition of p38 MAPK may be a mechanism by which minocycline exerts a wide range of anti-inflammatory effects in the brain and peripheral nervous system. Minocycline blocked the activation of p38 MAPK induced by different stimuli such as nitrous oxide (NO), 1-methyl-4-phenyl-1,2,3,6,-tetrahydropiridine (MPTP) and the up-regulation of glutamate family receptors by NMDA agonists or glutamate (Kumar et al. 2012; Tikka & Koistinaho 2001).
and occurred too rapidly to be amenable to therapeutic intervention (Chew et al. 2011). Moreover, in this study, surgical implantations of an avulsed root into the spinal cord inflicted an additional minor trauma to the cord apart from the avulsion injury itself. The surgical procedure involves a small longitudinal incision into the lateral portion of the lumbar spinal cord followed by the insertion of the avulsed root into the lateral funiculus and sutured in place with a monofilament suture. Special care is taken in a microsurgical way in order to minimise injury to the spinal cord. However, the injury from the surgery itself is imminent and may contribute to more local inflammatory reaction, leading to necrosis and neuronal death and formation of scar tissue.

Apart from microglia activation, a ventral root avulsion injury can also cause a marked astrogial reaction in the ventral horn as well as the activation of phagocytic macrophages in the setting of motoneuron death (Afshari et al. 2009). The presence of astrogial and macrophages in the ventral horn gray matter is consistent with the retrograde cell death of efferent spinal cord neurons, as demonstrated in previous studies (Afshari et al. 2009; Hoang et al. 2008).

The choice of peripheral nerve type is also a deciding factor. In this study, the autologous graft was used, which is the saphenous nerve. The saphenous nerve is a superficial sensory nerve harvested from the animal’s lower limb. A recent study had shown that reimplantation of the avulsed ventral root itself is superior in preserving motoneuron as compared to peripheral nerve graft (Su et al. 2013).

Implanted PN grafts and ventral roots both exert neuroprotective roles, possibly through the release of neurotrophic substances that act on the perikarya of the severed motoneurons and/or activation of specific survival genes (Chu et al. 2009). The ventral exit zone possesses natural conduits connecting with motoneurons. Repositioned ventral roots (which were attached to the ventrolateral surface of the spinal cord) may diffuse neurotrophic factors more efficiently than laterally implanted PN grafts, which do not connect with these natural conduits. Apart from that, another study reported that the ventral root preferentially supported motor axon regeneration since mRNA for pleiotrophin (PTN) and glial cell line-derived neurotrophic factor was upregulated to a greater degree in the ventral root (Hut et al. 2006). These may account for the stronger effect of superficially implanted ventral roots on promoting motoneuron survival and regeneration compared to PN grafts (Chu et al. 2009). One may also postulate that the PN graft contains lesser neurotrophic factors needed for the survival motoneuron. Thus, in this study, the use of a PN graft is insufficient to complement the effect of minocycline with respect to increasing the survival of motoneuron after implantation, even though minocycline significantly reduced the expression of microglia.

Motoneuron survival depends largely on the presence of neurotrophic factors. Studies also showed that motor nerves are superior to sensory nerves for promoting motoneuron survival and axonal regeneration after root avulsion (Chu et al. 2009) because of the higher expression of brain-derived neurotrophic factor (BDNF) and glial cell-line derived neurotrophic factor (GDNF) in motor nerves (Chu et al. 2009). Motoneurons require trophic factors for survival during embryonic development (Chu et al. 2009) and after injury in adult animals (Chu et al. 2009). For the present study, a plain PN graft was used without complementing with exogenous BDNF or GDNF. Therefore (as noted earlier), better survival for the motoneurons was not ensured even with the reduction in microglia expression.

The limitations of this study manifest in the surgical aspects of the procedure as well as with the evaluation of outcomes. The surgery involved in the avulsion model may have caused extensive injury to the spinal cord, resulting in massive loss of motoneurons and deprivation of neurotrophic factors from the initiation of the procedure. Apart from that, surgery itself causes formation of scar tissue in the surgical area, potentially impeding regeneration of motoneurons and axons and negatively impacting survival. Surgery related to the hemilaminectomy is complicated by the presence of bleeding arising from the bone. Additionally, avulsion of the spinal root causes massive bleeding (possibly from the site of the spinal venous plexus). The spinal artery may also sometimes be severed during the procedure; on more than one occasion, massive uncontrollable bleeding led to the animal’s death intraoperatively. In animals that survive, the disruption of vascular supply may cause cord ischaemia and neuronal loss may be due to ischaemia rather than to the neurotoxic cascade caused by upregulation of microglia. In that sense, the study’s aimed (examining the effect of minocycline on the survival of motoneurons) may have been impaired by this ischaemic condition.

Apart from that, minocycline in this study was introduced via the intraperitoneal route. This route may lead to reduced absorption of the active metabolites with accompanying reductions in effect on the injured spinal cord. Minocycline’s bioavailability is higher via gastrointestinal absorption and only a small amount diffuses into the CSF to reach the neural tissue (WHO Drug Information 1997). In order to overcome this limitation, in future studies, a different route of administration could be considered. One potential alternate route is intrathecal (through an intrathecal pump mechanism).

Outcomes of this study were assessed via histopathological examination only. Even though a significant survival effect was shown in the motoneuron in the implanted group (with reduction of minocycline), clinical outcomes for the study group were not assessed. A few parameters (such as paw grasping power, paw walking pressure and self-grooming) do exist for assessing the clinical outcomes of the animal models used in this study. Having a positive survival of motoneurons in the cervical spinal cord should correlate with improvement of clinical symptoms as well.
SUMMARY AND CONCLUSION

The present study investigates the efficacy of minocycline treatment and autologous peripheral nerve grafting on the survival of cervical spine motoneurons after peripheral nerve avulsion injury in adult rats.

On the basis of the experimental data, some conclusion can be made. Permanent avulsion of the peripheral nerve induces massive loss of motoneuron in the affected cervical spinal cord segment. Immediate repair of the injured cervical spinal cord using autologous peripheral nerve graft results in preservation and survival of motoneurons of that segment. However, surgery itself causes scar tissue formation and might impede motoneuron survival. The study also showed that the expression of microglia is significantly reduced after treatment with minocycline. However, the combination of autologous peripheral nerve grafting and minocycline treatment does not significantly affect motoneuron survival.

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