Research Paper

Therapeutic Effect of Fibroblast Growth Factor and Olfactory Ensheathing Cells in Rat Models of Spinal Cord Injury

Durai Murugan Muniswami, Bashir Ahmed, George Tharion

Department of Physical Medicine and Rehabilitation, Christian Medical College, Vellore, Tamil Nadu, India

Abstract

Objective: To compare the efficacy of individual and combined treatment of olfactory ensheathing cells (OECs) and acidic fibroblast growth factor (aFGF) in a rat model of spinal cord injury (SCI). **Materials and Methods:** Adult female Albino Wistar rats were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection and T10 laminectomy was performed to expose the spinal cord, before drop-weight injury. Following injury, 2 μg of aFGF was administered at the site of injury and cultured rat olfactory mucosal OEC were transplanted as a single dose of 1 × 106 cells on the 9th day after SCI, used individually as well as in combination therapy. The outcome of the treatments was assessed using the hind-limb motor recovery-Basso, Beattie, Bresnahan (BBB) scale, transcranial motor-evoked potential, and histological studies. **Results:** All the treated groups showed improvement in hind-limb motor recovery when compared to the control group in BBB ($P < 0.05$). There was increased electromyography amplitude in treated rats as compared to controls ($P < 0.05$). Retrograde and anterograde tract tracing showed an increase of preserved axons and possible regeneration. Combination of aFGF with OEC transplantation demonstrated more beneficial effects following SCI than with individual therapy. **Conclusion:** aFGF and OEC transplantation have neuroprotective and regenerative therapeutic potentials for the future clinical application.

Keywords: Basso, Beattie, Bresnahan score, electromyography amplitude, fibroblast growth factor acidic, histology, olfactory ensheathing cell transplantation, spinal cord injury

Introduction

Spinal cord injury (SCI) is a devastating condition with loss of motor and sensory function below the level of the injury. Morbidity is high for the individual, stressful for their family, and there is an enormous economic burden to the society.[1] Primary injury to the spinal cord is caused by mechanical trauma, which is followed by complex pathophysiological cascades such as inflammation, ischemia, free radical creation, and excitotoxicity which all contribute to secondary injury. These orchestrated events lead to cell death, and in addition, astrocytes proliferate, hypertrophy around the injury site, and form dense glial scars. Finally, SCI causes permanent neurological deficits because the injured cord lacks the spontaneous regenerative ability.[2]

There is no treatment option for SCI patients, which can restore the lost function. At present, there are two therapeutic strategies available: (1) To prevent the death of neuronal cells (neuroprotection) and (2) induce regeneration to restore functional recovery after SCI. Research studies focusing on preventing secondary damage are being conducted in various laboratories. They are using neuroprotective agents such as gangliosides,[3] naloxone,[4] lazaroids,[5] calcium channel blockers,[6] methyl-para-tyrosine,[7] and dimethyl sulfoxide.[8] The regenerative approach has gained intense momentum in the last decade. It uses transplantation of cells, such as olfactory ensheathing cell (OEC),[9] mesenchymal stem cells,[10] embryonic stem cells,[11] induced pluripotent stem cells,[12] Schwann cells,[13] neural stem cells,[14] as well as gene therapy.[15]

Following SCI, administration of neurotrophic factors to the injured spinal cord has been shown to promote axonal

Access this article online

Quick Response Code:

Website: www.jpharmacol.com

DOI: 10.4103/jpp.JPP_173_17

Address for correspondence: Durai Murugan Muniswami, Department of Physical Medicine and Rehabilitation, Christian Medical College, Vellore - 632 004, Tamil Nadu, India.
E-mail: durai.morgan@gmail.com

How to cite this article: Muniswami DM, Ahmed B, Tharion G. Therapeutic effect of fibroblast growth factor and olfactory ensheathing cells in rat models of spinal cord injury. J Pharmacol Pharmacother 2018;9:32-9.

Received: 30-12-2017 **Revised:** 17-03-2018 **Accepted:** 05-06-2018

regeneration and functional recovery.[16] Tsai *et al*. proposed that acidic fibroblast growth factor (aFGF) attenuates secondary injury cascades in rat spinal injury, acting as a neuroprotective agent and thus improving functional recovery.[17] Huang *et al*., reported that overexpression of aFGF in the spinal cord injured rats promotes functional recovery.[18] Goldshmit *et al*. demonstrated that adult zebrafish have a remarkable capacity to regenerate spinal cord after injury because of FGF signaling during glial cell morphogenesis causes axonal regeneration.[19] DePaul *et al*. suggest that the peripheral nerve grafting, supplemented with aFGF and chondroitinase, supports the recovery of the lower urinary tract functions in a mouse model of complete transected spinal cord.[20] Furthermore, OEC transplantation along with other strategies such as blockade of myelin inhibitory molecules[21] and neurotrophic factors[22] have shown beneficial effects.

Rats injured with the cervical spinal cord treated with OECs regained breathing and climbing abilities.[23] Moreover, OEC transplantation in spinal cord lesions has demonstrated remyelination,[24] axon sparing,[25] improved axonal conduction,[26] reduction of scar and cavity,[27] and regeneration and functional recovery.[28] Furthermore, OECs which were genetically engineered to produce BDNF elicited regeneration of rubrospinal tract regeneration and functional recovery in rat SCI.[29]

Peripheral nerve graft alone results in deposition of chondroitin sulfate proteoglycan at the junction of injured spinal cord and the graft. However, when aFGF treatment was combined with peripheral nerve graft, there was reduced expression of inhibitory molecules, as well as reduced microglial and astroglial activation. This was because of attenuation of blood–spinal cord barrier permeability in the injured rat spinal cord, suggesting that aFGF provided neuroprotection after SCI.[17,30]

There have been several reports of FGF as a mitogenic and a potent neurotrophic factor with the ability to enhance survival and outgrowth of spinal motor neurons upon axotomy or SCI.[31] To achieve robust functional recovery, aFGF was administered to rescue neuronal cells from death and OEC was transplanted to enhance regeneration after SCI. We aimed to evaluate the effect of acidic FGF and OEC in the SCI rat model individually as well as in combination. The outcome of these treatments was assessed using the motor-recovery scale (Basso, Beattie, Bresnahan [BBB] score), transcranial motor-evoked potential studies, and histological methods.

Materials and Methods

Thirty-six adult female Albino Wistar rats were used for the study. This study was approved by an Institutional Review Board and Institutions animal ethical committee of Christian Medical College, Vellore. The rats were divided into six groups (n = 6 rats each group) and each group received the following interventions:

- Group 1 received acidic FGF immediate after injury (FGF)
- Group 2 received OEC transplantation on the 9th day after SCI (OEC [9])
- Group 3 received aFGF immediate after injury and OEC transplantation on the 9th day (FGF + OEC [9])
- Group 4 received both aFGF and OEC immediate after injury (FGF + OEC)
- Group 5 received only Dulbecco's modified eagle's medium (DMEM) on the 9th day after SCI (Control-DMEM)
- Group 6 received only phosphate-buffered saline (PBS) immediate after SCI (Control-PBS).

Laminectomy and spinal cord injury

Female Albino Wistar rats weighing 100–250 g were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Anesthetized rats were middorsally shaved and cleaned with povidone-iodine. Tegaderm was applied to prevent fur contamination. Laminectomy was performed by a middorsal skin incision, made to expose the spinous process and laminae of T7–T9 vertebrae. The T10 spinal cord level was exposed by removing the spinous process and laminae. The drop-weight injury was created using an impactor device according to our previous study.[32] Contusive injury was created by a 10-g rod falling from 25-cm height on the exposed spinal cord. Following injury, paraspinal muscle and skin were sutured in layers. Neosporin was applied as a topical ointment on the sutured skin and routine postoperative care was given.

Administration of acidic fibroblast growth factor

Recombinant human acidic FGF was purchased from R&D Systems Inc. The aFGF was dissolved in PBS solution (1 μg/μl). A single dose of 2 μg/2 μl of acidic FGF[17] was injected into the injured spinal cord, at the site of the injury epicenter (single point) using a bevel needle attached to a 25-μl Hamilton syringe. This was done immediately after the drop-weight injury in Group 1 (FGF), Group 3 (FGF + OEC [9]), and Group 4 (FGF + OEC). The growth factor solution (2 μg/2 μl) was delivered slowly over 30 s into the injured spinal cord, then after waiting for another 30 s the syringe was pulled out.

Olfactory ensheathing cell transplantation

Ten adult Albino Wistar rats, weighing 100–250 g, were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Then, the olfactory mucosa was removed from the posterior region of nasal septum and OECs were cultured as described.[32] On the day of transplantation, the injured area of the spinal cord was exposed in the anesthetized rats, and the second passage cultured cells were harvested for allogeneic transplantation. Harvested cells (OEC) were injected into the injured spinal cord (2 μl/site in 5 points), in and around the site of injury epicenter at a dose of 1×10^6 cells with the aid of sterile 25-μl Hamilton syringe. The cell suspension was slowly delivered for 3–5 min into the injured spinal cord, and after waiting for another 1 min, the syringe was pulled out. OEC was transplanted on the 9thday after SCI

in the Group 2 (OEC [9]) rats. Group 3 (FGF + OEC [9]) rats received a combination of FGF immediate plus OEC on the 9th day after injury. Group 4 (FGF + OEC) received combined treatment of OEC with acidic FGF injected immediately after SCI. Group 5 and 6 rats served as control. Group 5 was given DMEM into the cord on the 9th day after injury, while Group 6 rats, received PBS immediately after the SCI.

Postoperative care

Following the surgery, the rats were monitored until they recovered from anesthesia. They were observed throughout the postinjury survival period for general health and mobility within the cage, with bladders being manually expressed twice daily. Ringer lactate (5 ml/100 g of body weight) was administered subcutaneously twice daily after each bladder expression for the first 7 postoperative days. The analgesic meloxicam (1 mg/kg) and the antibiotic enrofloxacin (2.5 mg/kg) were administered for the first 7 postoperative days.

Behavioral assessment-Basso, Beattie, Bresnahan score

The BBB scale[33] is an operationally defined 21-point scale, designed to assess functional hind-limb locomotor recovery after impact injury to the spinal cord in rats. This locomotor scale assesses combinations of rat hind-limb joint movements, trunk position and stability, stepping, coordination, paw placement, toe clearance, and tail position. It measures the sequential recovery stages that rats may attain after SCI. The motor assessments were performed for up to 8–10 weeks after injury/transplant. All the rats received bladder expression before open field testing to exclude behavior resulting from bladder fullness. Rats were allowed to walk in the open field (45 cm × 60 cm rectangular tray) and they were videographed. All the rats were assessed for BBB before transplant, that is, on the 9th day after SCI and every week posttransplant onward up to 8–9 weeks.

Motor-evoked potential studies

After 8–10 weeks postinjury/transplant, all the rats were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. The electromyography (EMG) stimulator device and recording software (custom-built data-acquisition system) designed by the Department of Bioengineering, Christian Medical College, Vellore, was used for the motor-evoked potential studies. First scalp fur was removed and then the superficial bipolar electrode was placed on the animal's scalp for transcranial electrical stimulation of its motor cortex and the EMG signals recorded from the hind-limb gastrocnemius muscle by placing needle electrode. The maximum peak attained in the wave pattern was taken as the amplitude and then the mean amplitude value of three random recorded waves was calculated for each animal. Single channel recorded EMG signals were analyzed for amplitude in mV and the data reported as mean ± standard error of the mean (SEM) in all the groups, and then the *P* values compared. This parameter was carried out to evaluate the functional integrity of the spinal cord.

Retrograde tract tracing

To investigate the presence of neurons having descending intact fibers in the injured spinal cord (i.e., across the injury epicenter), a retrograde tracer Fast Blue (FB) (Sigma)[34] was injected 2–4 mm caudal from the site of SCI at multiple sites. FB was administered 8–10 weeks after SCI/transplant ($n = 3$). All the injections were made with the help of pulled glass micropipettes. 5% aqueous FB was injected at about 0.7 μl/site at 8 different sites so that a total volume of 5.6 μl was injected. Paraspinal muscle and skin was sutured and routine postoperative care was given. After 7 days, rats were transcardially perfused with a 4% paraformaldehyde solution. The spinal cord was removed and postfixed in 30% sucrose/PBS at 4°C overnight. Twenty micrometers (μm) thick longitudinal cryosections were cut from the dorsal to the ventral side of the spinal cord, and mounted on poly-L-lysine-coated slides. The blue fluorescence of FB-labeled cell bodies and axons was visualized with confocal microscopy. FB-labeled cell bodies were counted 1.2 mm caudal to the injury epicenter and 1.2 mm rostral to the injury epicenter of both control – (DMEM) and FGF + OEC (9)-treated rats. Visible cell bodies were counted for the purpose of the analysis.

Anterograde tract tracing

To investigate the presence of preserved or regenerated descending fibers in the injured spinal cord (i.e., across the injury epicenter), anterograde tract tracing was performed. After 8–10 weeks postinjury/transplant, rats ($n = 3$) were anaesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. The scalp fur was shaved and cleaned with surgical spirit, and then a mid-sagittal incision was made on the skull. On the right side of the skull, a 1 cm × 1 cm burr hole was made over the corresponding area of hind-limb motor cortex. Biotinylated dextran amine 10% (BDA), MW 10000,[35] was injected in multiple sites along the following coordinates from bregma: 0 mm anterior/1 mm lateral, 0 mm anterior/1.5 mm lateral, 0.5 mm anterior/1 mm lateral, 0.5 mm anterior/1.5 mm lateral, 0.5 mm posterior/1.0 mm lateral, 0.5 mm posterior/1.5 mm lateral, 1 mm posterior/1 mm lateral, and 1 mm posterior/1.5 mm lateral. Three weeks after the injection, rats were transcardially perfused with a 4% paraformaldehyde solution. The spinal cord was removed and postfixed in 30% sucrose/PBS at 4°C overnight. Twenty micrometers (μm) thick cryosections were cut and mounted on poly-L-lysine-slides. Tissue was incubated with 0.3% Triton X-100 in PBS for overnight at 4°C, then washed with PBS and incubated with Streptavidin, Alexa Fluor 568 conjugate (1:400) for 2 h at room temperature. The sections were then washed with PBS and visualized in confocal microscope. This tracing method ensures continuity of the descending corticospinal tract fibers.

Electron microscopy

For transmission electron microscopy (TEM) carried out at the end of follow-up period, control (DMEM) ($n = 1$) and

treated (FGF + OEC [9]), FGF spinal cord tissues ($n = 1$) were dissected in a 1 cm length which contained the injury epicenter. This tissue was processed according to the standard procedure and observed using Bio-Twin TEM.

Statistical analysis

Data from each group were represented as the mean ± SEM statistical analysis was performed using SPSS version 16; Apple computer Inc, Chicago, USA. One-way ANOVA *post-hoc* Tukey was used for statistical analysis of BBB scores and amplitudes of motor-evoked potential. The Mann–Whitney test was used for the number of FB-labeled cell bodies. $P < 0.05$ was considered statistically significant.

Results

Behavioral assessment-Basso, Beattie, Bresnahan score

FGF-treated rats showed a motor recovery of BBB score 4 during the first week and 6.3 at the end of 9th week. In the OEC group, which received OEC transplantation on the 9th day, the BBB score progressed from 0 to 5.3 at the end of the 8th week after transplantation. The FGF + OEC group, which received both FGF and OEC soon after injury, attained a BBB score of 2.5 in the 1st week after SCI and by the end of the 8th week attained a score of 8. The control group did not show any motor recovery. Their BBB score of "0" showed that there was no spontaneous recovery after SCI [Figure 1a].

All treated groups progressed in hind-limb motor recovery. At the end of the follow-up period, the BBB score of OEC (9) was (5.3 ± 1.02), FGF (6.3 ± 1.56), FGF + OEC (9) group (8.3 ± 0.91), and FGF + OEC group (8.0 ± 1.84). The control group (0.16 ± 0.16) did not have an improvement in their BBB score. Control groups (PBS and DMEM) compared with treated groups showed statistical significance ($P < 0.05$) in functional recovery. Functional difference was observed in all the treated groups compared to control groups, but there was no statistically significant difference among the different treated groups [Figure 1b]. OEC and FGF, when used individually, improved functional recovery after SCI to a limited extent. However, in combination, they had a demonstrably better hind-limb motor recovery in spinal cord-injured rat models.

Motor-evoked potential study

Transcranial stimulation of the motor cortex elicited EMG responses in the gastrocnemius muscle of rats which received FGF with OEC [Figure 2b]. DMEM control rats did not show any significant response [Figure 2a]. There was a statistical significance ($P < 0.05$) in the EMG amplitude of the FGF group (1.0 ± 0.09), OEC (9) group (1.2 ± 0.19), FGF + OEC group (1.7 ± 0.33), and FGF + OEC (9) group (1.1 ± 0.19) when compared to the DMEM control group amplitude (0.1 ± 0.03). This indicated that the motor circuit was restored in the treated groups after SCI [Figure 2c]. However, statistical analysis did not show any significant difference among the different treatment groups.

Retrograde tract tracing analysis

There was a marked increase in degenerative cavities in the control cord [Figure 3a] as compared to treated spinal cord [Figure 3b]. The region 1.2 mm caudal to the injury epicenter and 1.2 mm rostral to the epicenter of both treated and control rat spinal cord longitudinal sections was assessed for FB labeled cell bodies [Figure 3]. The number of labeled cell bodies caudal to the injury epicenter was not significantly different ($P < 0.33$) between the control (280 ± 14.1) and the transplanted cord (310 ± 14.1) [Figure 3c]. But on the rostral side of the injury epicenter, the number of labeled cell bodies was greater in OEC + FGF treated (64.1 ± 43.1) cord as compared to control (10.3 ± 6.5). This was statistically significant ($P < 0.01$) [Figure 3d]. This shows that the neuronal tracts are intact and preserved or regenerated so that the dye migrated beyond the injury epicenter in the treated groups.

Anterograde analysis

BDA was injected into the motor cortex of the right hemisphere [Figure 4]. The tracer migrated caudally and was expressed on the contralateral side of the spinal cord; below the injury epicenter in treated FGF + OEC (9) spinal cord [Figure 4c]. There was no expression of BDA in control-DMEM spinal cord [Figure 4b]. A representative phase contrast image of spinal cord shows the grey and white matter [Figure 4a].

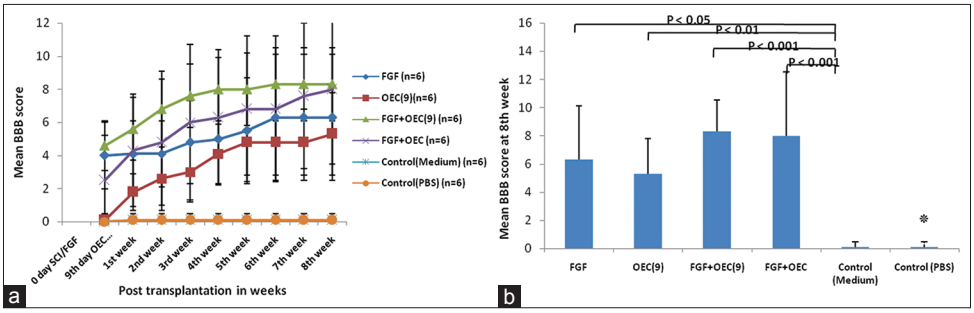

Figure 1: Hind-limb motor recovery following spinal cord injury – Basso, Beattie, Bresnahan score. (a) Mean Basso, Beattie, Bresnahan score of treated and control groups. The treated groups showed sequential hind-limb motor recovery. (b) Basso, Beattie, Bresnahan score at the end of experimental period. Significant differences were observed between treated and control groups, whereas there was no significant difference among treated groups. One-way ANOVA *post hoc* Tukey test, $P < 0.05$. (*) indicates not significant difference compared to Dulbecco's modified eagle's medium control group

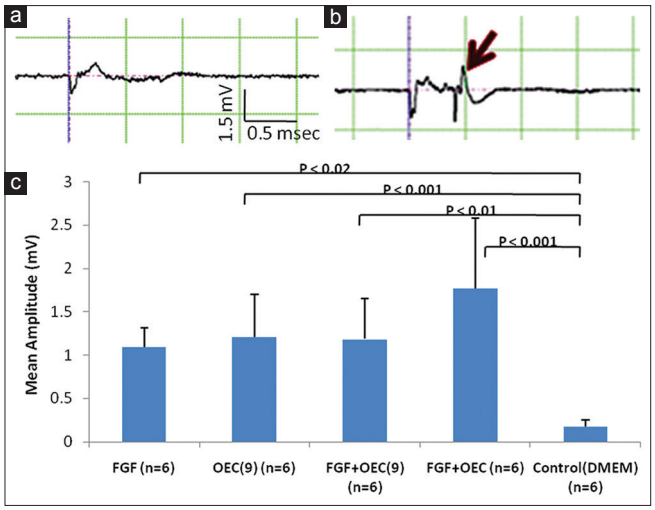

Figure 2: Transcranial electrical stimulation and hind-limb motor-evoked potentials following spinal cord injury. (a and b) Representative electromyography responses in control (Dulbecco's modified Eagle's medium; (a) And treated rats (fibroblast growth factor + olfactory ensheathing cell [9]); (b) At 8th-week posttransplant/spinal cord injury. The violet bars indicate the time of stimulation. Control rats indicated no clear amplitude (a), whereas the treated rats showed electromyography response (arrow, b). (c) Mean electromyography amplitude in treated and control groups. One-way ANOVA and *post hoc* Tukey test, $P < 0.05$

Electron microscopic study

EM showed that axons were surrounded by the nuclear and cytoplasmic processes of transplanted OECs in FGF + OEC (9) group, which could have played a role in enhancing motor recovery of the injured spinal cord [Figure 5b]. Preserved axons with myelin were seen in FGF-treated spinal cord [Figure 5c]. Whereas, untreated cord shows dissolved and demyelinated axons [Figure 5a].

Discussion

Acidic FGF is a potent neurotrophic factor of the spinal cord, which stimulates both survival and sprouting of neuronal cells.[36] After injury, adult central nervous system is able to produce a very limited amount of neurotrophic factors, however, exogenous neurotrophic factor delivery to the injured spinal cord has been shown to promote neuronal survival and regeneration.[37]

OEC has been used in a variety of spinal injury models, including complete transection,[38] hemisection,[23] tract lesion,[39] contusion,[25] and demyelination.[24] In our study, a drop-weight injury was created to mimic a road traffic accident, similar to the other study.[40] Earlier studies have shown that neural stem/progenitor and mesenchymal stem cells transplanted on the 9th-day post-SCI had improved survival and differentiation.[41,42] As a therapeutic window period, we preferred to use the 9th day after SCI for the transplantation of OEC in order to overcome the inflammatory response during the injury acute stage. Direct injection of cells/aFGF into the injured cord at the site of injury, rather than lumbar puncture, was employed to ensure the maximum benefit. Several studies have shown that administration of aFGF after SCI rescues neuronal cells from death by inhibition of astrocyte activation, inflammation, and scar formation.[17] Based on these findings, we decided to use aFGF during acute trauma to the spinal cord as a neuroprotective strategy. Both the FGF and FGF + OEC (9) group showed an acute response in BBB score of approximately 4 after 1 week of FGF administration. The result shows that aFGF administration rescues neuronal cells from some of the detrimental effects of SCI.

Locomotor recovery of the FGF group (6.3 ± 1.56) was better than that of the OEC (9) group (5.3 ± 1.02). The OEC alone-transplanted group showed only minimal recovery, when compared to other treated groups. The FGF + OEC (9) group showed the highest motor recovery 8.3 ± 0.91 compared to the other treated groups. Acute and subacute OEC transplantation in conjunction with aFGF gave a motor-BBB score of 8.0 ± 1.84 in the FGF + OEC group and 8.3 ± 0.91 in the FGF + OEC (9) group. Both combination strategies groups achieved approximately similar BBB scores at the end of the study. One week after aFGF treatment, there was an acute response in the BBB score, which may be due to protection of neuronal cells from death after SCI. Individual treatment strategies resulted in moderate therapeutic effects, but the combined effects of aFGF and OEC shows much more promise in hind-limb motor recovery. This motor behavior was dependent on intact descending spinal cord tracts. These transplanted rats also showed higher amplitude of motor-evoked potentials, which suggests that spinal motor circuits have been intact and regenerated. The amplitude of the FGF + OEC group (1.7 ± 0.33) showed a greater increase than the FGF + OEC (9) group (1.1 ± 0.19), but their in BBB scores were similar. This variation may be due to the number of fibers involved in conduction. However, the amplitude of treated groups was significantly increased as compared to DMEM controls. Since there was a similar BBB score both the control groups (DMEM, PBS), we did not evaluate motor evoked potential in the control (PBS) group. FB dye was injected below the injury epicenter and the dye migrated beyond the injury epicenter. The increased expression of dye on the rostral side indicates that the tracts are intact or have regenerated in treated spinal cord to support the retrograde tracing as compared to control group. Anterograde tracing demonstrated that corticospinal axons from the motor cortex of the brain had connections caudal to the injury epicenter in this group. This strongly suggests that descending corticospinal tracts are preserved and/or regenerated in treated rats. Both anterograde and retrograde tract-tracing study in all the groups and quantification was not done in all the groups. In this study, electron microscopy revealed that transplanted OEC wrapped around the axons of the injured spinal cord and that FGF preserved the axons with myelin. FGF alone-treated spinal cord showed preserved axons with myelin to a lesser extent than with the

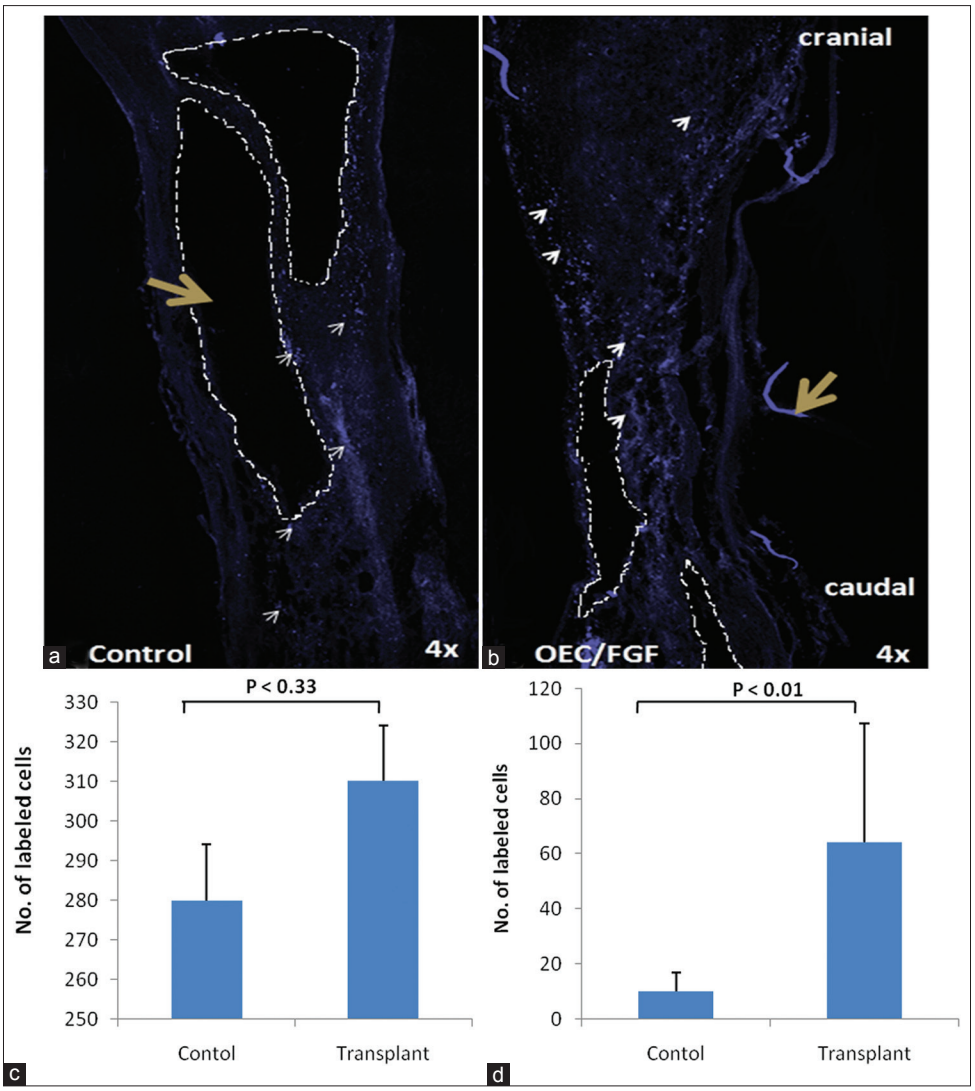

Figure 3: Retrograde analyses of preserved/regenerating neurons in control and fibroblast growth factor/olfactory ensheathing cell-treated rats. (a and b) Representative images of retrograde tracer Fast Blue-labeled rat spinal cord in control (Dulbecco's modified Eagle's medium; (a) and fibroblast growth factor + olfactory ensheathing cell (9) treated rats (b). white arrows, Fast Blue-positive blue fluorescent cell bodies; big arrows, injury epicenter. (c and d) The number of Fast Blue-labeled cell bodies in 1.2 mm caudal (c) and rostral (d) to the injury epicenter. The number of labeled cells were approximately similar in caudal (d, $P < 0.33$), while the number was increased in the rostral area of treated spinal cord (d, $P < 0.01$)

combined treatment. FGF + OEC (9)-treated cord exhibited a greater number of axons with myelin and survival of OEC, which contributes to the increased motor recovery after SCI. In addition to tract-tracing, EM demonstrated preserved axons with myelin and survival of OEC in treated spinal cord, which was not seen in injured control cord. It has been reported from earlier studies that following OEC transplantation into optic nerve lesions[43] and corticospinal tract lesions,[44] the arrangement of OEC is similar to that in the olfactory nerve. Transplantation studies have shown remyelination of demyelinated axons by the human OECs in rat spinal cord.[24]

Here, we report that OEC transplantation promoted functional repair to a limited extent after drop-weight SCI. The combination of OEC transplantation with acidic FGF enhanced functional recovery and axonal regeneration when compared to OECs alone or acidic FGF alone.

Conclusion

Functional recovery was achieved in rats treated with OEC and aFGF into the injured spinal cord by providing trophic support, restoring connectivity, and facilitating regeneration. Our present results suggest new directions for the treatment of spinal cord injuries. OEC can be obtained less invasively from patients for autologous transplantation.

Acknowledgment

Thankful to Christian Medical College, Vellore for all support.

Financial support and sponsorship

This study was financially supported by Department of

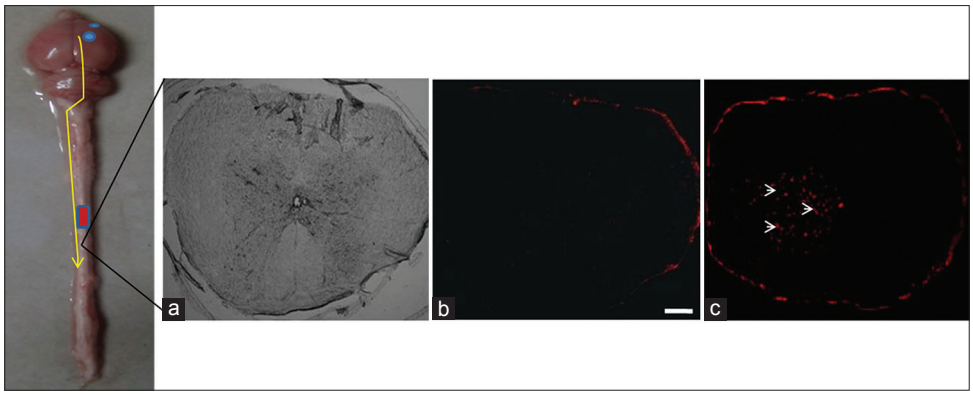

Figure 4: Anterograde tracing of corticospinal tract in control and fibroblast growth factor/olfactory ensheathing cell-treated rats. (a-c) Representative images of biotinylated dextran amine-labeled spinal cords below the injury epicenter of control (b) and treated rat (fibroblast growth factor + olfactory ensheathing cell [9]); (c). A control cord without biotinylated dextran amine staining is shown in (b). Blue color in the left panel indicates injection site of biotinylated dextran amine in the motor cortex and red color rectangle indicates the lesion site. Biotinylated dextran amine labeled axons were observed below the lesion of treated spinal cord on the contralateral side (white arrows) scale bar 200 μm

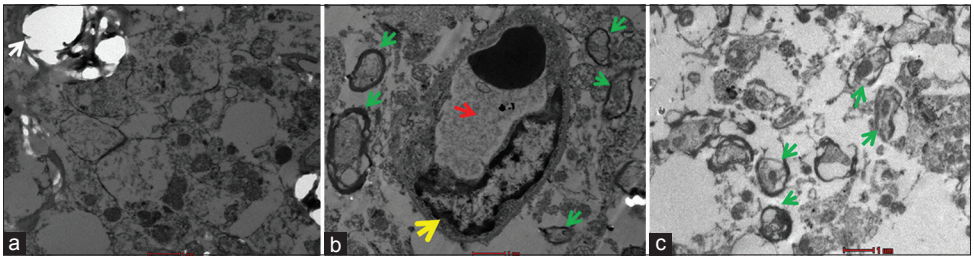

Figure 5: Electron microscopic photomicrographs of the spinal cord of control and fibroblast growth factor/olfactory ensheathing cell-treated rats. (a-c) Representative electron microscopic images of control (Dulbecco's modified Eagle's medium; (a), treated (fibroblast growth factor + olfactory ensheathing cell (9); (b) and fibroblast growth factor; (c)) spinal cords at the end of experimental period. Demyelinated and dissolved axons (white arrows) are observed in control injured spinal cord (a). Olfactory ensheathing cells wrap the axons in transplanted spinal cord (b). Preserved axons with myelin in fibroblast growth factor-treated spinal cord (c). Red arrow, single axon; yellow arrow, olfactory ensheathing cell; green arrow, axons with myelin scale bar 1 μm

Biotechnology, Ministry of Science and Technology, Government of India and Christian Medical College, Vellore.

Conflicts of interest

There are no conflicts of interest.

References

1. Singh A, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG. Global prevalence and incidence of traumatic spinal cord injury. Clin Epidemiol 2014;6:309-31.
2. Yılmaz T, Kaptanoğlu E. Current and future medical therapeutic strategies for the functional repair of spinal cord injury. World J Orthop 2015;6:42-55.
3. Chinnock P, Roberts I. Gangliosides for acute spinal cord injury. Cochrane Database Syst Rev 2005;18:CD004444.
4. Bracken MB, Holford TR. Effects of timing of methylprednisolone or naloxone administration on recovery of segmental and long-tract neurological function in NASCIS 2. J Neurosurg 1993;79:500-7.
5. Kunihara T, Sasaki S, Shiiya N, Ishikura H, Kawarada Y, Matsukawa A, *et al*. Lazaroid reduces production of IL-8 and IL-1 receptor antagonist in ischemic spinal cord injury. Ann Thorac Surg 2000;69:792-8.
6. Winkler T, Sharma HS, Stålberg E, Badgaiyan RD, Gordh T, Westman J, *et al*. An L-type calcium channel blocker, nimodipine influences trauma induced spinal cord conduction and axonal injury in the rat. Acta Neurochir Suppl 2003;86:425-32.
7. Hynes MD, Anderson CD, Gianutsos G, Lal H. Effects of haloperidol, methyltyrosine and morphine on recovery from lesions of lateral hypothalamus. Pharmacol Biochem Behav 1975;3:755-9.
8. Shi R, Qiao X, Emerson N, Malcom A. Dimethylsulfoxide enhances CNS neuronal plasma membrane resealing after injury in low temperature or low calcium. J Neurocytol 2001;30:829-39.
9. Mackay-Sim A, Féron F. Clinical trials for the treatment of spinal cord injury: Not so simple. Methods Mol Biol 2013;1059:229-37.
10. Neirinckx V, Agirman G, Coste C, Marquet A, Dion V, Rogister B, *et al*. Adult bone marrow mesenchymal and neural crest stem cells are chemoattractive and accelerate motor recovery in a mouse model of spinal cord injury. Stem Cell Res Ther 2015;6:211.
11. Lukovic D, Stojkovic M, Moreno-Manzano V, Bhattacharya SS, Erceg S. Perspectives and future directions of human pluripotent stem cell-based therapies: Lessons from Geron's clinical trial for spinal cord injury. Stem Cells Dev 2014;23:1-4.
12. All AH, Gharibani P, Gupta S, Bazley FA, Pashai N, Chou BK, *et al*. Early intervention for spinal cord injury with human induced pluripotent stem cells oligodendrocyte progenitors. PLoS One 2015;10:e0116933.
13. Oudega M, Xu XM. Schwann cell transplantation for repair of the adult spinal cord. J Neurotrauma 2006;23:453-67.
14. Mothe AJ, Tator CH. Review of transplantation of neural stem/progenitor cells for spinal cord injury. Int J Dev Neurosci 2013;31:701-13.
15. Uchida K, Nakajima H, Guerrero AR, Johnson WE, Masri WE, Baba H, *et al*. Gene therapy strategies for the treatment of spinal cord injury. Ther Deliv 2014;5:591-607.
16. Sharma HS. Neurotrophic factors in combination: A possible new therapeutic strategy to influence pathophysiology of spinal cord injury and repair mechanisms. Curr Pharm Des 2007;13:1841-74.

17. Tsai MC, Shen LF, Kuo HS, Cheng H, Chak KF. Involvement of acidic fibroblast growth factor in spinal cord injury repair processes revealed by a proteomics approach. Mol Cell Proteomics 2008;7:1668-87.
18. Huang WC, Kuo HS, Tsai MJ, Ma H, Chiu CW, Huang MC, *et al*. Adeno-associated virus-mediated human acidic fibroblast growth factor expression promotes functional recovery of spinal cord-contused rats. J Gene Med 2011;13:283-9.
19. Goldshmit Y, Sztal TE, Jusuf PR, Hall TE, Nguyen-Chi M, Currie PD, *et al*. Fgf-dependent glial cell bridges facilitate spinal cord regeneration in Zebrafish. J Neurosci 2012;32:7477-92.
20. DePaul MA, Lin CY, Silver J, Lee YS. Peripheral nerve transplantation combined with acidic fibroblast growth factor and chondroitinase induces regeneration and improves urinary function in complete spinal cord transected adult mice. PLoS One 2015;10:e0139335.
21. Rosner J, Avalos P, Acosta F, Liu J, Drazin D. The potential for cellular therapy combined with growth factors in spinal cord injury. Stem Cells Int 2012;2012:826754.
22. Liu Y, Gong Z, Liu L, Sun H. Combined effect of olfactory ensheathing cell (OEC) transplantation and glial cell line-derived neurotrophic factor (GDNF) intravitreal injection on optic nerve injury in rats. Mol Vis 2010;16:2903-10.
23. Li Y, Decherchi P, Raisman G. Transplantation of olfactory ensheathing cells into spinal cord lesions restores breathing and climbing. J Neurosci 2003;23:727-31.
24. Kato T, Honmou O, Uede T, Hashi K, Kocsis JD. Transplantation of human olfactory ensheathing cells elicits remyelination of demyelinated rat spinal cord. Glia 2000;30:209-18.
25. 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. J Neurotrauma 2003;20:1-6.
26. Imaizumi T, Lankford KL, Kocsis JD. Transplantation of olfactory ensheathing cells or Schwann cells restores rapid and secure conduction across the transected spinal cord. Brain Res 2000;854:70-8.
27. Ramer LM, Au E, Richter MW, Liu J, Tetzlaff W, Roskams AJ, *et al*. Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. J Comp Neurol 2004;473:1-5.
28. Mackay-Sim A. Olfactory ensheathing cells and spinal cord repair. Keio J Med 2005;54:8-14.
29. Ruitenberg MJ, Plant GW, Hamers FP, Wortel J, Blits B, Dijkhuizen PA, *et al*. Ex vivo adenoviral vector-mediated neurotrophin gene transfer to olfactory ensheathing glia: Effects on rubrospinal tract regeneration, lesion size, and functional recovery after implantation in the injured rat spinal cord. J Neurosci 2003;23:7045-58.
30. Lee YS, Zdunowski S, Edgerton VR, Roy RR, Zhong H, Hsiao I, *et al*. Improvement of gait patterns in step-trained, complete spinal cord-transected rats treated with a peripheral nerve graft and acidic fibroblast growth factor. Exp Neurol 2010;224:429-37.
31. Tassi E, Walter S, Aigner A, Cabal-Manzano RH, Ray R, Reier PJ, *et al*. Effects on neurite outgrowth and cell survival of a secreted fibroblast growth factor binding protein upregulated during spinal cord injury. Am J Physiol Regul Integr Comp Physiol 2007;293:R775-83.
32. Tharion G, Indirani K, Durai M, Meenakshi M, Devasahayam SR, Prabhav NR, *et al*. Motor recovery following olfactory ensheathing cell transplantation in rats with spinal cord injury. Neurol India 2011;59:566-72.
33. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995;12:1-21.
34. Salegio EA, Pollard AN, Smith M, Zhou XF. Macrophage presence is essential for the regeneration of ascending afferent fibres following a conditioning sciatic nerve lesion in adult rats. BMC Neurosci 2011;12:11.
35. Ueno M, Hayano Y, Nakagawa H, Yamashita T. Intraspinal rewiring of the corticospinal tract requires target-derived brain-derived neurotrophic factor and compensates lost function after brain injury. Brain 2012;135:1253-67.
36. Koshinaga M, Sanon HR, Whittemore SR. Altered acidic and basic fibroblast growth factor expression following spinal cord injury. Exp Neurol 1993;120:32-48.
37. Grill RJ, Blesch A, Tuszynski MH. Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells. Exp Neurol 1997;148:444-52.
38. Tabakow P, Raisman G, Fortuna W, Czyz M, Huber J, Li D, *et al*. Functional regeneration of supraspinal connections in a patient with transected spinal cord following transplantation of bulbar olfactory ensheathing cells with peripheral nerve bridging. Cell Transplant 2014;23:1631-55.
39. Li Y, Field PM, Raisman G. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 1997;277:2000-2.
40. Vijayaprakash KM, Sridharan N. An experimental spinal cord injury rat model using customized impact device: A cost-effective approach. J Pharmacol Pharmacother 2013;4:211-3.
41. Okano H. Stem cell biology of the central nervous system. J Neurosci Res 2002;69:698-707.
42. Parr AM, Kulbatski I, Wang XH, Keating A, Tator CH. Fate of transplanted adult neural stem/progenitor cells and bone marrow-derived mesenchymal stromal cells in the injured adult rat spinal cord and impact on functional recovery. Surg Neurol 2008;70:600-7.
43. Li Y, Sauvé Y, Li D, Lund RD, Raisman G. Transplanted olfactory ensheathing cells promote regeneration of cut adult rat optic nerve axons. J Neurosci 2003;23:7783-8.
44. Li Y, Field PM, Raisman G. Regeneration of adult rat corticospinal axons induced by transplanted olfactory ensheathing cells. J Neurosci 1998;18:10514-24.