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Research Paper: Minocycline Enhance the Restorative Ability of Olfactory Ensheathing Cells by the Upregulation of BDNF and GDNF Expression After Spinal Cord Injury

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ABSTRACT

Introduction: Spinal Cord Injury (SCI) is a global public health issue that results in extensive neuronal degeneration, axonal and myelin loss, and severe functional deficits. Neurotrophic factors are a potential treatment for reducing secondary damage, promoting axon growth; they are responsible for inducing myelination after injury. Olfactory Ensheathing Cells (OECs) and minocycline have promoted locomotor function after SCI. The present study investigated the neuroprotective effects of combined treatment with minocycline and OECs on spinal cord injury related to Brain-Derived Neurotrophic Factor (BDNF) and Glial Derived Neurotrophic Factor (GDNF) expressions after SCI.

Methods: Adult female rats were used to experimental SCI by weight compression method. Rats received an intraperitoneal minocycline injection (90 mg/kg) immediately after SCI and 24 h after injury. OECs were transplanted one week after the injury. The hindlimb function was assessed using Basso Beattie Bresnahan (BBB) locomotor rating scale and Electromyography (EMG). After 5 weeks, the spinal cord segment centered at the injury site was removed for histopathological analysis. Immunohistological and western blot assays were performed to observe the expression of NeuN, BDNF, GDNF, and Myelin Basic Protein (MBP).

Results: SCI induced the loss of locomotor function with decreased BDNF and GDNF expressions in the injury site. Minocycline+OECs increased the score of the BBB locomotor scale and increased spared tissue in the injury site. Immunohistochemical results suggested that NeuN expression significantly increased in the minocycline+OECs group than other groups. Moreover, electromyography amplitude in treated rats was increased compared to the control group. BDNF, GDNF, and MBP expressions and the number of ventral motor neurons increased further by minocycline+OECs in SCI rats.

Conclusion: The present study provides evidence that minocycline may facilitate recovery of locomotor function by OECs by increasing BDNF and GDNF expressions following SCI.

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Highlights

- Combined treatment with Minocycline and OECs increased the locomotor function.
- The results showed that BDNF and GDNF expression increased by combined treatment with minocycline and OECs.

Plain Language Summary

This study examined the effect of combined treatment with minocycline and olfactory ensheathing cell on the BDNF and GDNF expression after spinal cord injury model in rat. The results showed that injection of minocycline before transplantation of OECs enhances expression of neurotrophic factors that lead to an appropriate environment for transplanted OECs and increases neuronal survival that promotes tissue sparing and functional recovery.

1. Introduction

Injury to the spinal cord results in extensive neuronal death, axonal degeneration, and often severe functional deficits (Devivo, 2012). Little spontaneous axonal regeneration occurs following spinal cord injury (van Niekerk, Tuszynski, Lu, & Dulin, 2016). Several factors contribute to this lack of recovery, including glial scarring, myelin inhibition, the death of neurons and myelinating oligodendrocytes, the lack of permissive environment, and insufficient growth factor for axonal regeneration (Beattie, Li, & Bresnahan, 2000; Huber & Schwab, 2000). The restoration of axons depends on providing a permissive environment and enhancing neurotrophins at the injury site (Griffin & Bradke, 2020).

Recent attempts to decrease cell death and increase axonal regeneration by enhancing the level of neurotrophic factors and blocking inhibitory molecules and inflammation have elicited axonal regrowth after SCI (Lykissas, Batistatou, Charalabopoulos, & Beris, 2007). For instance, the beneficial effects of neurotrophic factors have been well documented on neuronal survival, axonal growth, and repair in the Central Nervous System (CNS) (Alsina, Ledda, & Paratcha, 2012). Of these, Brain-Derived Neurotrophic Factor (BDNF) and Glial Derived Neurotrophic Factor (GDNF) have been studied extensively to find whether they have a role in promoting motor axon outgrowth of spinal cord injury (Enomoto, 2016; Ortmann & Hellenbrand, 2018; Zhang et al., 2009). Exogenous administration of these Neurotrophic/growth factors have neuroprotective properties and limit secondary damages (Jones, Oudega, Bunge, & Tuszynski, 2001; Rosich, Hanna, Ibrahim, Hellenbrand, & Hanna, 2017). Previous studies reported that BDNF promotes developmental myelination in vivo. Furthermore, it promotes CNS myelination through TrkB signaling that could be a therapeutic target for demyelinating disorders, such as stroke, Multiple Sclerosis (MS), and traumatic injury (McTigue, Horner, Stokes, & Gage, 1998; Peckham et al., 2016; Ramos-Cejudo et al., 2015). BDNF and GDNF induce the proliferation and differentiation of oligodendrocyte progenitor cells to mature oligodandricytes. Moreover, they enhance axonal regrowth and expression of Myelin Basic Protein (MPB) in Spinal Cord Injury (SCI) (Fletcher, Jessica L, Murray, Simon & Xiao, Junhua, 2018; Iannotti, Li, Yan, Lu, Wirthlin, & Xu, 2003).

Minocycline, a tetracycline derivative, protects neurons against inflammation, oxidative stress, and apoptosis after spinal cord injury (Yong, Wells, Giuliani, Casha, Power, & Metz, 2004). Besides, minocycline reduced oligodendrocyte death and improved functional recovery following SCI (Wells, Hurlbert, Fehlings, & Yong, 2003). Recent studies indicated that minocycline is an interesting tool that reduces gliosis and promotes the production of neurotrophic factors from astrocytes, and markedly enhances the survival of injured motoneurons even when the treatment is delayed (Heydarizadi, Abbasi, Asadollahi, Rezaee, Moradipour, & Azizi, 2019; Stirling et al., 2004). Previous studies demonstrated that minocycline could upregulate the expression of brain-derived neurotrophic factors and nerve growth factors in the cerebral cortex and lumbar spinal cord; it also improve cognitive impairment via enhancing BDNF activity in the hippocampus (Zhao, Xiao, He, & Cai, 2015).

Another mechanism to alter this environment for axonal repair can take place is using cell transplantation, such as neural stem cell, Schwann cell, and Olfactory Ensheathing Cells (OECs) (Assinck, Duncan, Hilton, Plemel, & Tetzlaff, 2017; Heydarizadi et al., 2019).

Indeed, OECs transplanted into the injured spinal cord express cell surfaces molecules, such as N-CAM, Ncadherin, and laminin, also produce many growth factors, including several forms of neuregulins and some neurotrophins have resulted in a remarkable degree of axonal regeneration and functional recovery (Li, Field, & Raisman, 1998; Plant, Christensen, Oudega, & Bunge, 2003; Ramón-Cueto, Cordero, Santos-Benito, & Avila, 2000).

SCI is a complex complication A multifactorial molecular cascade of signals limits endogenous regenerative capacity. Accordingly, individual interventions induce small regeneration and functional recovery after SCI; a combination of neuroregenerative and neuroprotective strategies needs to overcome multiple factors to increase axonal growth (Pearse, Marcillo, Oudega, Lynch, Wood, & Bunge, 2004). In this regard, several studies reported the importance of a combination of cell graft and pharmacological strategies after SCI (Ahuja & Fehlings, 2016). Therefore, for further investigation on this issue, the present study investigated the effect of combined treatment with minocycline and OECs on the BDNF, GDNF, MBP, and neuronal survival after SCI.

2. Methods

Induction of spinal cord injury models and treatment schedule

In total, 50 female Wistar rats (220-250 g) were used in biochemical experiments. The experiment protocol in the present study was approved by the animal care and ethics in Baqiyatallah University of Medical Sciences, Tehran, Iran. All animals were allocated into the following 5 groups with 10 animals in each: (1) sham group, only laminectomy; (2) control group: laminectomy+injury+saline, (3) minocycline group: lam inectomy+injury+Intraperitoneal (IP) injection of minocycline (90 mg/kg) immediately and following 24 h after SCI, (4) OECs group: laminectomy+injury+OECs transplantation(450000 cells/6 µl) 7 days after injury, and (5) minocycline+OECs group: laminectomy+injury+the injection of minocycline immediately and 24 h after injury (90 mg/kg) and transplantation of OECs 7 days after injury. The laminectomy was performed at the T10-T12 vertebra, and the weight (10 g) was dropped at the height of 2.5 cm to induce contusive SCI (Mukhamedshina et al., 2016). The rats were allowed free access to food and water. The bladders were emptied by manual two times per day. After 5 weeks of the appropriate treatment schedule, the rats were sacrificed with chloral hydrate, and spinal cord tissues (2 cm) were removed for assay or stored at -80°C.

The preparation of OECs

OECs were obtained from the olfactory bulb, similar to the Nash method (Nash, Borke, & Anders, 2001). Briefly, the olfactory bulbs were dissected from meninges and blood vessels, then dissociated with mechanical trituration and incubated in 0.1% trypsin (Invitrogen) for 45 min at 37°C. The cell suspension was centrifuged at 1000 rpm for 5 minutes. After removal of trypsin, the cells resuspended in a mixture of DMEM/F12/10% FBS and 1% penicillin /streptomycin (P/S; Invitrogen; D/F-FBS); they were seeded to uncoated dishes twice, each for 24 h in an incubator at 37˚C and 5% CO_2. The cells were aspirated and transferred onto 25 cm2 poly L-lysine (Sigma-Aldrich)-coated plastic flasks following 18 h of culture. The medium was changed every 2 days. Dissociated OECs were maintained in vitro for 14 days and then immunopurified using p75nerve growth factor receptor (anti-p75-NGFR, sigma). The cells were incubated with rabbit anti p75NGFR antibody (1:100; Sigma, USA) for purification. OECs purity by immunolabeling against p75 was at least 75%.

Transplantation of OECs

The OECs were transplanted one week after injury. The laminectomy injury site was reexposed, and 6 µl cell suspension (450,000 cells/6µL for OECs) were injected by using a Hamilton syringe at a depth of 0.8 mm at the epicenter of the lesion and 1 mm rostral and caudal to the epicenter (2 µl per injection); it reminds in place for 5 minutes after each injection (Plant et al., 2003). SCI control animals were injected with an identical volume of DMEM at the same sites. After injection, the muscle and skin were closed.

Functional analysis

The locomotor function of rats after the SCI was assessed by open field scores such as the BassoBeattie-Bresnahan (BBB) score (Basso, Beattie, & Bresnahan, 1995). Two blind investigators measured scores before the injury and at 1, 7, 14, 21, 28, and 35 days after SCI and averaged them. This scale measures hindlimb movements with a score of 0 indicating no spontaneous movement, with increasing scores for using individual joints, coordinated joint movement, coordinated limb movement, weight-bearing, and so on to a maximum score of 21.

Electromyography (EMG) evaluation

One day before the sacrifice of the animal, rats were anesthetized, and the sciatic nerve was exposed. Electric

stimulation was applied to the proximal site of the injured nerve. The compound muscle action potential was recorded in the gastrocnemius muscle with a needle electrode and a reference cap electrode inserted at the knee joint. The stainless steel needle used as the ground electrode was inserted into the tail skin (Chen et al., 2007).

Histopathological evaluation

The effect of combined treatment with minocycline and OECs on morphology and number of surviving neurons was determined by staining of spinal cord sections (T 10/12, 3 mm rostral to the epicenter) with cresyl violet and H&E. Cross-sections at 10 µm thickness were dehydrated in 100% alcohol, 95% alcohol, 70% alcohol, and distilled water. Then slices were stained in 0.1% cresyl violet and hematoxylin and eosin (Sigma-Aldrich, Ph 3.0) for 10 minutes at 40°C, then washed very quickly in distilled water. Subsequently, the sections were differentiated in alcohol and xylene series and mounted. The Nissl-stained cells and motor neurons in the spinal cord tissue were observed under a light microscope. Quantification was performed by counting the number of survival neurons in sections was randomly selected from each group.

Immunofluorescence

Neuronal viability was evaluated using monoclonal antibody NeuN (He et al., 2017). Sections from the lesion epicenter were collected at 35 days after SCI. The transverse sections (7 µm thick) were deparaffinized and rehydrated to the standard protocol. Then sections were blocked for 60 min in 5% Bovine Serum Albumin (BSA) (PBS containing 5% BSA and 0.2% Triton X-100) and incubated with the appropriate primary antibodies, mouse monoclonal anti-NeuN (a neuronal marker) (1:1000, Chemicon, USA) at 4 °C overnight. After washing with PBS, the secondary antibody (FITC -conjugated IgG) was added, and the sections were incubated at room temperature for 1 h. The sections were mounted, and fluorescent labeling was visualized and captured using a fluorescence microscope.

Western blot analysis

Five weeks after injury (n=3/time point), a 5-mm spinal cord segment was dissected from each group's lesion epicenter and resuspended using RIPA lysis buffer. After centrifuging for 10 minutes (12,000 rpm/minutes, at 4˚C), for analysis by Western blot, equal amounts of each suspension sample (20 µg protein) waere separated in 12% SDS-PAGE were transferred to PVDF membranes. Then, the blots were blocked for 1 h at room temperature with 5% nonfat dry milk in Tris-Buffered Saline (TBS). After washing with TBST, the membranes were incubated with primary antibodies rabbit anti-rat antibodies against BDNF(1:200, Sigma), GDNF (1:200, Sigma), and MBP (1:200 Sigma) overnight at 4 °C. The membranes were then processed with HRP-conjugated goat anti-rabbit secondary antibody (1:500; Sigma) for 1 h at room temperature. Immunoreactive bands were quantified using ImageJ analysis software.

All data are expressed as Mean±SEM and analyzed using the Graph Prism Program, Version 5. Statistical differences were determined using a one-way Analysis of Variance (ANOVA). In Fisher's Least Significant Difference (LSD) post hoc analysis, a $P<0.05$, was considered to indicate a statistically significant difference.

3. Results

Evaluations of functional recovery

The sham, SCI, and treated animals were examined for locomotor activity on day 35 post-injury. It was observed that animals in the SCI and treated groups encountered hind limb paralysis and were unable to walk normally in the initial days after the saline or minocycline and OECs transplantation. In contrast, animals in the sham group walked normally. At 5 weeks after injury, the mean score of rats with minocycline and OECs graft were significantly higher than those for the SCI group ($P<0.01$, $P<0.05$) (Figure 1). In contrast, the BBB score was improved further in combined treatment with the minocycline and OECs group ($P<0.001$). There was a significant difference between OECs and minocycline +OECs groups ($P<0.05$).

Valuation of EMG

The results of the EMG tests comprised both amplitude and latency measures. The collected results indicated a statistically significant difference between control and experimental groups (OECs, minocycline, & combined treatment with minocycline & OECs). Moreover, results from the combined treatment group were significantly more favorable compared to the other groups ($P<0.05$) (Figure 2). There was not a significant difference between the study groups.

Histopathological results

The obtaiend data indicated that the mean cavity size was significantly less in minocycline and OECs treated groups than in the SCI group ($P<0.01$). Although the

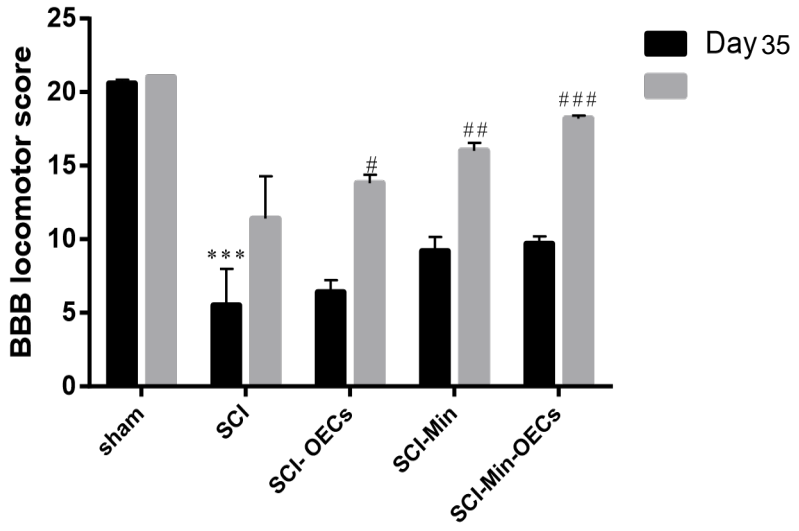

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Figure 1. Locomotor function in spinal cord injury after treatment with minocycline and OECs

Rats with combined treatment with minocycline and OECs transplantation showed significantly improved locomotor behavior up to 5 weeks after SCI (n=6). Data represent Mean values SEM. *** P<0.001 compared to sham group; # P<0.05; ## P<0.01; ### P<0.001 compared to SCI group.

percentage of cavitation in the OECs transplantation group showed a slight decrease compared to the minocycline group, this difference was not statistically significant (P>0.05). Moreover, the mean cavity area in the minocycline+OECs group was significantly reduced, comoared with SCI, OECs, and minocycline groups (P<0.001) (Figure 3). There was a significant difference between OECs and minocycline+OECs groups (P<0.05). Nissl and H&E staining of sections showed that the number of surviving neurons and quantities of Nissl bodies in the rat spinal cord was significantly reduced in the SCI group, compared with the sham group (P<0.001). The number of surviving neurons in the minocycline and minocycline+OECs groups was significantly higher than in the SCI group (P<0.01, P<0.001). Also, the OECs group revealed a significant increase in the number of neurons compared with the SCI group (P<0.05) (Figure 4). There was a significant difference between OECs and minocycline+OECs groups (P<0.05).

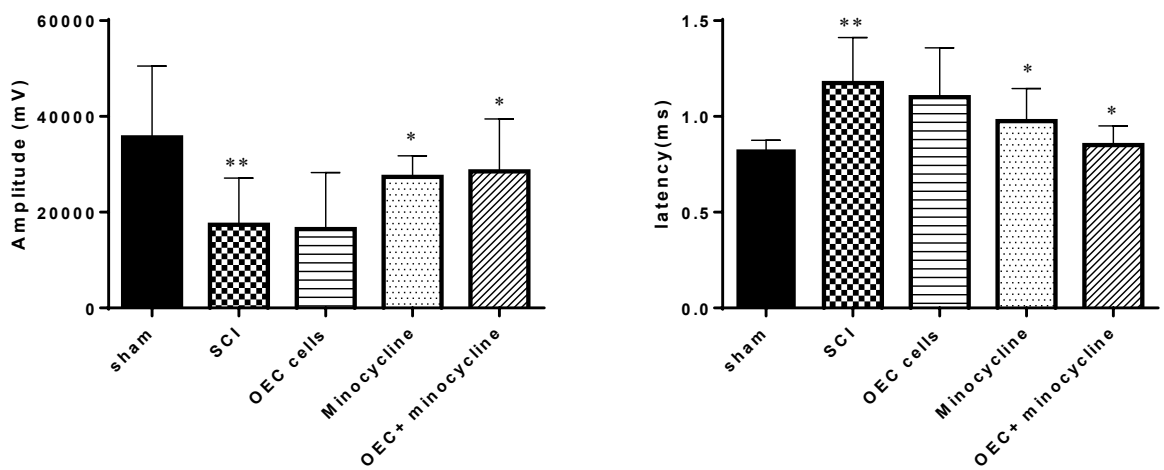

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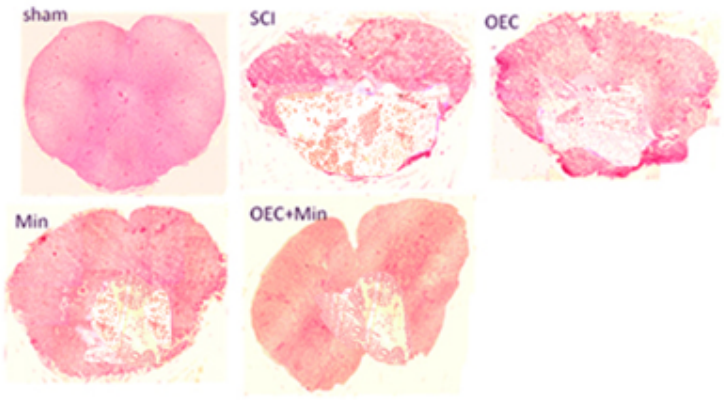
Figure 2. Results of electromyography tests of amplitude and latency

They showed there were saept statistically significant differences between the control and experimental groups and combined treatment resulted in more favorable results than the other groups (n=6). Data represent Mean±SEM. **P<0.01 vs. the sham group; *P<0.05 vs. the SCI group.



A

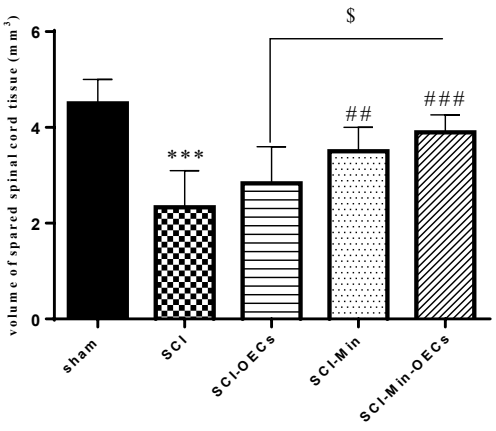


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Figure 3. Sparing of spinal cord tissue at 5 weeks after injury at the epicenter

A: Representative images of H$E stained sections at the epicenter in the different group (×10) (n=3); B: The volumes of spared spinal cord tissue compared between the four groups. Data represent Mean±SEM. *** P<0.001, in SCI versus sham; ##P<0.01 in minocycline versus SCI group; and ###P<0.001 in Min+OECs versus SCI group; $P<0.05 in OECs versus Min+OECs group.

Expression of NeuN

Minocycline could inhibit the inflammation and promote the mature neuron survival (P<0.01); when combined with OECs, the expressions of mature neuron marker NeuN were higher than other groups (P<0.001). Moreover, OECs alone suggested a protective effect on mature neuron survival (P<0.05); the lowest expression of NeuN was found in the OECs group (Figure 4). There was a significant difference between OECs and minocycline+OECs groups (P<0.05).

Expression of BDNF, GDNF, and MBP

BDNF and GDNF expression levels were significantly higher in the minocycline (P<0.01, P<0.05) and combined treatment with minocycline and OECs groups (P<0.001, P<0.01), compared with the SCI group. The expression level of BDNF was significant (P<0.05); however, GDNF was not significantly higher in the OECs group compared to the SCI control group. The expression levels were highest in the combined treatment with the minocycline and OECs group (Figure 5). There was a significant difference in BDNF level between OECs and minocycline+OECs groups (P<0.05).

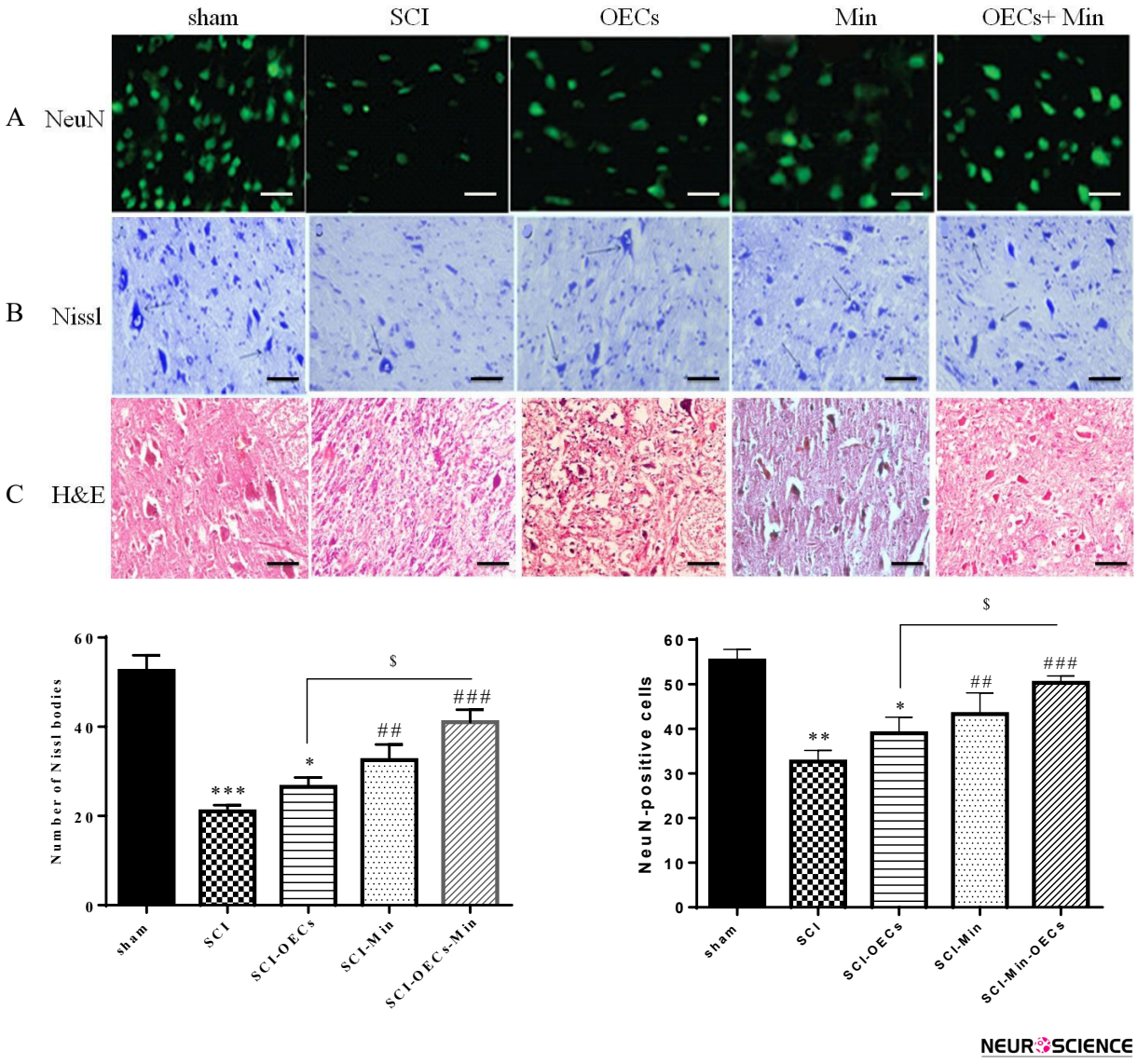

Figure 4. Combined treatment with minocycline and OECs increased motor neuronal survival

At 35 d after SCI, sections of the injured spinal cord were assessed via immunofluorescence staining, Nissl and H&E staining for NeuN (n=3) (Scale bars: 50 µm). A: Immunofluorescence staining for NeuN in the different groups; B: Nissl staining results in the ventral horn of the spinal cord in the different groups at 35 d after SCI; C: H&E staining results for the sections of the injured spinal cord in the experimental groups. D) The quantification analysis of the number of Nissl-stained cells; E: The quantification analysis of the number of NeuN-positive cells. Data represent Mean±SEM; ***P<0.001; **P<0.01 in SCI versus sham; *P<0.05 in OECs versus SCI group; $^{\#\#}$P<0.01 in Minocycline versus SCI group; and $^{\#\#\#}$P<0.001 in Min+OECs versus SCI group; $^{\$}$P<0.05 in OECs versus Min+OECs group.

The MBP expression was compared among experimental groups to determine the density of Myelin Basic Protein (MBP) as an axonal and myelin integrity marker. There was a strong level of white matter throughout all sections of the sham group. Statistical evaluations revealed that the SCI group significantly decreased MBP expression. However, it was significantly enhanced in the minocycline and minocycline+ OECs groups, whereas the OECs group had intermediate values. Data obtained revealed that the density of MBP in the ventral horn of the spinal cord was significantly reduced in the SCI group, compared to the sham group (P<0.001). Moreover, the density of MBP was significantly increased in minocycline (P<0.05) and minocycline+OECs (P<0.01) groups, compared with the SCI group (Figure 5). There was not a significant difference between the study groups.

4. Discussion

This study suggested that combined treatment with minocycline and OECs have more neuroprotective effects on the SCI. The beneficial effects of minocycline

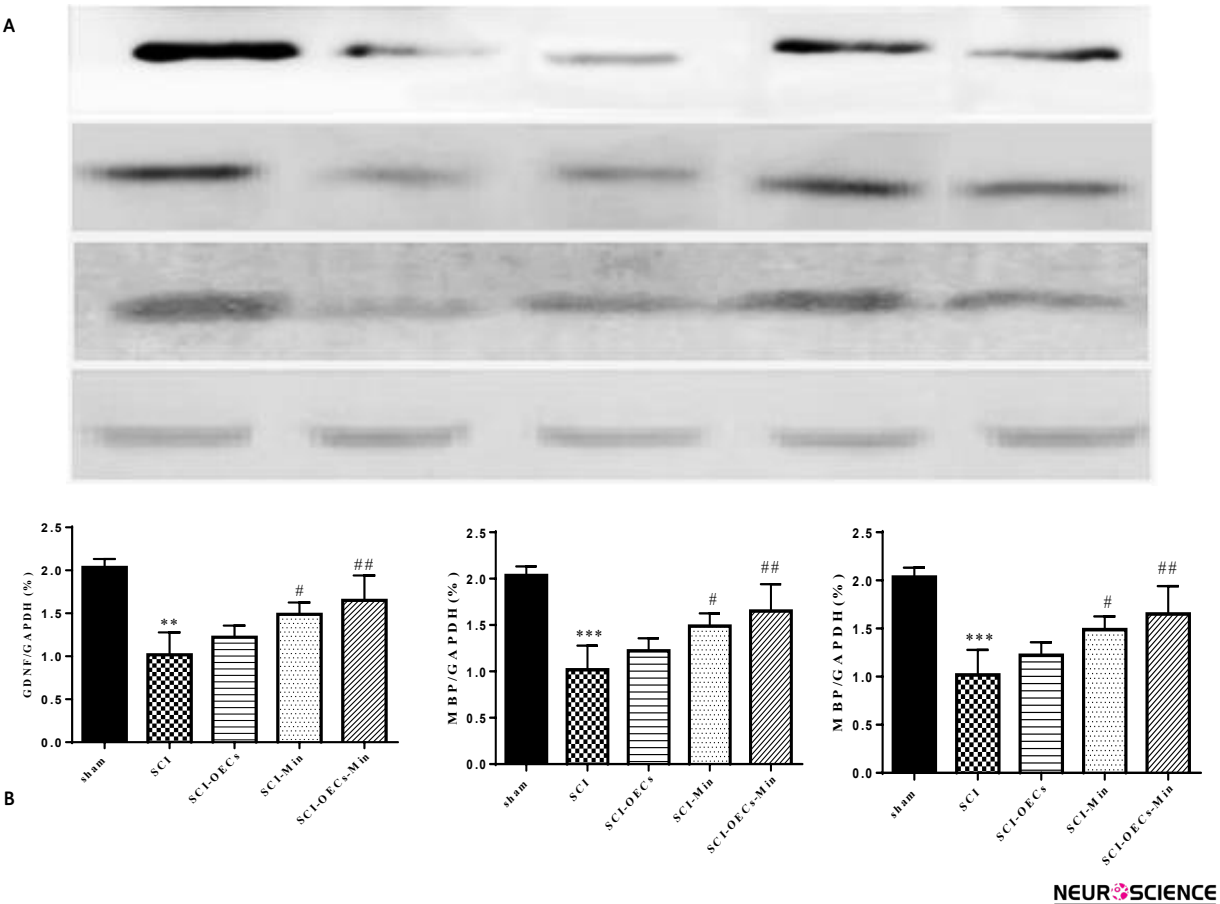

Figure 5. Effect of the combination therapy on BDNF, GDNF and MBP levels of the injured spinal cord

A: Western bloting for BDNF, GDNF and MBP in different groups (n=3); B: Quantification of protein expression of BDNF, GDNF MBP. Data are presented as Mean±SEM; **P<0.01; ***P<0.001 in SCI versus sham group; *P<0.05 in OECs versus SCI group; #P<0.05; ##P<0.01; in Minocycline versus SCI group, and P<0.01; ###P<0.001 in the Min+OECs versus SCI group; $$P<0.01 in SCI-Min versus SCI group.

and OECs on SCI may be related to multiple aspects. For the first time, we found that minocycline injection could upregulate the expression of BDNF and GDNF in a contusion model of spinal cord injury. Our results indicated that combined treatment with OECs and minocycline into the injured spinal cord significantly improved the recovery of locomotor function, reduced spinal cord damage, and resulted in significantly more spared tissue than individual treatment.

Additionally, BBB and EMG analyses found significant improvement in locomotor function in minocycline and minocycline+OECs groups at 5 weeks post-injury. Combined treatment with minocycline and OECs were more effective than individual treatment. In addition, minocycline+OECs significantly increased spared white matter and myelin at the injured epicenter.

Minocycline serves as an antiinflammation agent, protects motoneurons and oligodendrocytes against inflammation, oxidative stress, and apoptosis, and promotes neural regeneration (Ahmad, Zakaria, & Almutairi, 2016; Festoff, Ameenuddin, Arnold, Wong, Santacruz, & Citron, 2006). Furthermore, minocycline has been demonstrated to suppress astrocyte reactivation and reduce neuronal apoptosis following SCI (Festoff et al., 2006).

In this study, to further clarify the mechanism by which minocycline and OECs improved locomotor deficits, the expression of BDNF and GDNF were investigated. BDNF and GDNF are the neurotrophins that play a critical role in the development of the brain and are involved in structural remodeling, neuronal plasticity, and synaptic restructuring (Hellweg & JockersScherübl, 1994). BDNF and GDNF play essential roles in improving locomotor function after SCI through neural protection and neural regeneration by various cellular mechanisms (Jones, Oudega, Bunge, & Tuszynski, 2001).

In the present study, BDNF and GDNF were downregulated in the spinal cord tissue after contusive injury, whereas BDNF and GDNF were upregulated af-

ter minocycline and OECs transplantation. Moreover, statistical analysis sugegsted that the effects of OECs transplantation alone were better than those of SCI and increased BDNF. A combination of OECs and minocycline significantly bettered the above detection indexes compared with the SCI model group at 35 days. Thus, it is speculated that minocycline induces a neuroprotective effect in contusion models of SCI through enhancing the expression of BDNF and GDNF in the spinal cord. This result was consistent with the previous reports that minocycline could up-regulate the expression of BDNF and Nerve Growth Factor (NGF) both in the cerebral cortex and lumbar spinal cord of mice with experimental autoimmune encephalomyelitis (Chen et al., 2012). Besides, minocycline increased the expression of BDNF and improved cognitive suffered from impairment of permanent bilateral occlusion (Zhao et al., 2015).

Moreover, OECs may overcome the injury site inhibition and promote neurite sprouting and outgrowth by providing both an adhesive cellular substrate and permissive soluble factors (Ramón-Cueto & Valverde, 1995; Sonigra, Brighton, Jacoby, Hall, & Wigley, 1999; Barnett & Riddell, 2007) that may serve as the significant factors for promoting nerve growth, regeneration, and repair (Mills, Allchorne, Griffin, Woolf, & Costigan, 2007; Spencer, Mellado, & Filbin, 2008). It is reported that the therapeutic potential of olfactory ensheathing cells in spinal cord repair can enhance using neurotrophins (Wright et al., 2018). The obatined study determined that OECs increased BDNF one week after injury, reduced cell death, and induced neuroprotective effect against inflammation. This has been indicated in NeuN expression and nissl staining. Thus, it is speculated that minocycline and OECs have neuroprotective effects in contusive spinal cord injury by enhancing BDNF and GDNF in the SCI. Combined treatment induces more neuroprotective effects than individual treatment.

We used NeuN as an in vivo marker of mature neuronal survival and nissl staining following SCI to confirm these results. NeuN is an antigen used widely in research and diagnostics to identify postmitotic neurons (Gusel'Nikova & Korzhevskiy, 2015). Moroever, we observed that the NeuN expression and number of nissl staining in the minocycline+OECs group were more significant than in the minocycline and OECs groups. In other words, minocycline enhanced neurotrophic factor levels combined with OECs promote neuronal survival and have the best protective effect on mature neurons.

Besides, a combination of minocycline with OECs transplantation promoted MBP expression and preserved white matter after contusion spinal cord injury than other groups (Figure 4). Thus, up-regulation of MBP expression may reflect the axonal repair response that leads to recovery of the spinal cord function (Ruitenberg et al., 2003). This study has shown that minocycline increases BDNF and GDNF expressions, reducing oligodendrocyte apoptosis and promoting the upregulation of MBP (Ikeda et al., 2002; Koda et al., 2002). One study outlined that minocycline injection improves functional recovery after traumatic spinal cord injury by reducing oligodendrocytes death (Yune et al., 2007). Per previous studies (Li et al., 2011), the results of MBP expression in the OECs transplantation group showed a partial increase in MBP expression and remyelination than SCI group. Several studies have reported that OECs can remyelination of axons; however, it remains unclear whether OECs are directly or indirectly responsible for this process (Franssen, de Bree, & Verhaagen, 2007).

5.Conclusion

In conclusion, the injection of minocycline before OECs graft could increase neurotrophic factors such as BDNF and GDNF that result in a favorable environment for OECs graft and contribute to the ability of OECs to enhance axon regeneration after SCI.

Ethical Considerations

Compliance with ethical guidelines

All experimental protocols were in strict accordance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals.

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Authors' contributions

All authors contributed to preparing this study equally.

Conflict of interest

The authors declared no conflict of interest.

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