Chapter

Effects of Cyclosporin-A, Minocycline, and Tacrolimus (FK506) on Enhanced Behavioral and Biochemical Recovery from Spinal Cord Injury in Rats

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Abstract

Spinal cord injury (SCI) results into an immediate primary injury (physical damages) followed by secondary damages (prolonged posttraumatic inflammatory disorder) resulting into severe motor dysfunction including paralysis. The present chapter discusses and investigates the neuroprotective effects of cyclosporin-A (CsA), minocycline, and tacrolimus (FK506) and their therapeutic effectiveness in recovery from the animal model of SCI. Based on the available recent literature on these three drugs, as well as in perspective of the results obtained on some experimental behavioral, biochemical, and oxidative stress parameters in the present study, the therapeutical potential of these three drugs has been discussed. Furthermore, the animal model of SCI used herein has been reviewed and compared with other reported animal models for understanding the utility, suitability, and reproducibility of the methodology of the present model for screening purposes in quest of searching ideal therapeutic compounds for maximum recovery from SCI.

Keywords: cyclosporin-A, minocycline, tacrolimus (FK506), rats, spinal cord injury, behavior, oxidative stress

1. Introduction

Spinal cord injury (SCI) is prevalent worldwide [1, 2] and often incapacitates the victims for life resulting in disability. Injury to the spinal cord results in processes that occur in three phases: the first phase is immediate physical phase also known as acute phase comprising affected spinal shock and initial trauma (primary injury) followed by the second phase known as secondary phase which is a prolonged cascade of damaging processes over a time period of minutes to weeks after the injury (secondary injury). Such damages include ischemia, vascular alterations, biochemical alterations, and cellular responses that lead to peripheral posttraumatic inflammatory cell infiltration and cell death (secondary injury) [1, 3–5]. The third phase that sustains between days and years after SCI trauma is characterized by proapoptotic degeneration and scarring that establishes permanent functional impairment [6, 7]. Secondary injury leads to the key pathophysiological response

to SCI causing severe and permanent functional deficits. Most of the clinical trials and experimental studies are conducted for intense research to unfold the underlying pathophysiological processes and for searching ideal and potential therapy for recovery from secondary SCI injuries [8]. Besides motor dysfunction, some of the other important SCI-related biochemical and immunological impairments that get involved are serum tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interleukin-6, nuclear factor (NF)-κB p65, p38 mitogen-activated protein kinase (MAPK), inducible nitric oxide synthase (iNOS), caspase-3, superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) [9]. SCI trauma causes a devastating effect not only to the individual patient, but it also incurs heavy expensive burden to the society in general and to the family members, due to substantial long-term healthcare expenditures [10].

Despite considerable therapeutic studies, no proven drugs or techniques are available for satisfactory treatment of SCI. Much of these therapeutic studies have been reported from animal models, and it needs to be understood that a successful clinical trial in humans can only be initiated based on previously available preclinical data reported from animal model studies that closely mimic the losses as in human SCI functions [11]. Although rats are the animal model of choice for SCI studies, the major anatomical differences in axonal tracts and sensory motor pathways between quadrupeds and bipeds need to be taken into careful account to improve the targets of human SCI treatments [12].

Currently, methylprednisolone is the only recognized treatment for human SCI; however, it has significant adverse effects, including respiratory complications, sepsis, and gastrointestinal hemorrhage [13]. Furthermore, other important evidence-based therapies that have potential neuroprotective and neural reparative therapeutic properties and are undergoing clinical trials for human SCI include surgical decompression, blood pressure augmentation, riluzole, granulocyte colony-stimulating factor, minocycline, glibenclamide, cerebrospinal fluid drainage, magnesium, therapeutic hypothermia, Cethrin (VX-210), anti-NOGO antibody, cell-based approaches, and bioengineered biomaterials [5, 14, 15].

Some other experimental drugs that have been studied for therapeutical use in animal SCI are recombinant human erythropoietin [10], tetrodotoxin [16], BCL-2 [17], cyclosporin-A [18], edaravone [19], atorvastatin [20], calpain inhibitors [21] FK506, and minocycline [22]. Also, some natural products like eugenol oil [9], curcumin [23], and melatonin [24] have shown promising effects in animal SCI functional recovery. It sounds reasonable that instead of using a highly selective treatment that targets a specific molecule or pathway, a compound with multifunctional properties that targets several mediators involved in spinal cord pathology may be more effective for recovery from SCI [25]. In our earlier study [22], the promising potential of FK506 and minocycline has been reported for their effectiveness in rat SCI model. Thus, in the present study, besides these two multifactorial effective compounds minocycline and FK506, a third compound cyclosporin-A (CsA) was also included, and all the three compounds minocycline, FK506 (tacrolimus), and cyclosporine-A were chosen to evaluate in a comparative manner for their therapeutical potential using some important and reliable parameters that are most commonly used in rat SCI model [22]. Before discussing the outcome of our present results, we review the multifactorial effects of these three compounds that have been reported in literature using rat SCI model.

1.1 Minocycline

Minocycline, a semisynthetic second-generation tetracycline, has robust neuroprotective effects in rodent models of neurodegenerative diseases [26] and provides

neuroprotection in experimental models of neurological diseases, including SCI [27]. In a broad range of secondary injury mechanisms via its anti-inflammatory, antioxidant, and antiapoptotic properties, minocycline is effective in reducing secondary injury and promoting locomotor functional recovery [28–31]. Minocycline prevents N-methyl-D-aspartate (NMDA)-induced excitotoxicity by diminishing NMDA-induced Ca^{2+} influx and mitochondrial Ca^{2+} uptake [32] and protects gray and white matter from SCI [33]. Minocycline also inhibits p38 mitogen-activated protein kinase (p38 MAPK) activation and microglial pro-nerve growth factor (proNGF) expression resulting from inflammatory reactions due to SCI and improves oligodendrocyte survival [34]. Inflammation due to SCI also upregulates and activates a class of enzymes like phospholipase A2s (PLA2s), and minocycline reduces cPLA2s [35]. It also inhibits monocyte and microglial expression of cyclooxygenase 2 (COX2) and production of proinflammatory prostaglandins E2 [36] and suppresses 5-lipoxygenase (5-LOX) action in SCI tissue [37]. Minocycline also eliminates free radicals in the post-SCI microenvironment and protects from oxidative stress [38]. It inhibits malondialdehyde, a by-product of lipid peroxidation [39, 40], and increases glutathione (GSH) [39], superoxide dismutase, and glutathione peroxidase [40], suggesting the powerful antioxidative mechanisms of minocycline to recover from secondary injury in SCI. Minocycline is reported to inhibit matrix metalloproteinases (MMPs) that are upregulated following SCI and are involved in injury and recovery processes [41, 42]. Furthermore, minocycline improves functional outcome, reduces lesion size and cell death, and alters cytokine expression after SCI [43–45]. Minocycline reduces the lesion area, increases the number of descending sympathoexcitatory axons traversing the injury site, and ultimately reduces the severity of autonomic dysreflexia [46]. In a murine model of SCI, minocycline treatment was superior to methylprednisolone in promoting functional improvement [44] and had neuroprotective effects on the SCI epicenter [47], motor neuron recovery, and neuropathic pain [48]. Minocycline has recently been reported to be effective in reducing secondary injury and promoting locomotor functional recovery in experimental SCI [28].

It has also been reported to attenuate reactive astrocytosis in SCI which directly damages cell bodies and triggers endogenous processes including neuroinflammation and reactive astrocytosis [49, 50]. In combination studies also, minocycline has been reported for better recovery from SCI when used in combination with other drugs like FK506 [22] and bone marrow mesenchymal cells (BMSCs) [51] showing a very significant recovery in behavioral function, oxidative stress, and reduction in lesion size from SCI in rats warranting further research on this drug.

1.2 Cyclosporin-A

Cyclosporin-A is an immunosuppressive cyclic undecapeptide that inhibits T cells and depresses both cellular and humoral immune responses to prevent graft rejection and reduces the inflammatory responses [52]. CsA significantly decreases the expression levels of interleukin-10, tumor necrosis factor-α, cyclophilin-D (Cyp-D), and apoptosis-inducing factor (AIF) [53]. CsA does not readily cross the blood-spinal cord barrier (BSCB), which restricts the clinical application of CsA for SCI treatment. Thus, polyethylene glycol (PEG)-transactivating-transduction protein (TAT)-modified CsA-loaded cationic multifunctional polymeric liposome-poly (lactic-co-glycolic acid) (PLGA) core/shell nanoparticles (PLGA/CsA NPs) to transport and deliver CsA across the BSCB have a new potential to treat SCI [54]. CsA inhibits primarily the inflammatory reaction and the synthesis of constitutive nitric oxide (NO) and inducible nitric oxide synthases (NOS), well-known neurotoxic agents for SCI diminishing overproduction of free radicals, and secondarily

lipid peroxidation (LP) observed after SCI [55, 56]. CsA may also induce other non-immunological effects that could be beneficial for treatment of neurological disorders [57]. CsA has been widely used in the treatment of various diseases including aplastic anemia, nephritic syndrome, rheumatoid arthritis, psoriasis, and cerebral ischemic injuries [53]. CsA promotes neuroprotection by diminishing both demyelination and neuronal cell death, resulting in a better motor outcome after SCI [52, 58, 59]. CsA in combination with FK506 had a neuroprotective treatment against SCI hypoxia-induced damage mediated via their antioxidant actions on mitochondrial ATP, tissue-reduced glutathione, tissue LPO level, and myeloperoxidase (MPO) activity [60]. Administration of CsA in combination with olfactory ensheathing cell (OEC) transplantation results in augmented functional improvements and promotes axon regeneration after SCI [61].

1.3 FK506

FK506 (tacrolimus), a macrolide lactane antibiotic, was introduced as an immunosuppressive agent [62] with virtually no side effects [63]. FK506, a potent calcineurin inhibitor, exhibits neuroprotective actions in several experimental models of central nervous system trauma, including stroke, and improved neurological recovery following peripheral and spinal cord injuries [47, 63–67]. It is reported that FK506 has beneficial effects in SCI recovery involving various mechanisms such as neuroregeneration and neuroprotection [67], promotion of axonal outgrowth [68], and suppression of oxidative stress [60]. FK506 improves the functional outcome of SCI [67–69] and has an in vivo neurotrophic action, whereby it enhances the rate of axon regeneration, leading to more rapid neurological recovery [70–73]. Significant functional recovery from SCI due to FK506 treatment has been reported in rat models [22, 67, 74]. Activation of NF-κB and proinflammatory cytokines (TNF-a, IL-1b, and IL-6) expression levels in SCI animals is reversed by FK506 treatment involving microglial activation after SCI [7]. FK506 upregulates epidermal growth factor (EGF)-level expression of astrocytes that have an important role as mediators for SCI functional recovery promoting axonal regeneration [74]. FK506 in combination as a cocktail with other drugs like minocycline [22], CSA [60], RhoA inhibitors [75], nerve growth factor (NFG) [76], and methylprednisolone (MP) [77] has shown significant therapeutic recovery from SCI in rats.

Considering the above-discussed multifactorial effects of CsA, minocycline, and FK506, the present study was undertaken to investigate the neuroprotective effects of these three compounds in a comparative manner on recovery from experimental SCI, as these three drugs target multiple processes involved in mediating cell death and the development of secondary injury in SCI. Furthermore, our earlier findings on FK506 and minocycline [22] prompted us to include CsA (another promising drug for SCI recovery) and compare their effectiveness in rat model of SCI, using the behavioral and biochemical parameters as in earlier [22].

2. Utility of experimental animal models for SCI studies

For SCI studies, animal models are used because of their easy accessibility, convenience, and capability of the researchers to explore them at several levels (simulated to human clinical SCI levels) for motor functional, biochemical, and oxidative stress and genetic, therapeutic, and pathophysiological evaluations [78]. Over the last decade, a variety of animal models have been used for experimental SCI studies, including rats, mice, gerbils, guinea pigs, hamsters, rabbits, dogs, goats, pigs, and nonhuman primates [79]. Among these animals, rodents in general

and rats in particular are the most widely and commonly studied SCI models [80]. In the present study also, we have used young adult male Sprague-Dawley rats with all similar specifications of breeding, housing facilities, and experimental handlings, as described in our earlier study [22].

To establish an ideal SCI animal model for research purposes, various models have been tried and reported till to date in quest of searching methodology to obtain maximum recovery from SCI. These experimental animal models include spinal cord traumatic injury model [81], photochemical-induced SCI model [82], spinal cord transection model [83], bidirectional distraction SCI model [84], and the spinal cord ischemia-reperfusion injury model [85]. For traumatic injury model, the contusive SCI model is used by inducing contusion on the dorsal spinal cord by dropping a desired weight either from a computer-controlled impact device [86] or from a customized impact device [87]. Another traumatic injury model known as compressive SCI model is also very commonly used where instead of dropping the weight, it is placed on the exposed spinal cord segment in the dorsoventral direction to induce a compressive SCI [88, 89]. However, since SCI caused by impact and compression is more common in clinical patients [79], in the present study also, we have used the compressed SCI model induced in the rats as described in our earlier study [22]. Briefly, the SCI was induced in the rats following the modified method of Nystrom and Berglund [89]. Laminectomy was performed at the T 7–8 level, and spinal cord compression injury was produced by placing a load with a total weight of 35 g, for 5 min over the exposed extradural area.

All experimental rats were randomly divided into the following six groups with eight animals in each as described earlier [22]:

Group I: The normal control group without laminectomy or compression injury

Group II: Sham group with laminectomy alone but no spinal compression injury

Group III: SCI control group with laminectomy and spinal compression injury

SCI-treated groups were the same as the SCI control group (Group III) and consisted of three groups in which the effect on the recovery from SCI using the same parameters is mentioned in our earlier study [22]. Doses of the three drugs CSA, minocycline, and FK506 were selected on the basis of our pilot screening of these drugs at low, medium, and high doses, and the best effective dose in each was used in the present study as follows:

Group IV: Cyclosporin-A 5 mg/kg

Group V: Minocycline 50 mg/kg

Group VI: FK506 (tacrolimus) 1 mg/kg

All protocols for the drug administration, follow-ups, care, and experimental handlings of the animals for various evaluation parameters were the same as described earlier [22].

3. Behavioral evaluations in SCI animals

To analyze the therapeutic recovery from induced SCI in animal models, several behavioral outcome measures have been developed and widely used, such as the catwalk [90], the Basso-Beattie-Bresnahan (BBB) locomotor scale [91], the horizontal ladder test, and the cylinder rearing test [92]. From the literature review of the recent years, it is found that BBB locomotor scale has been most widely used in SCI rat models to evaluate motor functional recovery from SCI [9, 12, 22–24, 53, 58, 74, 77, 93]. However, in the present study, besides BBB locomotor scale [94], a battery of some more behavioral motor functions was included like Tarlov scoring [95], inclined plane test [96], and some functional deficit scorings like toe spread, platform hang, wire mesh descent, and hind foot bar grab [97, 98]. Our pilot study showed that a naive control group of animals treated with CsA, minocycline, and FK506 without SCI

showed no different behaviors than the naïve control untreated groups (data not shown in behavioral results) for all the observed behavioral parameters. Thus, the results of the drug treatments alone were not included in all behavioral results (**Figures 1–4**).

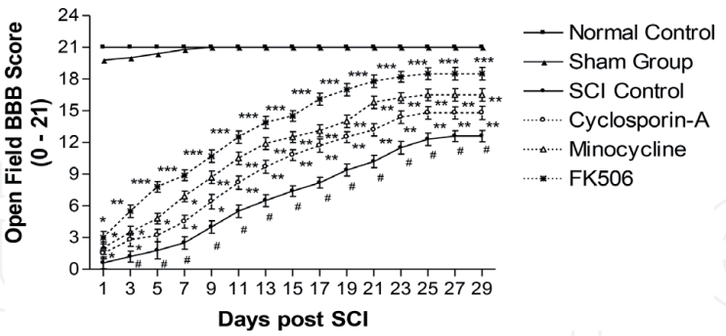

Figure 1.
*Effect of CSA, FK506, and minocycline on gait performance tunnel (GPT) behavioral motor performance activities (BBB Score) of hind limbs of rats subjected to SCI. The graph shows the comparative functional recovery from SCI over a period of 29 days. Animals were treated with the drugs daily after SCI for 3 weeks. Abbreviations: CSA, cyclosporin-A; FK506, tacrolimus; SCI, spinal cord injury; BBB, Basso, Beattie, and Bresnahan. Drug doses used are cyclosporin (5 mg/kg), FK506 (1 mg/kg), and minocycline (50 mg/kg); the drugs are effective in the order FK506 > minocycline > cyclosporin-A. # shows the SCI group is significantly ($p < 0.001$) different from the SCI uninjured control group. *, **, and *** represent the SCI-treated groups are significantly different at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, compared to the SCI group by ANOVA with post hoc testing using Tukey-Kramer or Student-Newman-Keuls Multiple Comparison Tests.*

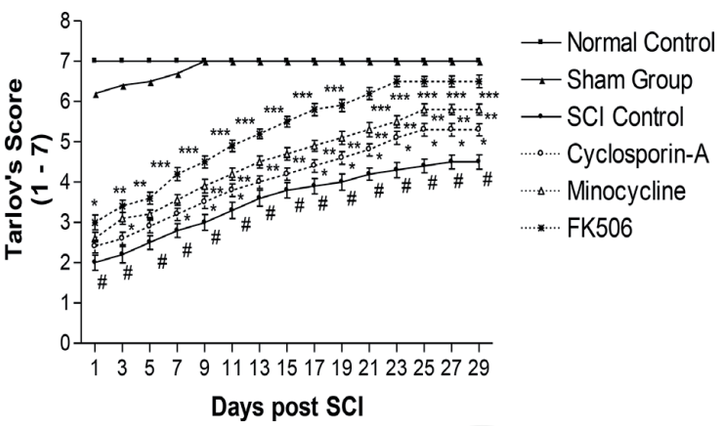

Figure 2.
The effect of cyclosporin-A, FK506, and minocycline on the behavioral motor performance activity (Tarlov's Score) of hind limbs of rats subjected to SCI. The graph shows the comparative functional recovery from SCI over a period of 29 days. Animals were treated with drugs daily after SCI for 3 weeks. Abbreviations, drugs used and their doses, and all statistical significances are the same as in **Figure 1**.

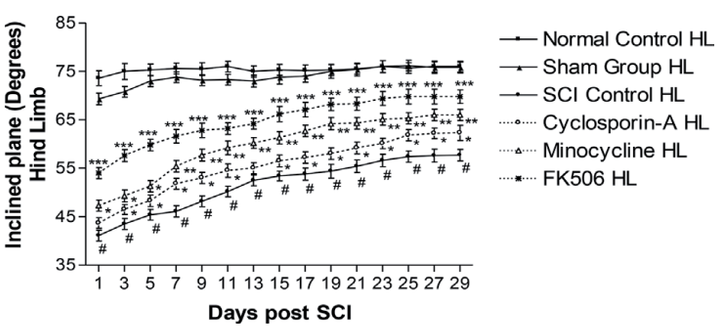

Figure 3.
The effect of cyclosporin-A, FK506, and minocycline on the behavioral motor performance activity (Inclined Plane Test) of hind limbs (HL) of rats subjected to SCI. The graph shows the comparative functional recovery from SCI over a period of 29 days. Animals were treated with drugs daily after SCI for 3 weeks. Abbreviations, drugs used and their doses, and all statistical significances are the same as in **Figure 1**.

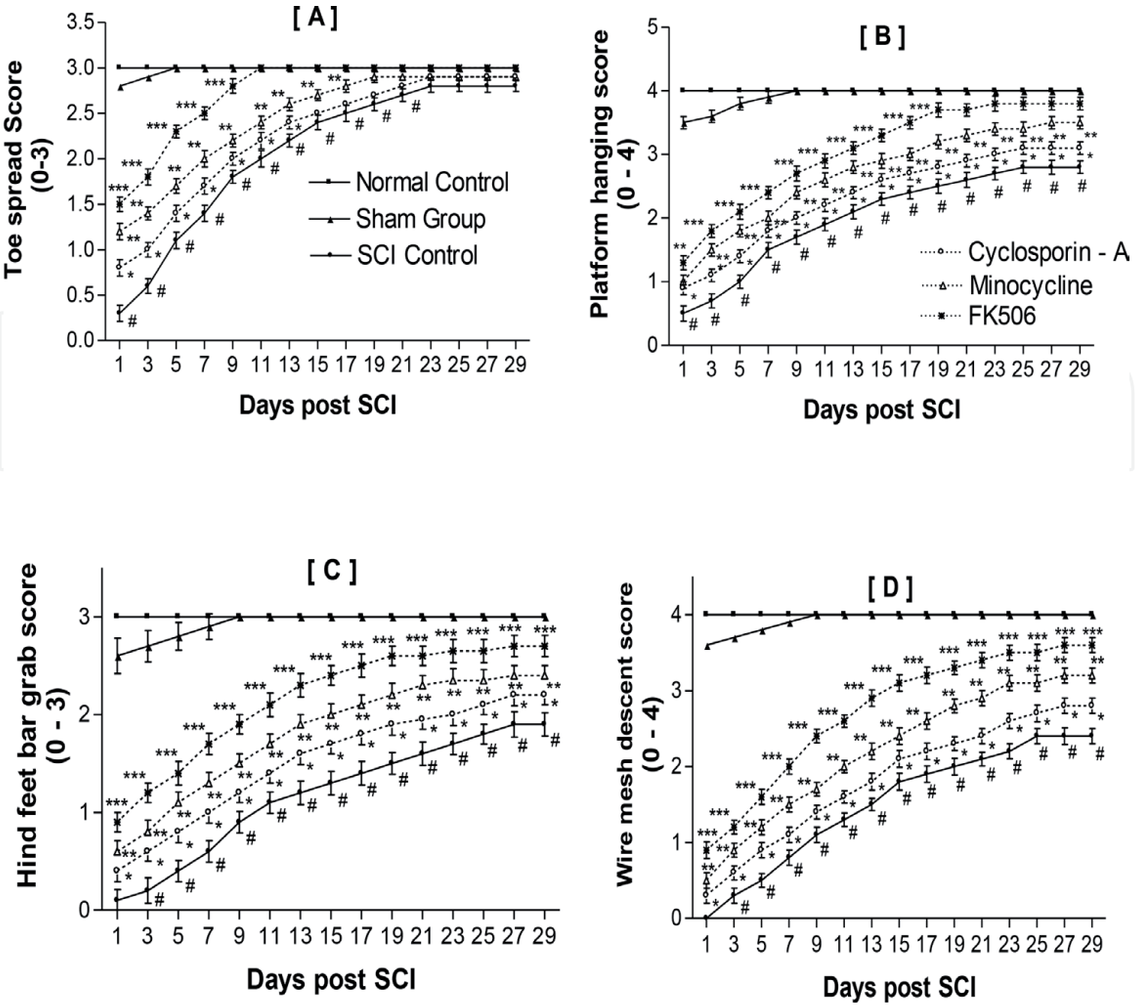

Figure 4.
(A–D) The effect of cyclosporin-A, FK506, and minocycline on the behavioral motor functional scoring of toe spread (A), platform hanging (B), hind foot bar grab (C), and wire mesh decent (D) of rats subjected to SCI. The graph shows the comparative functional recovery from SCI over a period of 29 days. Animals were treated with drugs daily after SCI for 3 weeks. Abbreviations, drugs used and their doses, and all statistical significances are the same as in **Figure 1**.

The present results of behavioral observations indicated that treatment with all the three drugs in this study induced significant recovery from SCI with respect to time in all behavioral activities compared to the SCI control group, and the drugs were effective in the order of FK506 > minocycline > CsA ($F = 13.49$, $F = 5.82$, and $F = 3.14$; $df = 3$; $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively) throughout (**Figures 1–4**).

4. Biochemical evaluations in SCI animal models

Biochemical evaluations have a vast list of parameters that exist as biomarkers for assessing recovery from SCI in animal models. Some of the most important biochemical parameters include oxidative stress indices like lipid peroxidation and total glutathione, nitric oxide synthase, myeloperoxidase, mitochondrial permeability, inflammatory responses, autonomic dysreflexia, cerebrospinal fluid biomarkers, immune responses, astrocyte modulations, etc., and all of these have been reviewed in detail earlier in this chapter, especially for the three drugs, CsA, minocycline, and FK506, that have been evaluated in the present study.

The biochemical parameters evaluated in this study included determination of monoamines 5-hydroxy-indoleacetic acid (5-HIAA) and serotonin or 5-hydroxy

tryptamine (5-HT) [99], lipid peroxides determined as thiobarbituric acid-reactive substances (TBARS) [100, 101], total glutathione [102, 103], and myeloperoxidase [104] and have been described for their methods in our earlier study [22].

The present biochemical results showed significant ameliorating effect of all three drugs on the levels of 5-HT (**Figure 5A**, 5-HIAA; **Figure 5B**, on the ratio of 5-HIAA; and **Figure 5C**, 5-HT). TBARS was significantly stimulated (**Figure 6A**), whereas GSH was significantly inhibited (**Figure 6B**), and MPO level was significantly diminished toward the normal level (**Figure 6C**). Overall, the entire biochemical parameters evaluated in the present study were significantly affected by the three drugs effectively in the order FK506 > minocycline > CsA throughout.

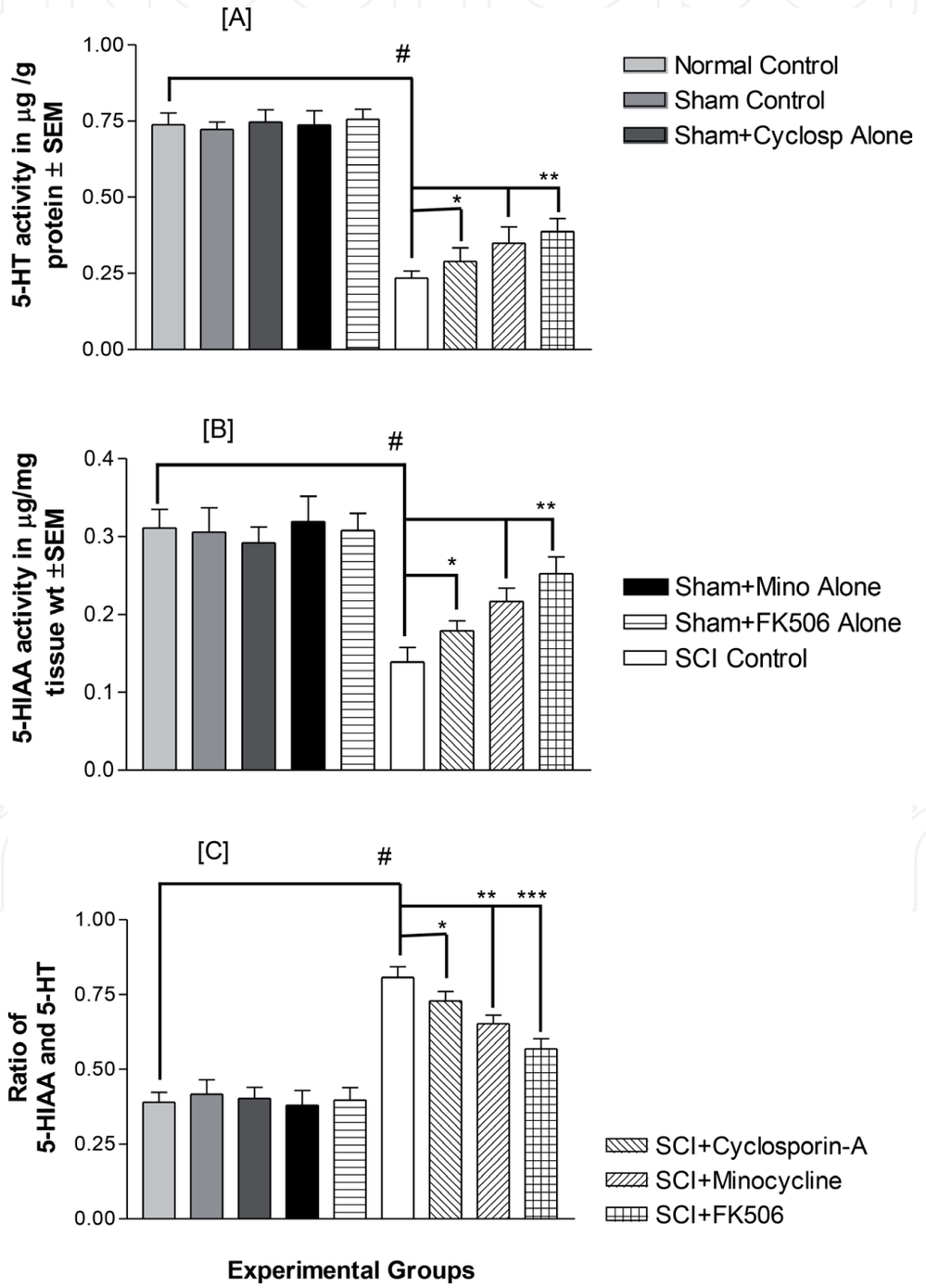

Figure 5.
(A–C) Levels of (A) 5-HT (5-hydroxytryptamine), (B) 5-HIAA (5-hydroxy-indoleacetic acid), and (C) the ratio of 5-HIAA and 5-HT activities in the spinal cord tissue of rats 29 days post-SCI and the effects of treatment with various drugs. Abbreviations, drugs used and their doses, and all statistical significances are the same as in **Figure 1.**

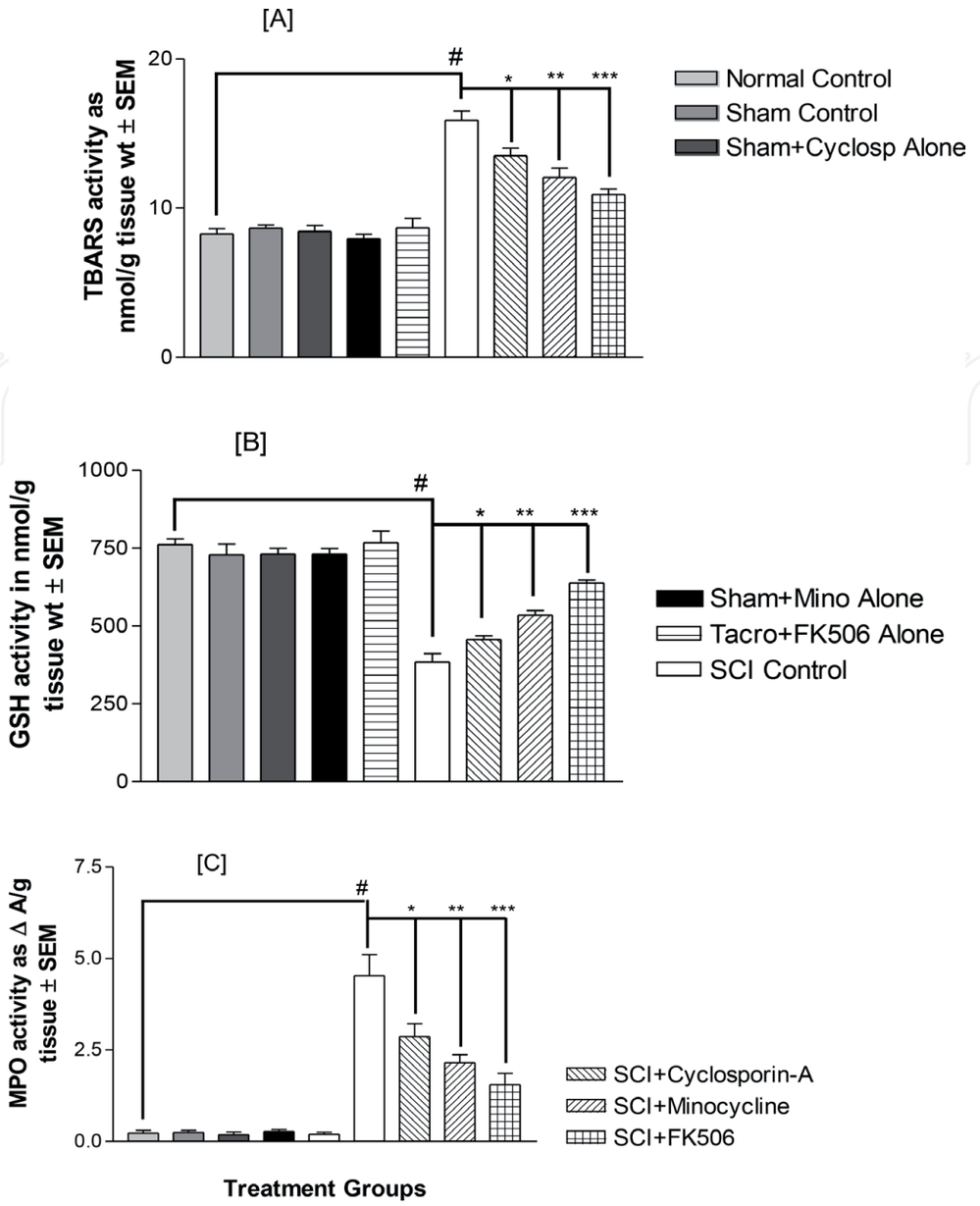

Figure 6.
Levels of (A) thiobarbituric acid, (B) glutathione, and (C) myeloperoxidase activities in the injured spinal cord tissue of rats 29 days post-SCI and the effects of treatment with various drugs. Abbreviations, drugs used and their doses, and all statistical significances are the same as in **Figure 1**.

5. Discussion

SCI leads to persistent pain and motor dysfunction, both of which lack effective therapeutics [105]. Therapeutic approaches that promote both neuroprotection and neuroregeneration are valuable for SCI therapies [52]. From the present discussed literature, the potential agents that have generated interest in SCI studies in the recent past include the multifactorial drugs minocycline, FK506, and CsA [18, 22, 52, 106].

In the present chapter also, the treated rats showed recovery in their hind limb reflexes rapidly regaining responses comparable with those of uninjured control rats (**Figure 1**). Although all drug-treated groups showed improved recovery in BBB and all behavioral activities, the best and the most significant recovery was observed with FK506 treatment. The drugs were effective in the order FK506 > minocycline > CsA throughout. Earlier studies have also used BBB scoring along with other behavioral parameters and have shown significant behavioral functional outcome in the

SCI animals treated with FK506 [7, 22, 60, 67, 69, 70, 74, 76], minocycline [22, 28, 43–45], and CsA [18, 52, 53, 58, 61]. In addition to the therapeutic effects of the three drugs, it has been also suggested that the daily routine behavioral assessment procedures may also assist the animals as equivalent to their exercises that help them to recover from SCI [107]. However, more studies are required to confirm this presumption. Additionally, no notable side effects were noted at the dosing regimen of the drugs (selected from the pilot studies) used in the present chapter. However, it has been suggested particularly for FK506 [63] that the therapeutic dosing regimen is a key factor that can affect efficacy as a neuroprotectant for CNS injuries.

FK506 has been reported by others also for being a potential therapy for SCI recovery through various mechanisms [7, 60, 63, 68, 108]. It is evidently proven that FK506 prevents the activation of NF-κB in microglia which reduces production of proinflammatory cytokines like TNF-a, IL-1b, and IL-6 in the SCI responses for effective recovery [7, 109]. Furthermore, it is suggested that inhibition of inflammatory reaction in SCI by FK506 could be due to its inhibitory action on decreasing the free radical formation and lipid peroxidation preventing calcineurin-mediated dephosphorylation of NOS activity in a Ca^{2+}-dependent manner [77]. FK506 also enhances neurite outgrowth and improves functional recovery from SCI by stimulating astrocytes to secrete epidermal growth factor (EGF) for neural repair [74].

CsA has also been reviewed and reported as a potent neuroprotectant for functional recovery from SCI [5, 55]. The significant protective role of CsA has been reported for recovery from SCI through inhibiting the apoptosis of spinal cord cells [53], improving locomotor function [58], increasing mean arterial pressure [110], inhibiting NOS [56], diminishing demyelination and neuronal cell death [60], attenuating reactive astrocytosis due to injury improved neurologic outcome [50], and reducing pain [111].

Minocycline has also been reviewed recently for its effectiveness through multiple mechanisms for functional recovery from SCI [38]. The multiple targets that minocycline works for SCI functional recovery include upregulation of the protein VEGF and BDNF expressions; downregulation of protein p-38MAPK, proNGF, p75NTR, and RhoA expressions and suppressed caspase-3 activity [51]; and improved antioxidant activity through amelioration in oxidative stress in the SCI tissue [40].

Monoamines such as norepinephrine (NE), dopamine (DA), and serotonin (5-HT) can activate the spinal neurons involved in walking [112–114]. Thus, the decrease in the level of 5-HT and 5-HIAA in the SCI animals in the present chapter clearly indicates that SCI inevitably affects the normal functioning of these spinal neurotransmitters involved in locomotor function. SCI-injured animals treated with the drugs herein improved levels of 5-HT and 5-HIAA (**Figure 5A–C**, respectively). Our present behavioral findings also showed an overall correlation and significant improvement in the functional deficits of the hind limbs after treatment with these drugs, indicating the presence of potential mechanisms of serotonergic agents in these drugs, as present in indorenate (5-methoxytryptamine, beta-methyl carboxylate hydrochloride), a 5-HT1A agonist that improved motor function in rats with chronic SCI [115].

The oxidant/antioxidant balance was clearly reflected by the increased level of TBARS (**Figure 6A**) and decreased level of GSH (**Figure 6B**) in the contused tissue of SCI control animals. However, treatment of SCI animals with the drugs interfered with the formation of free radicals following traumatic SCI. The comparative behavioral restorative effects of these drugs in the formation of free radicals in injured SC were in the order of FK506 > minocycline > CsA.

Spinal cord injury in mice results in severe trauma characterized by edema and neutrophil infiltration (measured as an increase in myeloperoxidase activity), and

these neutrophils are thought to be involved in tissue injury through the release of various inflammatory mediators [116, 117]. The MPO levels in the present SCI animals were also significantly increased in the injured spinal cord tissue (**Figure 6C**). However, administration of minocycline, FK506, and CsA interfered significantly with the formation of MPO following traumatic SCI. The comparative restorative effects of these drugs in the formation of MPO in injured SC were in the order of FK506 > minocycline > CsA.

The pathophysiological events resulting from SCI are reported to involve free radical production; lipid peroxidation; excitotoxic molecules such as glutamate, eicosanoid, and prostaglandin production; protease activity; and intracellular increases in Ca^{2+} [118]. Furthermore, the primary auto-destructive event is initiated by the hydrolysis of fatty acids from membrane phospholipids, leading to cellular damage [119], and microglia becomes activated [120], which in turn may release neurotoxic molecules that further damage nearby neurons [121].

The hind limb functional deficits in the model of SCI (like the one as in the present chapter) are largely due to the loss of white matter axonal tracts [16, 122]. The white matter degeneration is caused by the primary injury (i.e., mechanical lesion), and there is also evidence that post-SCI demyelination caused by oligodendrocyte death/malfunction contributes significantly to chronic SCI functional deficits [123, 124].

The secondary injury is reported to result from several proposed auto-destructive events, including reactive oxygen species-induced lipid peroxidation [125], activation of non-NMDA ionotropic glutamate receptors [126], and caspase-3 activation [127, 128]. Secondary injury events include Na^{+} influx-mediated intra-axonal Ca^{+} accumulation leading to proteinase activation, which destroys the cytoskeleton [16, 129], as well as the induction of oligodendroglial apoptosis with subsequent demyelination of the surviving axons [79, 130]. Lipid peroxidation is one of the main pathological mechanisms involved in secondary damage after SCI [79]. Another key factor in the secondary injury mechanism is Ca^{2+} ions. Following trauma or ischemia, Ca^{2+} influx plays an important role in the pathogenesis of neural injury [130, 131]. Many drugs, including steroids, gangliosides, ion channel blockers, antioxidants, and free radical scavengers, have mild therapeutic effectiveness in experimental spinal cord injury [74, 119]. Another mechanism to promote functional recovery after spinal cord injury is enhancing axonal regeneration. Several strategies, including blocking myelin or glial scar inhibitors, delivery of neurotrophic factors, and cell transplantation, induce axonal outgrowth after experimental spinal cord injury. Among them, olfactory ensheathing cell grafts promote neuroprotection, axonal regeneration, and functional recovery after incomplete spinal cord injury [132, 133]. Furthermore, a regular enforced movement activity may additionally help provide faster functional restoration and recovery after SCI [134].

Studies on combinatorial effects of CsA, minocycline, and FK506 in various combinations with each other or with other compounds may prove to be more effective in recovery from SCI. Earlier combined treatments like FK506 and NGF [76], FK506 and minocycline [22], FK506 and methylprednisolone [77], FK506 and RhoA inhibitor [76, 77], minocycline and bone marrow mesenchymal stem cells [51], and CsA with PEG-TAT [54] have all shown significant functional recovery from SCI as compared to these compounds individually.

6. Conclusions

From the overall literature review on the multifactorial effects of CsA, minocycline, and FK506 and from the discussion of the present findings, it can be

concluded that the drugs CsA, minocycline, and FK506 induce good recovery from experimentally induced SCI in rats. However, these drugs significantly improve functional restoration, replenish 5-HT and 5-HIAA levels, and restore the oxidant/antioxidant balance in the contused tissue after moderate SCI in rats in the order FK506 < minocycline < CsA. Furthermore, it is suggested that the present compressive SCI model of rats could still serve as the most convenient model for therapeutic screenings of various drugs in search of ideal therapy for SCI. CsA, minocycline, and FK506 appear to have gained support in a multifactorial effective manner through ample research work and should be considered as ideal therapeutical agents for the treatment of acute SCI. These drugs should be supported for clinical trials with further studies and tests. Although FK506 appears to be the most promising among the three drugs, more work is needed to screen all three compounds as cocktails in various combinations with better expected outcomes in SCI recovery possibly due to their cumulative multifactorial beneficial effects.

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Conflict of interest

The authors have no conflict of interests.

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References

[1] Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal injury. Spine (Phila Pa 1976). 2001;**26**:S2-S12

[2] Saunders LL, Clarke A, Tate DG, Forchheimer M, Krause JS. Lifetime prevalence of chronic health conditions among persons with spinal cord injury. Archives of Physical Medicine and Rehabilitation. 2015;**96**:673-679. DOI: 10.1016/j.apmr.2014.11.019

[3] O'Brien MF, Lenke LG, Lou J, Bridwell KH, Joyce ME. Astrocyte response and transforming growth factor-beta localization in acute spinal cord injury. Spine (Phila Pa 1976). 1994;**19**:2321-2329

[4] Gorman PH. The review of systems in spinal cord injury and dysfunction. Continuum (Minneapolis, Minn.). 2001;**17**:630-634. DOI: 10.1212/01.CON.0000399092.88866.1c

[5] Ahuja CS, Nori S, Tetreault L, Wilson J, Kwon B, Harrop J, et al. Traumatic spinal cord injury-repair and regeneration. Neurosurgery. 2017;**80**: 9-22. DOI: 10.1093/neuros/nyw080

[6] Profyris C, Cheema SS, Zang D, Azari MF, Boyle K, Petratos S. Degenerative and regenerative mechanisms governing spinal cord injury. Neurobiology of Disease. 2004;**15**:415-436. DOI: 10.1016/j.nbd.2003.11.015

[7] Liu G, Fan G, Guo G, Kang W, Wang D, Xu B, et al. FK506 attenuates the inflammation in rat spinal cord injury by inhibiting the activation of NF-κB in microglia cells. Cellular and Molecular Neurobiology. 2017;**37**:843-855. DOI: 10.1007/s10571-016-0422-8

[8] Thuret S, Moon LD, Gage FH. Therapeutic interventions after spinal cord injury. Nature Reviews. Neuroscience. 2006;**7**:628-643. DOI: 10.1038/nrn1955

[9] Ma L, Mu Y, Zhang Z, Sun Q. Eugenol promotes functional recovery and alleviates inflammation, oxidative stress, and neural apoptosis in a rat model of spinal cord injury. Restorative Neurology and Neuroscience. 2018;**36**:659-668. DOI: 10.3233/RNN-180826

[10] Gorio A, Gokmen N, Erbayraktar S, Yilmaz O, Madaschi L, Cichetti C, et al. Recombinant human erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**:9450-9455. DOI: 10.1073/pnas.142287899

[11] Lukovic D, Moreno-Manzano V, Lopez-Moncholi E, Rodriguez-Jime'nez FJ, Jendelova P, Sykova E, et al. Complete rat spinal cord transection as a faithful model of spinal cord injury for translational cell transplantation. Scientific Reports. 2015;**5**:9640. DOI: 10.1038/srep09640

[12] Filipp ME, Travis BJ, Henry SS, Idzikowski EC, Magnuson SA, Loh MY, et al. Differences in neuroplasticity after spinal cord injury in varying animal models and humans. Neural Regeneration Research. 2019;**14**:7-19. DOI: 10.4103/1673-5374.243694

[13] Fu ES, Tummala RP. Neuroprotection in brain and spinal cord trauma. Current Opinion in Anaesthesiology. 2005;**18**:181-187. DOI: 10.1097/01.aco.0000162838.56344.88

[14] Witiw CD, Fehlings MG. Acute Spinal Cord Injury. Journal of Spinal Disorders & Techniques. 2015;**28**:202-210. DOI: 10.1097/BSD.0000000000000287

[15] Uindreaj A, Badner A, Fehlings MG. Promising neuroprotective strategies for traumatic spinal cord injury with a focus on the differential effects among anatomical levels of injury. F1000Res. 2017;**6**:1907. DOI: 10.12688/f1000research.11633.1

[16] Teng YD, Wrathall JR. Local blockade of sodium channels by tetrodotoxin ameliorates tissue loss and long-term functional deficits resulting from experimental spinal cord injury. The Journal of Neuroscience. 1997;**17**:4359-4366

[17] Seki T, Hida K, Tada M, Koyanagi I, Iwasaki Y. Role of the bcl-2 gene after contusivespinal cord injury in mice. Neurosurgery. 2003;**53**:192-198

[18] Ibarra A, Correa D, Willms K, Merchant MT, Guizar-Sahagun G, Grijalva I. Effects of cycolsporin-A on immune response, tissue protection and motor function of rats subjected to spinal cord injury. Brain Research. 2003;**979**:165-178

[19] Ohta S, Iwashita Y, Takada H, Kuno S, Nakamura T. Neuroprotection and enhanced recovery with edaravone after acute spinal cord injury in rats. Spine (Phila Pa 1976). 2005;**30**:1154-1158

[20] Pannu R, Barbosa E, Singh AK, Singh I. Attenuation of acute inflammatory response by atorvastatin after spinal cord injury in rats. Journal of Neuroscience Research. 2005;**79**: 340-350. DOI: 10.1002/jnr.20345

[21] Arataki S, Tomizawa K, Moriwaki A, Nishida K, Matsushita M, Ozaki T, et al. Calpain inhibitors prevent neuronal cell death and ameliorate motor disturbances after compression-induced spinal cord injury in rats. Journal of Neurotrauma. 2005;**22**: 398-406. DOI: 10.1089/neu.2005.22.398

[22] Ahmad M, Zakaria A, Almutairi KM. Effectiveness of minocycline and FK506 alone and in combination on enhanced behavioral and biochemical recovery from spinal cord injury in rats. Pharmacology, Biochemistry, and Behavior. 2016;**145**:45-54. DOI: 10.1016/j.pbb.2016.04.003

[23] Yao M, Yang L, Wang J, Sun YL, Dun RL, Wang YJ, et al. Neurological recovery and antioxidant effects of curcumin for spinal cord injury in the rat: A network meta-analysis and systematic review. Journal of Neurotrauma. 2015;**32**:381-391. DOI: 10.1089/neu.2014.3520

[24] Yang L, Yao M, Lan Y, Mo W, Sun YL, Wang J, et al. Melatonin for spinal cord injury in animal models: A systematic review and network meta-analysis. Journal of Neurotrauma. 2016;**33**:290-300. DOI: 10.1089/neu.2015.4038

[25] Yong VW, Wells J, Giullani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. Lancet Neurology. 2004;**3**:744-751. DOI: 10.1016/S1474-4422(04)00937-8

[26] Kim HS, Suh YH. Minocycline and neurodegenerative diseases. Behavioural Brain Research. 2009;**196**:168-179. DOI: 10.1016/j.bbr.2008.09.040

[27] Garrido-Mesa N, Zarzuelo A, Galvez J. Minocycline: Far beyond an antibiotic. British Journal of Pharmacology. 2013;**169**:337-352. DOI: 10.1111/bph.12139

[28] Wang Z, Nong J, Shultz RB, Zhang Z, Kim T, Tom VJ, et al. Local delivery of minocycline from metal ion-assisted self-assembled complexes promotes neuroprotection and functional recovery after spinal cord injury. Biomaterials. 2017;**112**:62-71. DOI: 10.1016/j.biomaterials.2016.10.002

[29] Plane JM, Shen Y, Pleasure DE, Deng W. Prospects for minocycline neuroprotection. Archives of Neurology.

2010;**67**:1442-1448. DOI: 10.1001/archneurol.2010.191

[30] Ghazali H, Wu W, Abdullah MA. Histological analysis of motoneuron survival and microglia inhibition after nerve root avulsion treated with nerve graft implantation and minocycline: An experimental study. Sains Malaysiana. 2016;**45**:1641-1648

[31] Chin TY, Kiat SS, Faizul HG, Wu W, Abdullah JM. The effects of minocycline on spinal root avulsion injury in rat model. Malaysian Journal of Medical Sciences. 2017;**24**:31-39. DOI: 10.21315/mjms2017.24.1.4

[32] Garcia-Martinez EM, Sanz-Blasco S, Karachitos A, Bandez MJ, Fernandez-Gomez FJ, Prez-Alvarez S, et al. Mitochondria and calcium flux as targets of neuroprotection caused by minocycline in cerebellar granule cells. Biochemical Pharmacology. 2010;**79**:239-250. DOI: 10.1016/j.bcp.2009.07.028

[33] Takeda M, Kawaguchi M, Kumatoriya T, Horiuchi T, Watanabe K, Inoue S, et al. Effects of minocycline on hind-limb motor function and gray and white matter injury after spinal cord ischemia in rats. Spine (Phila Pa 1976). 2011;**36**:1919-1924. DOI: 10.1097/BRS.0b013e3181ffda29

[34] Yune TY, Lee JY, Jung GY, Kim SJ, Jiang MH, Kim YC, et al. Minocycline alleviates death of oligodendrocytes by inhibiting pro-nerve growth factor production in microglia after spinal cord injury. The Journal of Neuroscience. 2007;**27**:7751-7761. DOI: 10.1523/JNEUROSCI.1661-07.2007

[35] Ma L, Nagai J, Ueda H. Microglial activation mediates de novo lysophosphatidic acid production in a model of neuropathic pain. Journal of Neurochemistry. 2010;**115**:643-653. DOI: 10.1111/j.1471-4159.2010.06955.x

[36] Pang T, Wang J, Benicky J, Saavedra JM. Minocycline ameliorates LPS-induced inflammation in human monocytes by novel mechanisms including LOX-1, Nur77 and LITAF inhibition. Biochimica et Biophysica Acta. 1820;**2012**:503-510. DOI: 10.1016/j.bbagen.2012.01.011

[37] Chu LS, Fang SH, Zhou Y, Yin YJ, Chen WY, Li JH, et al. Minocycline inhibits 5-lipoxygenase expression and accelerates functional recovery in chronic phase of focal cerebral ischemia in rats. Life Sciences. 2010;**86**:170-177. DOI: 10.1016/j.lfs.2009.12.001

[38] Shultz RB, Zhong YZ. Minocycline targets multiple secondary injury mechanisms in traumatic spinal cord injury. Neural Regeneration Research. 2017;**12**:702-713. DOI: 10.4103/1673-5374.206633

[39] Sonmez E, Kabatas S, Ozen O, Karabey G, Turkoglu S, Ogus E, et al. Minocycline treatment inhibits lipid peroxidation, preserves spinal cord ultrastructure, and improves functional outcome after traumatic spinal cord injury in the rat. Spine (Phila Pa 1976). 2013;**38**:1253-1259. DOI: 10.1097/BRS.0b013e3182895587

[40] Aras M, Altas M, Motor S, Dokuyucu R, Yilmaz A, Ozgiray E, et al. Protective effects of minocycline on experimental spinal cord injury in rats. Injury. 2015;**46**:1471-1474. DOI: 10.1016/j.injury.2015.05.018

[41] Zhang H, Chang M, Hansen CN, Basso DM, Noble-Haeusslein LJ. Role of matrix metalloproteinases and therapeutic benefits of their inhibition in spinal cord injury. Neurotherapeututics. 2011;**8**:206-220. DOI: 10.1007/s13311-011-0038-0

[42] Modheji M, Olapour S, Khodaya MJ, Jalili A, Yaghooti H. Minocycline is more potent than tetracycline and doxycline in inhibiting MMP-9

in vitro. Jundishapur Journal of Natural Pharmaceutical Products. 2016;**11**:e273377

[43] Lee S, Yune TY, Kim SI, Paark W, Lee YK, Km YC, et al. Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat. Journal of Neurotrauma. 2003;**10**:1017-1027. DOI: 10.1089/089771503770195867

[44] Stirling DP, Khodarahmi K, Liu J, McPhail LT, McBride CB, Steeves JD, et al. Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. The Journal of Neuroscience. 2004;**24**:2182-2190. DOI: 10.1523/JNEUROSCI.5275-03.2004

[45] Wells JE, Hurlbert RJ, Fehlings MG, Yong VW. Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. Brain. 2003;**126**:1628-1637. DOI: 10.1093/brain/awg178

[46] Squair JW, Ruiz I, Phillips AA, Zheng MMZ, Sarafis ZK, Sachdeva R, et al. Minocycline reduces the severity of autonomic dysreflexia after experimental spinal cord injury. Journal of Neurotrauma. 2018;**35**:2861-2871. DOI: 10.1089/neu.2018.5703

[47] Saganova K, Orendacova J, Cizkova D, Vanickv I. Limited minocycline neuroprotection after balloon-compression spinal cord injury in the rat. Neuroscience Letters. 2008;**433**:246-249. DOI: 10.1016/j.neulet.2008.01.041

[48] Cho DC, Cheong JH, Yang MS, Hwang SJ, Kim JM, Kim CH. The effect of minocycline on motor neuron recovery and neuropathic pain in a rat model of spinal cord injury. Journal of Korean Neurosurgical Association. 2011;**49**:83-91. DOI: 10.3340/jkns.2011.49.2.83

[49] Su Z, Yuan Y, Cao L, Zhu Y, Gao L, Qiu Y, et al. Triptolide promotes spinal cord repair by inhibiting astrogliosis and inflammation. Glia. 2010;**58**:901-915. DOI: 10.1002/glia.20972

[50] Drenger B, Blanck TJJ, Jaffery BPE, Recino-Pinto E, Sideris A. Minocycline before aortic occlusion reduces hindlimb motor impairment, attenuates spinal cord damage and spinal astrocytosis, and preserve neural cytoarchitecture in the rat. Journal of Cardiothoracic and Vascular Anesthesia. 2019;**33**:1003-1011. DOI: 10.1053/j. jvca.2018.07.028

[51] Chen D, Zeng W, Fu Y, Gao M, Lv G. Bone marrow mesenchymal stem cells combined with minocycline improve spinal cord injury in a rat model. International Journal of Clinical and Experimental Pathology. 2015;**8**:11957-11969

[52] Ibarra A, Hernandez E, Lomeli J, Pineda D, Buenrostro M, Martinon S, et al. MadrazoI: Cyclosporin-A enhances non-functional axonal growing after complete spinal cord transaction. Brain Research. 2007;**1149**:200-209. DOI: 10.1016/j.brainres.2007.02.056

[53] Chen ZR, Ma Y, Guo HH, Lu ZD, Jin QH. Therapeutic efficacy of cyclosporin a against spinal cord injury in rats with hyperglycemia. Molecular Medicine Reports. 2018;**17**:4369-4375. DOI: 10.3892/mmr.2018.8422

[54] Gao SJ, Liu Y, Wang HJ, Ban DX, Cheng SZ, Ning GZ, et al. New approach to treating spinal cord injury using PEG-TAT-modified, cyclosporine-A-loaded PLGA/polymeric liposomes. Journal of Drug Targeting. 2017;**25**:75-82. DOI: 10.1080/1061186X.2016.1191082

[55] Ibarra A, Diaz-Ruiz A. Protective effect of cyclosporin-A in spinal cord injury: An overview. Current Medicinal Chemistry. 2006;**13**:2703-2710

[56] Diaz-Ruiz A, Vergara P, Perez-Severiano F, Segovia J, Guizar-Sahagun G, Ibarra A, et al. Cyclosporin-A inhibits constitutive nitric oxide synthase activity and neuronal and endothelial nitric oxide synthase expressions after spinal cord injury in rats. Neurochemical Research. 2005;**30**:245-251

[57] Gold BG. Neuroimmunophillin ligands: Evaluation of their therapeutic potential for the treatment of neurological disorders. Expert Opinion on Investigational Drugs. 2000;**9**:2331-2342. DOI: 10.1517/13543784.9.10.2331

[58] Roozbehi A, Joghataie MT, Mehdizadeh M, Mirzaei A, Delaviz H. The effects of cyclosporine-A on functional outcome and axonal regrowth following spinal cord injury in adult rats. Acta Medica Iranica. 2012;**50**:226-232

[59] Ibarra A, Correa D, Willms K, Merchant MT, Guizar-Sahagun G, Grijalva I, et al. Effects of cyclosporin-A on immune response, tissue protection and motor function of rats subjected to spinal cord injury. Brain Research. 2003;**979**:165-178

[60] Yousuf S, Atif F, Kesharwani V, Agrawal SK. Neuroprotective effects of Tacrolimus (FK-506) and Cyclosporin (CsA) in oxidative injury. Brain and Behavior: A Cognitive Neuroscience Perspective. 2011;**1**:87-94. DOI: 10.1002/brb3.16

[61] Khankan RR, Griffis KG, Haggerty-Skeans JR, Zhong H, Roy RR, Edgerton VR, et al. Olfactory ensheathing cell transplantation after a complete spinal cord transection mediates neuroprotective and immunomodulatory mechanisms to facilitate regeneration. The Journal of Neuroscience. 2016;**36**:6269-6286. DOI: 10.1523/JNEUROSCI.0085-16.2016

[62] Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK506 for liver, kidney and pancreas transplantation. Lancet. 1989;**2**:1000-1004

[63] Sosa I, Reyes O, Kuffler DP. Immunosuppressants: Neuroprotection and promoting neurological recovery following peripheral nerve and spinal cord lesions. Experimental Neurology. 2005;**195**:7-15. DOI: 10.1016/j.expneurol.2005.04.016

[64] Bochelen DMR, Sauter A. Calcineurin inhibitors FK506 and SDZASM981 alleviate the outcome of focal cerebral ischemic/reperfusion injury. The Journal of Pharmacology and Experimental Therapeutics. 1999;**288**:653-659

[65] Butcher SP, Henshall DC, Teramura Y, Iwasaki K, Sharkey J. Neuroprotective actions of FK506 in experimental stroke: In vivo evidence against an antiexcitotoxic mechanism. The Journal of Neuroscience. 1997;**17**:6939-6946

[66] Madsen JR, McDonald P, Irwin N, Goldberg DE, Yao GL, Meiri KF, et al. Tacrolimus (FK506) increases neuronal expression of GAP-43 and improves functional recovery after spinal cord injury in rats. Experimental Neurology. 1998;**154**:673-683. DOI: 10.1006/exnr.1998.6974

[67] Saganova K, Galik J, Blasko J, Korimova A, Racekova E, Vanicky I. Immunosuppressant FK506: Focusing on neuroprotective effects following brain and spinal cord injury. Life Sciences. 2012;**91**:77-82. DOI: 10.1016/j.lfs.2012.06.022

[68] Pan F, Chen A, Guo F, Zhu C, Tao F. Effect of FK506 on expression of hepatocyte growth factor in murine spinal cord following peripheral nerve injury. Journal of Huazhong University of Science and Technology. Medical

Sciences. 2008;**28**:159-162. DOI: 10.1007/s11596-008-0211-9

[69] Yamaji T, Yamazaki S, Li J, Price RD, Matsuoka N, Mutoh S. FK1706, a novel non-immunosuppressant neurophilin ligand, ameliorates motor dysfunction following spinal cord injury through its neurodegenerative action. European Journal of Pharmacology. 2008;**591**:147-152. DOI: 10.1016/j.ejphar.2008.06.064

[70] Gordon T, Sulaiman O, Boyd JG. Experimental strategies to promote functional recovery after peripheral nerve injuries. Journal of the Peripheral Nervous System. 2003;**8**:236-250

[71] Navarro X, Udina E, Ceballos D, Gold BG. Effects of FK506 on nerve regeneration and re innervation after graft or tube repair of long nerve gaps. Muscle & Nerve. 2001;**24**:905-915

[72] Sulaiman OA, Voda J, Gold BG, Gordon T. FK506 increases peripheral nerve regeneration after chronic axotomy but not after chronic Schwann cell denervation. Experimental Neurology. 2002;**175**:127-137. DOI: 10.1006/exnr.2002.7878

[73] Udina E, Rodrigues FJ, Verdu E, Espejo M, Gold BG, Navarro X. FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogenic Schwann cells. Glia. 2004;**47**:120-129. DOI: 10.1002/glia.20025

[74] Cai J, Sun Y, Yin Z, Wang D, Shi K, Fu Y, et al. Analysis of FK506-mediated functional recovery and neuroprotection in a rat model of spinal cord injury indicates that EGF is modulated in astrocytes. Experimental and Therapeutic Medicine. 2018;**16**: 501-510. DOI: 10.3892/etm.2018.6283

[75] Devaux S, Cizkoya D, Mallah K, Karnoub MA, Laouby Z, Kobeissv F, et al. RhoA inhibitor treatment at acute phase of spinal cord injury may induce neurite outgrowth and synaptogenesis. Molecular & Cellular Proteomics. 2017;**16**:1394-1415. DOI: 10.1074/mcp.M116.064881

[76] Chen G, Zhang Z, Wang S, Lv D. Combined treatment with FK506 and nerve growth factor for spinal cord injury in rats. Experimental and Therapeutic Medicine. 2013;**6**:868-872. DOI: 10.3892/etm.2013.1254

[77] Kaymaz M, Emmez H, Bukan N, Dursun A, Kurt G, Pasaoglu A. Effectiveness of FK506 on lipid peroxidation in the spinal cord following experimental traumatic injury. Spinal Cord. 2005;**43**:22-26. DOI: 10.1038/sj.sc.3101621

[78] Sharif-Alhoseini M, Rahimi-Movaghar V. Animal models in traumatic spinal cord injury. In: Dionyssiotis Y, editor. Topics in Paraplegia. UK: Intechopen Limited; 2014. pp. 209-228. DOI: 10.5772/57189

[79] Zhang N, Fang M, Chen H, Gou F, Ding M. Evaluation of spinal cord injury animal models. Neural Regeneration Research. 2014;**9**:2008-2012. DOI: 10.4103/1673-5374.143436

[80] Lee DH, Lee JK. Animal models of axon regeneration after spinal cord injury. Neuroscience Bulletin. 2013;**29**:436-444. DOI: 10.1007/s12264-013-1365-4

[81] Koozekanani SH, Vise WM, Hashemi RM, McGhee RB. Possible mechanisms for observed pathophysiological variability in experimental spinal cord injury by the method of Allen. Journal of Neurosurgery. 1976;**44**:429-434

[82] Piao MS, Lee JK, Jang JW, Kim SH, Kim HS. A mouse model of photochemically induced spinal cord injury. Journal of Korean Neurosurgical Association. 2009;**46**:479-483. DOI: 10.3340/jkns.2009.46.5.479

[83] Min SH, Lee SH, Shim H, Park JS, Lee YI, Kim HW, et al. Development of complete thoracic spinal cord transection model in rats for delayed transplantation of stem cells. Spine (Phila Pa 1976). 2011;**36**:155-163. DOI: 10.1097/BRS.0b013e3181d8b92a

[84] Seifert JL, Bell JE, Elmer BB, Sucato DJ, Romero MI. Characterization of a novel bidirectional distraction spinal cord injury animal model. Journal of Neuroscience Methods. 2011;**197**:97-103. DOI: 10.1016/j.jneumeth.2011.02.003

[85] Lacifi G, Gedik HS, Korkmaz K, Erdem H, Cicek OF, Nacar OA, et al. Efficacy of iloprost and montelukast combination on spinal cord ischemia/reperfusion injury in a rat model. Journal of Cardiothoracic Surgery. 2013;**4**:8-64. DOI: 10.1186/1749-8090-8-64

[86] Yeo SJ, Hwang SN, Park SW, Kim YB, Min BK, Kwon JT, et al. Development of a rat model of graded contusive spinal cord injury using a pneumatic impact device. Journal of Korean Medical Science. 2004;**19**:574-580. DOI: 10.3346/jkms.2004.19.4.574

[87] Vijayaprakash KM, Sridharan N. An experimental spinal cord injury rat model using customized impact device: A cost-effective approach. Journal of Pharmacology and Pharmacotherapeutics. 2013;**4**:211-213. DOI: 10.4103/0976-500X.114607

[88] Blight AR. Morphometric analysis of a model of spinal cord injury in Guinea pigs, with behavioral evidence of delayed secondary pathology. Journal of the Neurological Sciences. 1991;**103**:156-171

[89] Nystrom B, Berglund JE. Spinal cord restitution following compression injuries in rats. Acta Neurologica Scandinavica. 1998;**78**:467-472

[90] Van Meeteren NL, Eggers R, Lankhorst AJ, Gispen WH, Hamers FP. Locomotor recovery after spinal cord contusion injury in rats is improved by spontaneous exercise. Journal of Neurotrauma. 2003;**20**:1029-1037. DOI: 10.4103/1673-5374.143436

[91] Zeman RJ, Wen X, Ouyang N, Rocchio R, Shih L, Alfieri A, et al. Stereotactic radiosurgery improves locomotor recovery after spinal cord injury in rats. Neurosurgery. 2008;**63**:981-987. DOI: 10.1227/01.NEU.0000330404.37092.3E

[92] Lee JH, Tigchelaar S, Liu J, Stammers AM, Streijger F, Tetzlaff W, et al. Lack of neuroprotective effects of simvastatin and minocycline in a model of cervical spinal cord injury. Experimental Neurology. 2010;**225**:219-230. DOI: 10.1016/j.expneurol.2010.06.018

[93] McMahon SS, Albermann S, Rooney GE, Moran C, Hynes J, Garcia Y, et al. Effect of cyclosporin A on functional recovery in the spinal cord following contusion injury. Journal of Anatomy. 2009;**215**:267-279. DOI: 10.1111/j.1469-7580.2009.01107.x

[94] Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. Journal of Neurotrauma. 1995;**12**:1-21. DOI: 10.1089/neu.1995.12

[95] Tarlov IM, Klinger H. Spinal cord compression studies. AMA Archives of Neurology and Psychiatry. 1954;**71**:275-290

[96] Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. Journal of Neurosurgery. 1977;**47**:577-581

[97] Gale K, Kerasidis H, Wrathall JR. Spinal cord contusion in the rat: Behavioral analysis of functional neurologic impairment. Experimental Neurology. 1985;**88**:123-134

[98] Kerasidis H, Wrathall JR, Gale K. Behavioral assessment of functional deficit in rats with contusive spinal cord injury. Journal of Neuroscience Methods. 1987;**20**:167-179

[99] Patrick OE, Hirohisa M, Masahira K, Koreaki M. Central nervous system bioaminergic responses to mechanic trauma. Surgical Neurology. 1991;**35**:273-279

[100] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979;**95**:351-358

[101] Jo C, Ahn DU. Fluorometric analysis of 2-thiobarbituric acid reactive substances. Poultry Science. 1998;**77**:475-480. DOI: 10.1093/ps/77.3.475

[102] Mangino MJ, Murphy MK, Glabar GG, Anderson CB. Protective effects of glycine perfusion injury. The American Journal of Physiology. 1991;**261**:F841-F848. DOI: 10.1152/ajprenal.1991.261.5.F841

[103] Tariq M, Morais C, Kishore PN, Biary N, Al Deeb S, Al Moutaery K. Neurological recovery in diabetic rats following spinal cord injury. Journal of Neurotrauma. 1998;**15**:239-251. DOI: 10.1089/neu.1998.15.239

[104] Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. The Journal of Investigative Dermatology. 1982;**78**:206-209

[105] Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part I: Pathophysiologic mechanisms. Clinical Neuropharmacology. 2001;**24**:254-264

[106] Kaptanoglu E, Tuncel M, Palaoglu S, Konan A, Demirpence E, Kilinc K. Comparison of the effects of melatonin and methylprednisolone in experimental spinal cord injury. Journal of Neurosurgery. 2000;**93**:77-84

[107] Antri M, Orsal D, Barthe JY. Locomotor recovery in the chronic spinal rat: Effects of long-term treatment with a 5-HT2 agonist. The European Journal of Neuroscience. 2002;**6**:467-476

[108] Zhang J, Zhang A, Sun Y, Cao X, Zhang N. Treatment with immunosuppressants FTY720 and tacrolimus promotes functional recovery after spinal cord injury in rats. The Tohoku Journal of Experimental Medicine. 2009;**219**:295-302

[109] Vafadari R, Kraaijeveld R, Weimar W, Baan CC. Tacrolimus inhibits NF-κB activation in peripheral human T cells. PLoS One. 2013;**8**:e60784. DOI: 10.1371/journal.pone.0060784

[110] Romero SE, Bravo G, Hong E, Rojas G, Ibarra A. Acute, subacute and chronic effect of cyclosporin-A on mean arterial pressure of rats with severe spinal cord contusion. Neuroscience Letters. 2008;**445**:99-102. DOI: 10.1016/j.neulet.2008.08.063

[111] Zhou YQ, Liu DQ, Chen SP, Sun J, Wang XM, Tian YK, et al. Minocycline as a promising therapeutic strategy for chronic pain. Pharmacological Research. 2018;**134**:305-310. DOI: 10.1016/j.phrs.2018.07.002

[112] Noga BR, Pinzon A, Mesigil RP, Hentall ID. Steady-state levels of monoamines in the rat lumbar spinal cord: Spatial mapping and the effect of acute spinal cord injury. Journal of Neurophysiology. 2004;**92**:567-577. DOI: 10.1152/jn.01035.2003

[113] Bravo G, Ibarra A, Guizar-Sahagun G, Rojas G, Hong E. Indorenate improves motor function in rats with chronic spinal cord injury.

Basic & Clinical Pharmacology & Toxicology. 2007;**100**:67-70. DOI: 10.1111/j.172-7843.2007.00004.x

[114] Taoka Y, Okajima K, Uchiba M, Murakami K, Kushimoto S, Johno M, et al. Role of neutrophils in spinal cord injury in rats. Neuroscience. 1997;**79**:1177-1182

[115] Genovese T, Mazzon E, Di Paola R, Cannavo G, Muia C, Bramanti P, et al. Role of endogenous ligands for the peroxisome proliferators activated receptors alpha in the secondary damage in experimental spinal cord trauma. Experimental Neurology. 2005;**194**:267-278. DOI: 10.1016/j.expneurol.2005.03.003

[116] Voda J, Hama A, Sagen J. FK506 reduces the severity of cutaneous hypersensitivity in rats with a spinal cord contusion. Neuroscience Research. 2007;**58**:95-99. DOI: 10.1016/j.neures.2007.02.004

[117] Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vaccaro AR. Pathophysiology and pharmacologic treatment of acute spinal cord injury. The Spine Journal. 2004;**4**:451-464. DOI: 10.1016/j.spinee.2003.07.007

[118] Lee VM, Hartley RS, Trojanowski JQ. Neurobiology of human neurons (NT2N) grafted into mouse spinal cord: Implications for improving therapy of spinal cord injury. Progress in Brain Research. 2000;**128**:299-307. DOI: 10.1016/S0079-6123(00)28027-8

[119] Anthes DL, Theriault E, Tator CH. Ultrastructural evidence for arteriolar vasospasm after spinal cord trauma. Neurosurgery. 1996;**39**:804-814

[120] Blight AR. Cellular morphology of chronic spinal cord injury in the cat: Analysis of myelinated axons by line-sampling. Neuroscience. 1983;**10**:521-543

[121] Beattie MS, Li Q, Bresnahan JC. Cell death and plasticity after experimental spinal cord injury. Progress in Brain Research. 2000;**128**:9-21. DOI: 10.1016/S0079-6123(00)28003-5

[122] Kakulas BA. A review the neuropathology of human spinal cord injury with emphasis on special features. The Journal of Spinal Cord Medicine. 1999;**22**:119-124

[123] Iwasa K, Ikata T, Fukuzawa K. Protective effect of vitamin E on spinal cord injury by compression and concurrent lipid peroxidation. Free Radical Biology & Medicine. 1989;**6**:599-606

[124] Agrawal SK, Fehlings MG. The effect of the sodium channel blocker QX-314 on recovery after acute spinal cord injury. Journal of Neurotrauma. 1997;**14**:81-88. DOI: 10.1089/neu.1997.14.81

[125] Springer JE, Azbill RD, Knapp PE. Activation of the caspase-3 apoptic cascade in traumatic spinal cord injury. Nature Medicine. 1999;**5**:943-946. DOI: 10.1038/11387

[126] Citron BA, Smirnova IV, Arnold PM, Festoff BW. Upregulation of neurotoxic serine proteases, prothrombin, and protease-activated receptor 1 early after spinal cord injury. Journal of Neurotrauma. 2000;**17**:1191-1203. DOI: 101089/neu.2000.17.1191

[127] Stys PK, Waxman SG, Ransom BR. Ionic mechanisms of anoxic injury in mammalian CNS white matter: Role of Na+ channels and Na(+)-Ca2+ exchanger. The Journal of Neuroscience. 1992;**12**:430-439

[128] Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS. Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nature Medicine. 1997;**73**:73-76

[129] Liu XZ, Xu XM, Hu R, Du C, Zhang SX, McDonald JW, et al. Neuronal and glial apoptosis after traumatic spinal cord injury. The Journal of Neuroscience. 1997;**17**:5395-5406

[130] Hall ED, Wolf DL. A pharmacological analysis of the pathophysiological mechanisms of posttraumatic spinal cord ischemia. Journal of Neurosurgery. 1986;**64**:951-961. DOI: 10.3171/jns.1986.64.6.0951

[131] Young W, Koreh I. Potassium and calcium changes in injured spinal cord. Brain Research. 1986;**365**:42-53

[132] Plant GW, Christensen CL, Oudega M, Bunge MB. Delayed transplantation of olfactory ensheathing glia promotes sparing/regeneration of supraspinal axons in the contused adult rat spinal cord. Journal of Neurotrauma. 2003;**20**:1-16. DOI: 10.1089/08977150360517146

[133] Verdu E, Garcia-Alias G, Fores J, Lopez-Vales R, Navarro X. Olfactory ensheathing cells transplanted in lesioned spinal cord prevent loss of spinal cord parenchyma and promote functional recovery. Glia. 2003;**42**: 275-286. DOI: 10.1002/glia.10217

[134] Ahmad M, Abo Shaiqah A, Alshehri AS, Alotaibi AM. Accentuated rehabilitation recovery from spinal cord injury in rats through increased behavioral activity besides minocycline treatment: A nursing care perspective. JOJ Nurse Health Care. 2018;**5**:555674. DOI: 10.19080/JOJNHC.2018.05.555674