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ABOUT COVER

Editorial Board Member of *World Journal of Stem Cells*, Dr. Mohammed Grawish is a Distinguished Professor at Mansoura University and Vice-Dean for Community Services and Environmental Affairs at Delta University for Science and Technology (Egypt). Dr. Grawish received his Bachelor's degree (1990), Master's degree in Oral Biology (1998), and his PhD (2003) from the Faculty of Dentistry, Mansoura University. After, he worked as Lecturer in the Al-Gabl Al-Garby University (2005-2008; Gehrian, Libya) and as Associate Professor in the King Saud University (2011-2013; Riyadh, Saudi Arabia). His ongoing research interests focus mainly on the appropriate therapeutic use of stem cells in dentistry, the design and characterization of biomaterials as scaffold materials for loading stem cells, and the application of complementary and alternative medicine as an adjunctive treatment to traditional medicine for oral diseases. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells (WJSC, World J Stem Cells)* is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, etc.

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Basic Study

Spinal cord injury regeneration using autologous bone marrow-derived neurocytes and rat embryonic stem cells: A comparative study in rats

Mir Sadat-Ali, Dakheel A Al-Dakheel, Ayesha Ahmed, Haifa A Al-Turki, Abdallah S Al-Omran, Sadananda Acharya, Methal I Al-Bayat

ORCID number: Mir Sadat-Ali 0000-0001-8590-0830; Dakheel A Al-Dakheel 0000-0002-3100-3381; Ayesha Ahmed 0000-0002-2036-7067; Haifa A Al-Turki 0000-0002-7472-7304; Abdallah S Al-Omran 0000-0002-4578-2769; Sadananda Acharya 0000-0003-2106-0136; Methal I Al-Bayat 0000-0002-8514-8957.

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Mir Sadat-Ali, Department of Orthopedic Surgery, Imam Abdulrahman Bin Faisal University and King Fahd Hospital of the University, Dammam 31952, Saudi Arabia

Dakheel A Al-Dakheel, Department of Orthopedic Surgery, Imam Abdulrahman Bin Faisal University, AlKhobar 31952, Saudi Arabia

Ayesha Ahmed, Methal I Al-Bayat, Department of Pathology, Imam Abdulrahman Bin Faisal University, Dammam 31952, Saudi Arabia

Haifa A Al-Turki, Department of Obstetrics and Gynecology, Imam Abdulrahman Bin Faisal University, Dammam 31142, Saudi Arabia

Abdallah S Al-Omran, Department of Orthopedic Surgery, Imam Abdulrahman Bin Faisal University and King Fahd Hospital of the University, AlKhobar 31952, Saudi Arabia

Sadananda Acharya, Department of Public Health, College of Public Health, Imam Abdulrahman Bin Faisal University, Dammam 31441, Saudi Arabia

Corresponding author: Mir Sadat-Ali, BM BCh, FRCS (Gen Surg), MBBS, MSc, PhD, Full Professor, Doctor, Department of Orthopedic Surgery, Imam Abdulrahman Bin Faisal University and King Fahd Hospital of the University, PO Box 40071, Dammam 31952, Saudi Arabia. drsadat@hotmail.com

Abstract

BACKGROUND

Spinal cord injury (SCI) is an important cause of traumatic paralysis and is mainly due to motor vehicle accidents. However, there is no definite treatment for spinal cord damage.

AIM

To assess the outcome of rat embryonic stem cells (rESC) and autologous bone marrow-derived neurocytes (ABMDN) treatment in iatrogenic SCI created in rats, and to compare the efficacy of the two different cell types.

METHODS

authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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The study comprised 45 male Wistar rats weighing between 250 and 300 g, which were divided into three groups, the control, rESC and ABMDN groups. The anesthetized animals underwent exposure of the thoracic 8th to lumbar 1st vertebrae. A T10-thoracic 12th vertebrae laminectomy was performed to expose the spinal cord. A drop-weight injury using a 10 g weight from a height of 25 cm onto the exposed spinal cord was conducted. The wound was closed in layers. The urinary bladder was manually evacuated twice daily and after each evacuation Ringer lactate 5 mL/100 g was administered, twice daily after each bladder evacuation for the first 7 postoperative days. On the 10th day, the rats underwent nerve conduction studies and behavioral assessment [Basso, Beattie, Brenham (BBB)] to confirm paraplegia. Rat embryonic stem cells, ABMDN and saline were injected on the 10th day. The animals were euthanized after 8 wk and the spinal cord was isolated, removed and placed in 2% formalin for histopathological analysis to assess the healing of neural tissues at the axonal level.

RESULTS

All the animals tolerated the procedure well. The BBB scale scoring showed that at the end of the first week no recovery was observed in the groups. Post-injection, there was a strong and significant improvement in rats receiving rESC and ABMDN as compared to the control group based on the BBB scale, and the Train-of-four-Watch SX acceleromyography device exhibited statistically significant ($P < 0.0001$) regeneration of neural tissue after SCI. Histological evaluation of the spinal cord showed maximum vacuolization and least gliosis in the control group compared to the rESC and ABMDN treated animals. In the ABMDN group, limited vacuolization and more prominent gliosis were observed in all specimens as compared to the control and rESC groups.

CONCLUSION

This study provided strong evidence to support that transplantation of rESC and ABMDN can improve functional recovery after iatrogenic SCI. The transplanted cells showed a beneficial therapeutic effect when compared to the control group.

Key Words: Spinal cord injury; Regeneration; Embryonic stem cells; Autologous bone marrow-derived neurocytes; Iatrogenic; Stem cells

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Core Tip: We performed a comparative study of rats with iatrogenic SCI treated with control, rat embryonic stem cells and autologous bone marrow-derived neurocytes. Regeneration was assessed using three classic parameters and it was found that neurocytes were superior in the regeneration process.

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INTRODUCTION

Spinal cord injury (SCI) is an important cause of traumatic paralysis and is mainly due to motor vehicle accidents. It is reported that in United States over 200000 patients suffer injury to the spinal cord and over 50% of spinal cord injured individuals are considered paraplegic and about 48% quadriplegic. Approximately > 10000 new injuries occur each year of which 82% occur in males and 56% of injuries occur in young people < 30 years^[1]. The total direct cost for care of spinal cord injured patients in the United States is approximately \$7.736 billion^[2]. The yearly incidence of traumatic

SCI worldwide is about 15-40 cases *per million*^[3]. Despite serious preventive measures to reduce the injuries causing traumatic paraplegia, the incidence of such injuries is increasing. In addition, although many treatments to reverse total paralysis have been tried, we currently do not have effective therapies to reverse this disabling condition.

Spinal cord bypass surgery using peripheral nerves^[4], olfactory ensheathing cells^[5,6] Schwann cells for transplantation^[7] and neural stem cells (NSC)^[8] have been performed. Studies in animals have shown improvements in injured spinal cord function when cells derived from the embryonic central nervous system of mice were transplanted^[9]. In another report, adult stem cells from the central nervous system were also used with limited success^[10-12]. Recently Syková *et al.*^[13] (2006) injected mesenchymal stem cells (MSCs) into the spinal cord in rats with iatrogenic injury and found acceptable functional recovery, which was confirmed histologically. These results were similar to those reported by Kang *et al.*^[14]. In a recent review, Han *et al.*^[15] (2019) reported the extraction of MSCs from various sources, which included bone marrow, adipose tissue, synovium, umbilical cord and human blood. In animal models, MSCs-based therapies had a beneficial effect on locomotor recovery^[16,17], but the results could not be replicated or *in vitro*.

Expanded MSCs have a short life span and they disappear within 24 h^[18], while terminal cells such as neurocytes remain alive for about 90 d.

Our objective was to study the outcome of rat embryonic stem cells (rESC) and autologous bone marrow-derived neurocytes (ABMDN) in iatrogenic SCI created in rats, and to compare the efficacy of the two different cell types.

MATERIALS AND METHODS

This study was carried out following approval by the Institutional Review Board of the University of Dammam, which was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Surgical steps

A drop weight device was standardized, which consisted of a 10 g weight dropped from a height of 25 cm. The study included 45 male Wistar rats weighing between 250 and 300 g (Figure 1). The animals were kept in the similar cages and had similar feeding habits. They were divided into the following three groups: Group I was the control group, Group II was the rESC group and Group III was the ABMDN group. rESC were obtained from Celprogen Inc., Torrance, CA, United States and ABMDN were harvested at the university's stem cell laboratory. The animals were anesthetized with ketamine 50 mg/kg body weight and xylazine 35 mg/kg). Sterile eye drops were instilled to keep the eyes moist during surgery. The fur was clipped and the surgical area was cleaned with betadine. An incision was made over the lower thoracic area (Figure 2), muscle and connective tissues were bluntly dissected to expose the thoracic 8th to lumbar 1st vertebrae. A T10-thoracic 12th vertebrae laminectomy was performed, avoiding any injury to the spinal cord. A 10 g weight was dropped from a height of 25 cm onto the exposed spinal cord (Figure 3A and B). The wound was closed in layers. Diclofenac 1 mg/kg body weight as an analgesic and floxacillin 3 mg/kg body weight were given. Postoperatively, Ringer lactate 5 mL/100 g was given subcutaneously. Following surgery, the rats were monitored thrice a day for one week. The urinary bladder was manually evacuated twice daily and Ringer lactate 5 mL/100 g was administered for the first week after surgery.

On the 10th day after creation of the SCI all the rats underwent nerve conduction studies and behavioral assessment (Basso, Beattie, Bresnahan [BBB])^[19] to confirm paraplegia. The BBB scale, which was described in 1995, is a 21-point scale which assesses trunk stability, hind limb movements, stepping with coordination and clearance of toes, and tail position. Following the drop weight SCI, injection of rESC, ABMDN or saline was administered on the 10th day. Behavioral assessment using the BBB scale was conducted prior to cell transplantation. Once paraplegia was confirmed the injured site was reopened and in the controls 1 mL of normal saline (Group I), 4 million rESC (Group II) and 4 million ABMDN (Group III) was injected. The wound was closed in layers. At the end of the 8-wk period nerve conduction tests were performed using Train-of-four (TOF)-Watch SX, which is the current "clinical gold standard", for measuring recovery of nerve blockade. In TOF nerve stimulation, the ratio of the fourth muscle contraction (twitch) to the first twitch is recorded; when this ratio is 90% (or 0.9) or greater, full recovery of muscle strength is present indicating nerve recovery.



Figure 1 Surgical marking to identify lower thoracic and upper lumbar spine.

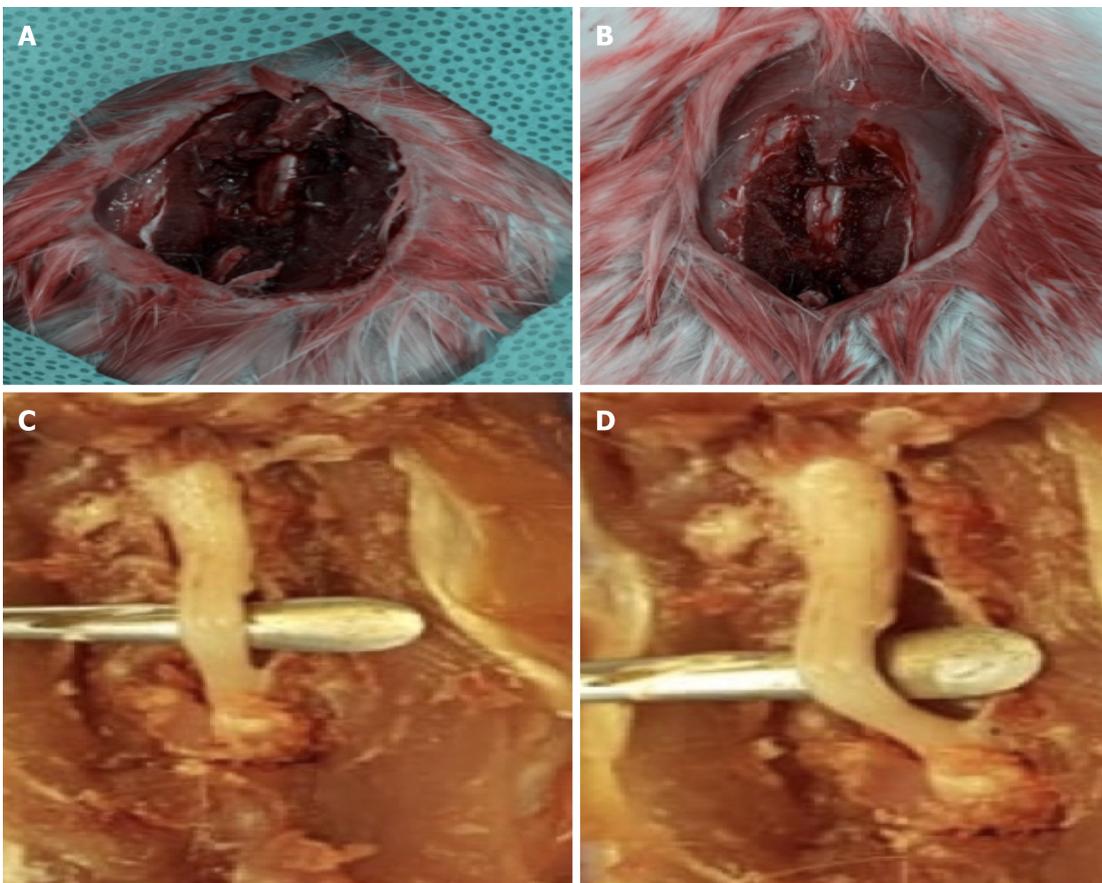


Figure 2 Surgical images. A and B: Surgical image of the spinal cord; C and D: The spinal cord before removal.

The animals were euthanized after 8 wk and spinal cords were isolated (Figure 4A and B) and placed in 2% formalin for histopathological analysis to assess healing of neural tissues at the axonal level.

RESULTS

All the animals tolerated the procedure well. Eight weeks after implantation, the assessment of locomotion by BBB and electromyographic studies was carried out and animals were then euthanized and spinal cord was dissected out and sent for histopathological studies.

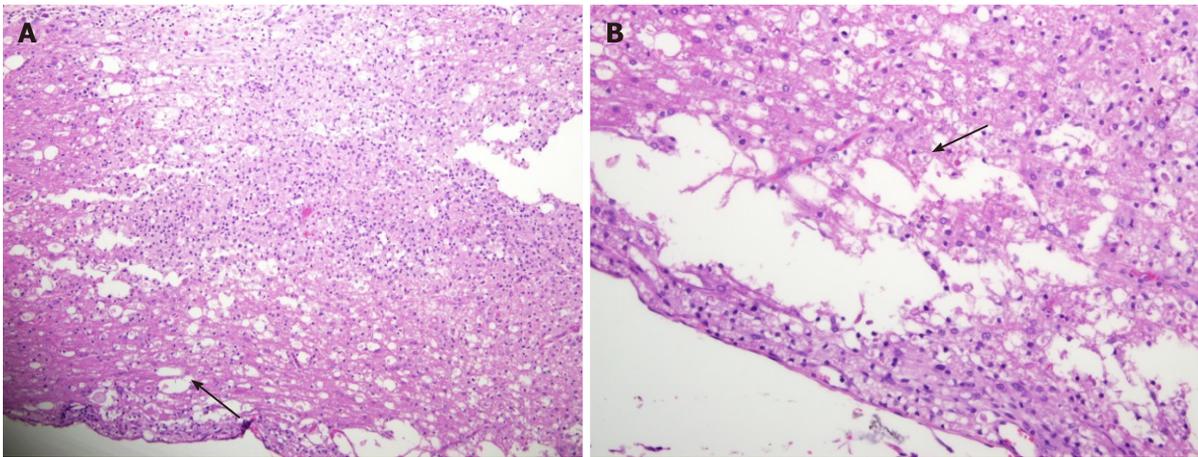


Figure 3 Control group showing extensive vacuolization. A: H&E × 200; B: H&E × 400. Highlighted by a single arrow.

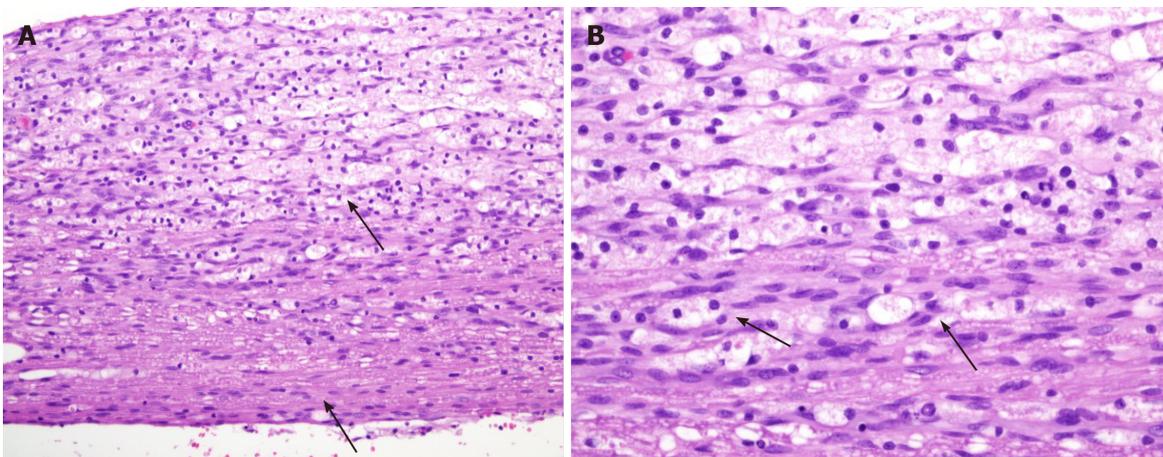


Figure 4 Control group showing minimum gliosis. A: H&E × 200; B: H&E × 400. Highlighted by a single arrow.

Recovery of locomotion

BBB scale scoring showed that at the end of the first week, no recovery was observed in the groups and by the 1st week there was recovery in the rESC and ABMDN groups. There was a significant improvement in rats receiving ESC and ABMDN. In the rESC group the final score was 11/21, was 13/21 in the ABMDN group and was 2/21 in the control group (Table 1). A statistically significant difference between the control group and the rESC group was observed ($P < 0.0001$), between the control group and the ABMDN group ($P < 0.0001$) and between the rESC group and the ABMDN group ($P < 0.01$). Overall, the transplanted cells resulted in a remarkable recovery at 8 wk.

Electromyography studies

The TOF-Watch SX acceleromyography device (Organon, Ireland) is accepted as the standard monitor for use in clinical trials, and was used to assess the improvement in neural function in the lower limbs. In the rESC group, the amplitude was statistically significant in the rESC and ABMDN groups as compared with the control group ($P < 0.0001$), exhibiting regeneration of neural tissue after SCI (Tables 2-4). Table 5 shows the results of a comparison between the three groups, which revealed that recovery was much better in the ABMDN group followed by the rESC group.

Histological studies

Cross-section of the spinal cord showed dissolved and degenerated cells with the formation of varying extents of vacuolization and reactive gliosis in the control group (Figures 3 and 4), whereas the spinal cords in rESC transplanted animals were smaller and further apart with more reactive gliosis and no tumor formation (Figures 5 and 6). In the ABMDN group, limited vacuolization and more prominent gliosis were

Table 1 Basso, Beattie, Brenham scale following treatment with control, rat embryonic stem cells and autologous bone marrow-derived neurocytes

Week	Control	rESC	ABMDN
1	0	0	0
2	0	0	3
3	0	3	3
4	1	3	4
5	1	5	6
6	1	7	8
7	2	7	10
8	2	11	13

rESC: Rat embryonic stem cells; ABMDN: Autologous bone marrow-derived neurocytes.

Table 2 Train-of-four-Watch record for the control group

	Mode	Tw1 (%)	Tw2 (%)	Tw3 (%)	Tw4 (%)	TOF (%)	Temp (°C)	Stim (mA)	T (μs)	Sens.
1	TOF	4	0	0	1	25	33.6	50	200	157
2	TOF	0	0	0	0		33.1	50	200	157
3	TOF	0	0	0	0		32.6	50	200	157
4	TOF	0	0	0	0		33.3	50	200	157
5	TOF	0	0	0	0		32.7	50	200	157
6	TOF	0	0	0	0		32.7	50	200	157
7	TOF	6	3	3	2	33.3	32.9	50	200	157
8	TOF	4	3	0	3	52	33.8	50	200	157
9	TOF	0	0	0	0		32.4	50	200	157
10	TOF	0	0	0	0		32.0	50	200	157
11	TOF	0	0	0	0		32.7	50	200	157
12	TOF	5	0	2	3	60	33.1	50	200	157
13	TOF	4	3	3	0		32.9	50	200	157
14	TOF	0	0	0	0		33.1	50	200	157
15	TOF	7	3	4	3	42	33.6	50	200	157

TOF: Train-of-four.

observed in all specimens compared to the controls (Figures 7 and 8). Overall, the specimens exposed to ABMDN demonstrated less pronounced vacuolization and enhanced reactive gliosis as compared to specimens exposed to rESC. This was further confirmed by glial fibrillary acidic protein staining (Figure 9).

DISCUSSION

Our study found that iatrogenic SCI can functionally and neurologically recover after rESC and ABMDN transplantation compared with the control group. When the outcome in the rESC and ABMDN groups was compared, the latter showed better results on the BBB scale, electromyographic and histological analysis. Ye *et al*^[20] (2018) conducted a trial using bone mesenchymal stem cells (BMSCs) and BMSC neural-like cells and showed that human cerebrospinal fluid-derived neural-like cells fared better

Table 3 Train-of-four-Watch record for the rat embryonic stem cells group

	Mode	Tw1 (%)	Tw2 (%)	Tw3 (%)	Tw4 (%)	TOF (%)	Temp (°C)	Stim (mA)	T (μs)	Sens.
1	TOF	85	81	67	86	96	32.8	50	200	157
2	TOF	87	43	56	60	86	33.1	50	200	157
3	TOF	76	57	52	42	68	33.2	50	200	157
4	TOF	79	71	78	65	82	32.8	50	200	157
5	TOF	65	77	43	59	46	33.1	50	200	157
6	TOF	52	57	52	42	80	32.2	50	200	157
7	TOF	43	77	75	75	89	31.8	50	200	157
8	TOF	33	34	34	30	90	31.1	50	200	157
9	TOF	52	57	52	42	81	31.2	50	200	157
10	TOF	66	48	69	24	88	32.8	50	200	157
11	TOF	33	34	34	65	90	33.1	50	200	157
12	TOF	52	57	52	42	80	33.2	50	200	157
13	TOF	75	71	72	24	88	32.8	50	200	157
14	TOF	39	34	55	70	76	32.1	50	200	157
15	TOF	56	57	52	42	75	33.6	50	200	157

(Twitch 4/Twitch 1) × 100 (%). TOF: Train-of-four.

Table 4 Train-of-four-Watch record for the autologous bone marrow-derived neurocytes group

	Mode	Tw1 (%)	Tw2 (%)	Tw3 (%)	Tw4 (%)	TOF (%)	Temp (°C)	Stim (mA)	T (μs)	Sens.
1	TOF	45	61	66	44	67	32.1	50	200	157
2	TOF	39	24	34	28	71	32.5	50	200	157
3	TOF	58	53	42	32	55	33.2	50	200	157
4	TOF	62	61	87	45	72	32.8	50	200	157
5	TOF	49	67	35	34	57	32.9	50	200	157
6	TOF	60	59	51	50	69	32.7	50	200	157
7	TOF	61	23	29	43	70	33.2	50	200	157
8	TOF	66	43	61	39	59	32.6	50	200	157
9	TOF	52	49	51	44	84	31.9	50	200	157
10	TOF	67	31	27	37	55	32.3	50	200	157
11	TOF	39	43	54	29	74	32.8	50	200	157
12	TOF	65	64	57	35	45	32.7	50	200	157
13	TOF	58	61	59	61	67	32.9	50	200	157
14	TOF	53	60	51	49	77	32.1	50	200	157
15	TOF	62	59	56	61	79	32.6	50	200	157

(Twitch 4/Twitch 1) × 100 (%). TOF: Train-of-four.

when the cells were transplanted into the lateral ventricles. Our results also confirmed that ABMDN resulted in a better outcome than rESC. Muniswami *et al*^[21] (2018) used bone marrow-derived MSCs and found that these cells improved healing of the injured spinal cord.

The question arises whether to use MSCs or rESC or the terminal neurocytes. When

Table 5 Comparison of electromyographic recording in the three groups

Variable	Control	rESC	ABMDN
Twitch1 height	2	49.5	52.9
Twitch2 height	0.8	50	48.7
Twitch3 height	0.8	51	43.7
Twitch4 height	0.8	42	38.9
TOF ratio = (Twitch 4/Twitch 1) × 100 (%)	47.6 ± 20.3	69.33 ± 13.9	80.2 ± 11.9
Stimulation current (60 mA)	50	50	50
Pulse width (200 μs)	200	200	200

rESC: Rat embryonic stem cells; ABMDN: Autologous bone marrow-derived neurocytes; TOF: Train-of-four.

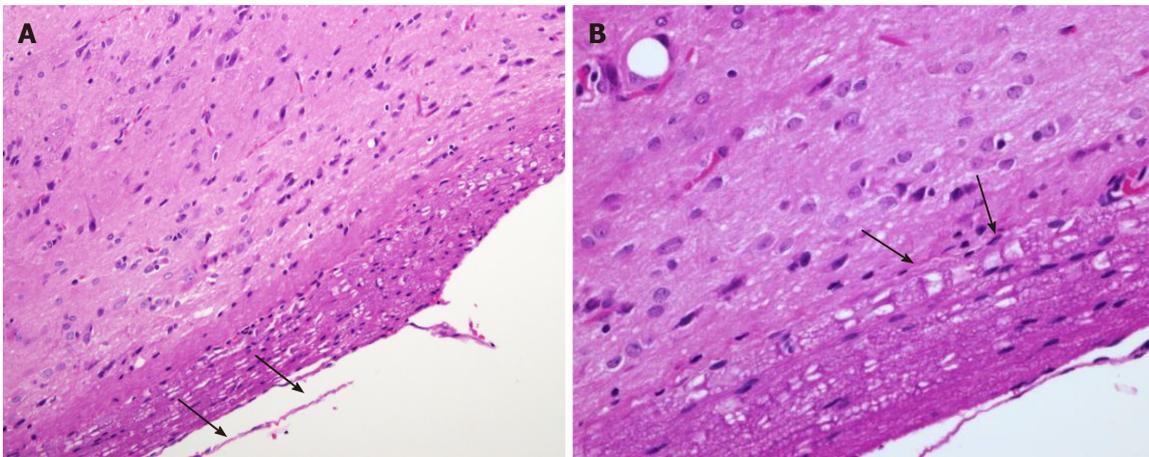


Figure 5 Spinal cord injury treated with rat embryonic stem cells showing vacuolization at the peripheral area of a longitudinal section of the spinal cord. A: H&E × 200; B: H&E × 400.

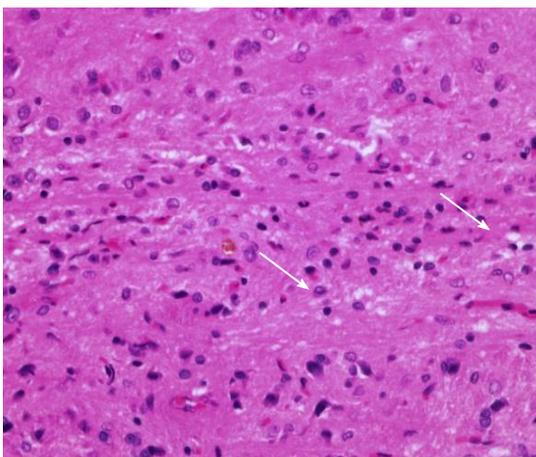


Figure 6 Section (× 400 HPF) shows reactive gliosis in rat embryonic stem cells-treated spinal cord injury.

MSCs enter the circulation, they need to be transformed to terminal cells for reasonable function. During the course of transformation to neurocytes, transformation can be arrested at any time. Secondly, rESC when given intravenously can differentiate into any cell type with a short life span and carry the risk of teratoma formation^[22]. The life span of neurocytes has been reported to be between 90-120 d with no risk of tumor formation as these are autologous derived cells.

Jung *et al*^[23] (2009) compared allogeneous, autogenous, and control stem cells, and

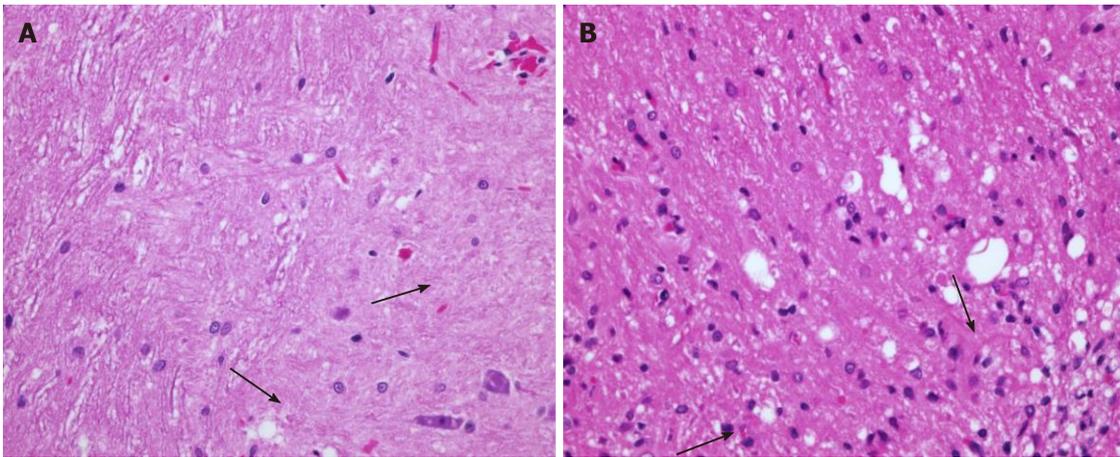


Figure 7 Spinal cord injury treated with autologous bone marrow-derived neurocytes showing limited vacuolization. A: H&E × 200; B: H&E × 400.

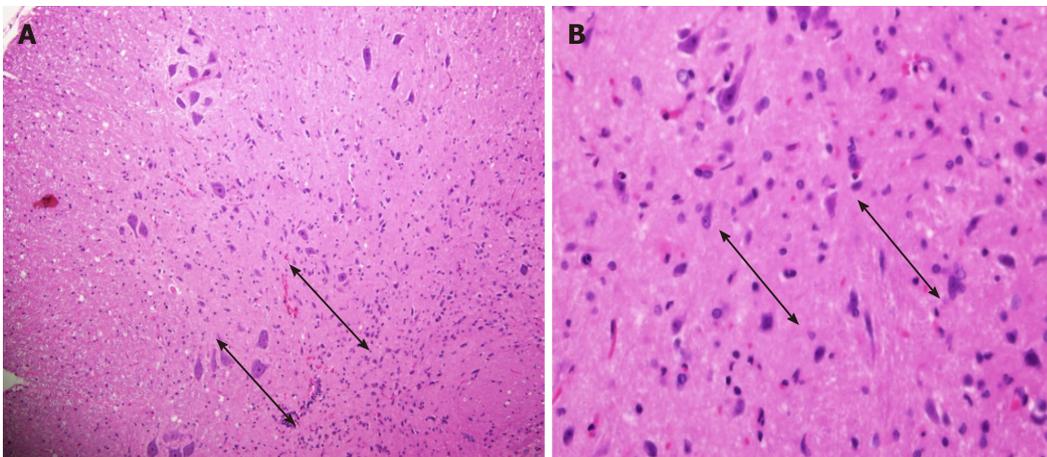


Figure 8 Autologous bone marrow-derived neurocytes-treated spinal cord injury showing prominent gliosis. A: H&E × 200; B: H&E × 400.

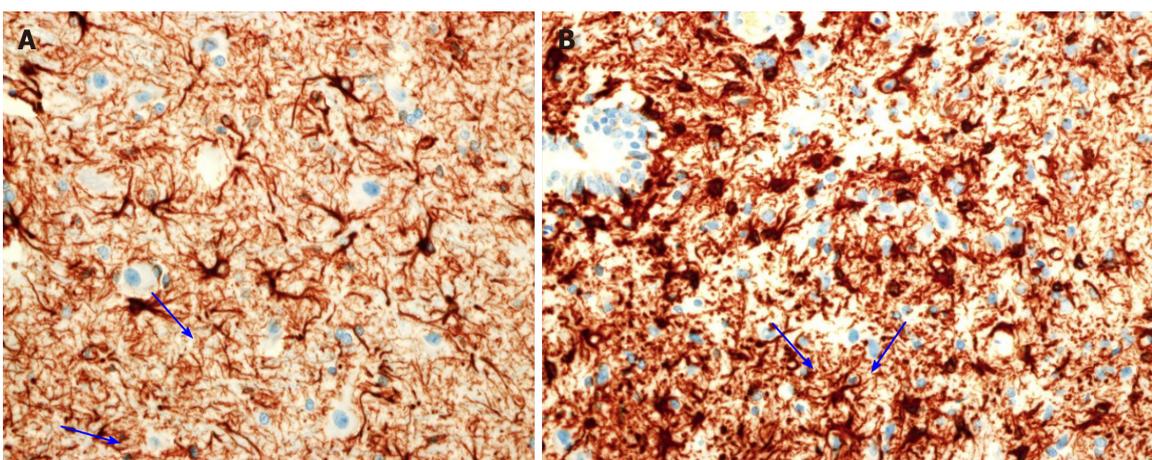


Figure 9 Comparison of glial fibrillary acidic protein staining. A: Rat embryonic stem cells; B: Autologous bone marrow-derived neurocytes.

reported that in the control group the recovery was minimal compared to either the allogeneous or autogenous stem cell group. This indicates that allogeneous stem cells do work but the immune reactions need to be overcome.

Different routes of administration for cell therapies in SCI have been tried. Amemori *et al*^[24] (2015) injected NSCs either intraspinally or directly into the subarachnoid space

of rats and found that both methods resulted in recovery. The report by Cheng *et al*^[25] (2012) further supported this method of injection with acceptable recovery.

In the present study, we transplanted the cells directly at the injury site with improved results. Our results are similar to those reported by other researchers in the field. Intravenous administration of hNSCs has been reported to have good therapeutic potential, is less invasive, and avoids a second surgery. This type of therapy needs to be investigated further^[26,27]. The literature on stem cell therapy has shown that it is quite promising and can be used in many diseases after appropriate controlled trials. Of these diseases, SCI has shown slow recovery following many modes of treatment. The use of stem cell therapy in SCI in recent years has shown improvement in neural function, which was once believed to be unachievable^[28,29].

Recently, trials to heal SCI have used different types of cells for transplantation, including neural stem/progenitor cells (NS/PCs)^[16,30], Schwann cells^[31], olfactory ensheathing glia^[32], MSCs^[33] and oligodendrocyte progenitor cells^[34] with varying success. The use of scaffolds especially biological scaffolds is emerging as a potential game changer for reducing necrosis, local inflammatory reactions, otherwise could increase necrosis at the site of SCI^[35]. Recently, Koffler *et al*^[36] (2019) convincing showed that 3D biomimetic scaffolds with the use of neural progenitor cells enhanced growth and regeneration in host cells. For the first time we used ABMDN in rats and these cells provided better results than rESCs even without a scaffold. It is probable that recovery using a scaffold could lead to further improvement.

CONCLUSION

In conclusion, the present study provides strong evidence, based on the BBB scale, electromyographic and histopathological studies, to support that transplantation of ABMDN could improve functional recovery after iatrogenic SCI. The transplanted ABMDNs showed a beneficial therapeutic effect. As recovery was $\geq 60\%$ in 8 wk, this is sufficient to warrant large-scale, randomized controlled studies to evaluate their efficacy in larger animals and in humans.

ARTICLE HIGHLIGHTS

Research background

Different methods have been used to heal damaged neural tissue and the results have not been promising.

Research motivation

Many young people have been paralyzed due to trauma and adequate treatment to heal neural tissue is unavailable.

Research objectives

To heal damaged neural tissue using stem cells.

Research methods

Wistar rats were paralyzed using direct trauma and then treated with rat embryonic stem cells (rESC), autologous bone marrow-derived neurocytes (ABMDN) and saline and the results were observed using electromyography and histology.

Research results

ABMDN resulted in significantly better recovery than rESC.

Research conclusions

ABMDN led to significantly better recovery than rESC in rats with iatrogenic SCI.

Research perspectives

These findings provide adequate evidence for conducting trials in larger animals followed by phase I human trials.

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